Bulgarian Journal of Veterinary Medicine, 2015, **18**, No 3, 239–247
ISSN 1311-1477; DOI: 10.15547/bjvm.836

Original article

DETERMINATION OF BETA-HAEMOLYTIC ACTIVITY AND MINIMUM INHIBITORY CONCENTRATIONS OF ANTIMICROBIAL DRUGS AGAINST *AEROMONAS HYDROPHILA* STRAINS ISOLATED FROM FISH PRODUCTS

D. STRATEV[1], I. VASHIN[1] & H. DASKALOV[2]

[1]Department of Food Hygiene and Control, Veterinary Legislation and Management, Faculty of Veterinary Medicine, Stara Zagora, Bulgaria;
[2]NDRVMI, Bulgarian Food Safety Agency, Sofia, Bulgaria

Summary

Stratev, D., I. Vashin & H. Daskalov, 2015. Determination of beta-haemolytic activity and minimum inhibitory concentrations of antimicrobial drugs against *Aeromonas hydrophila* strains isolated from fish products. *Bulg. J. Vet. Med.,* **18**, No 3, 239–247.

The purpose of the study was to investigate the prevalence of β-haemolytic *Aeromonas hydrophila* strains in 83 samples from cooled horse mackerel, rainbow trout, Black sea roach, silver carp, vacuum-packed rainbow trout fillets and 20 samples frozen rainbow trout and to establish the minimum inhibitory concentrations (MIC) of several antimicrobial drugs against these strains. Forty-five *Aeromonas* spp. strains were isolated, 36 (80%) of them with β-haemolytic activity. Identification of biochemical properties and determination of MICs were performed with 20 β-haemolytic *A. hydrophila* strains. The MICs of cefoxitin (≤0.5–32 μg/mL), azithromycin (≤0.5–16 μg/mL), chloramphenicol (≤2–16 μg/mL), tetracycline (≤4–16 μg/mL), ceftriaxone (≤0.25–32 μg/mL), amoxicillin/clavulanic acid (≤8/4–32/16 μg/mL), ciprofloxacin (≤0.015–1 μg/mL), gentamicin (≤1–4 μg/mL), nalidixic acid (≤0.5–8 μg/mL), ceftiofur (≤1–8 μg/mL), sulfisoxazole (≤64–256 μg/mL), trimethoprim/ sulfamethoxazole (≤0.12/2.38–1/19 μg/mL), kanamycin (≤8–16 μg/mL) and streptomycin (≤32 μg/mL) were determined using Sensititre® CMV2AGNF microtitre plates (Trek Diagnostic Systems, USA). Tested strains were resistant to ampicillin concentrations in the plate: >32 μg/mL.

Key words: *Aeromonas hydrophila*, β-haemolysis, fish products, minimum inhibitory concentrations

INTRODUCTION

Microorganisms of genus *Aeromonas* are a part of the Aeromonadaceae family (Janda & Abbott, 1998). They have been isolated from various foods as fish, milk and dairy products, meat and meat products, vegetables (Palu *et al.*, 2006; Kaskhedikar & Chhabra, 2010). Some strains are enteropathogenic and possess virulence factors as enterotoxins, cytotoxins, haemolysins and invasins. A relationship

between haemolysin production and enterotoxicity is reported, as 97% of enterotoxigenic strains are haemolysin producers (Burke *et al.*, 1982). *Aeromonas* spp. could grow and release enterotoxin and haemolysin at fridge temperature conditions (Sharma & Kumar, 2011). A number of researchers have confirmed the presence of β-haemolytic aeromonads in fish products (Tsai & Chen, 1996; Wang & Silva, 1999; Radu *et al.*, 2003; Thayumanavan *et al.*, 2003; Yucel *et al.*, 2005; Erdem *et al.*, 2010). A special attention is paid on *A. hydrophila* as a causative agent of foodborne gastroenterites, and fish products as the commonest vector of this microbial pathogen (Thayumanavan *et al.*, 2003; Hatha *et al.*, 2005; Paniagua *et al.*, 2006). The wide spread of these bacteria is considered public health threat due to the fact that *Aeromonas* infections are provoked by consumption of contaminated water and food (Kirov, 2001; Ottaviani *et al.*, 2006). Gastroenterites are the commonest infections provoked by *Aeromonas* spp., which may appear as diarrhoea, cholera-like disease or colitis (Janda & Abbott, 1998). According to Parker & Shaw (2010) 85% of human *Aeromonas*-related gastroenterites are caused by *A. hydrophila*, *A. caviae* and *A. veronii* bv *sobria*.

Various systems for biochemical identification of members of genus *Aeromonas* are commercially available. These are API 20E, API ZYM, API 20NE, API 50 CH, API Rapid ID 32 (bioMérieux, France), Biolog MicroPlates GN2, GP2, AN (Biolog, Inc., USA), Enterotubes, BBL Crystal E/NF (Becton-Dickinson & Company, USA), Minitek (Becton-Dickinson & Company, USA), Bionor Aqua (Bionor, Skien, Norway) (Popovic *et al.*, 2007). In the view of Wilcox *et al.* (1992) API 20NE could not distinguish *A. hydrophila* from *A. caviae*, and therefore, a test for β-haemolysis, which is positive for *A. hydrophila* and negative for *A. caviae,* is recommended.

Aeromonas spp. are sensitive to cephalosporins, aminoglycosides, chloramphenicol, tetracycline, trimethoprim-sulphamethoxazole, aztreonam and fluoroquinolones (Ko *et al*., 2003) and resistant to penicillins (Awan *et al.*, 2009). In these bacteria, antimicrobial resistance is chromosomally mediated but occasionally, β-lactamases produced by aeromonads could be encoded by plasmids or integrons (Aravena-Roman *et al.*, 2012). The continuous increase of resistance to antimicrobial drugs is a serious concern which requires its periodic monitoring with respect to *A. hydrophila* in the different geographical regions in order to select a proper drug for therapy (Kaskhedikar & Chhabra, 2010).

At the background of the increasing role of microorganisms from the genus *Aeromonas* as agents of foodborne infections in men, we aimed at establishing the prevalence of β-haemolytic *A. hydrophila* strains in cooled and frozen fish products and to determine the minimum inhibitory concentrations of some antimicrobial drugs against these isolates.

MATERIALS AND METHODS

Sample collection

The study included 103 samples cooled and frozen fish products. Cooled fish products consisted of horse mackerel (20 samples), rainbow trout (11 samples), Black sea roach (12 samples), silver carp (20 samples), vacuum-packed rainbow trout fillets (20 samples). Frozen fish products included 20 rainbow trout samples. Samples were collected from retail

stores for fish and fish products and transported to the lab in a cooler bag.

Aeromonas spp. isolation

The isolation procedure was performed as per Evangelista-Barreto *et al.* (2006). Ten g muscle tissue with skin were collected aseptically from cooled fish and weighed in a Stomacher® 400 Circulator (Seward, England) bag. Ninety mL sample diluent (MRD, Merck) were added, and samples were homogenised at 256 rpm for 1 min. Tenfold dilutions were performed in tubes with 9 mL sample diluent (MRD, Merck). From each dilution, 0.1 mL were inoculated on two GSP agar plates (Merck, Germany). Plates were incubated at 28 °C over 24 h.

For isolation of *Aeromonas* spp. from frozen fish, tryptic soy broth (Merck, Germany) was employed for enrichment of samples. From the initial dilution, 10 mL homogenate were inoculated in 10 mL TSB at 28 °C for 24 h. After the incubation, inoculation on GSP agar (Merck, Germany) were performed and plates were incubated for another 24 h at 28 °C. Typical colonies were yellow, 2–3 mm in diameter, surrounded by a yellow zone. The reference *A. hydrophila* ATCC 7965 strain was used as positive control in the different tests with fish isolates.

Haemolytic activity determination

The haemolytic activity tests were performed on blood agar containing 5% sheep red blood cells. Typical colonies of each sample were grown for 4–5 h in brain heart infusion broth (Merck, Germany). The presence of haemolysis in agar plates was detected after 24-hour incubation at 37 °C according to Singh & Sanyal (1992).

Identification of β-haemolytic Aeromonas spp.

Isolates exhibiting β-haemolytic activity were identified by API 20 NE (bioMérieux, France).

Determination of minimum inhibitory concentrations of antimicrobial drugs

The minimum inhibitory concentrations of antimicrobial drugs against β-haemolytic *A. hydrophila* isolates were determined with Sensititre® CMV2AGNF microtiter plates (Trek Diagnostic Systems, USA) according to manufacturer's instructions.

RESULTS

Table 1 presents the results from the haemolytic activity of *Aeromonas* spp. isolates. A total of 45 *Aeromonas* spp.

Table 1. Haemolytic activity of *Aeromonas* spp.

Origin	Number of isolates	Haemolytic acitivity		
		α	β	γ
Cooled horse mackerel	6	–	5	1
Cooled rainbow rout	3	–	3	–
Cooled Black sea roach	0	–	–	–
Cooled silver carp	17	1	13	3
Cooled rainbow trout fillets	17	2	13	2
Frozen rainbow trout	2	–	2	–
Total	45	3	36	6

strains were isolated from cooled and frozen fish samples, and 36 of them (80%) exhibited β-haemolysis. The number of isolates showing α-haemolysis (3; 6.7%) and γ-haemolysis (6; 13.3%) was relatively small.

Identification of biochemical properties and determination of minimum inhibitory concentrations were performed with 20 β-haemolytic *A. hydrophila* strains. All tested isolates were identified as *A. hydrophila* in API 20 NE tests. Seven different profile index patterns were determined, most prevalent among which were 7577755 in eight and 7577754 in six out of the 20 tested β-haemolytic *A. hydrophila* isolates. The other 6 *A. hydrophila* strains were from another five different profile index patterns.

Tables 2 and 3 present the results from the determination of antimicrobial drug MICs against β-haemolytic *A. hydrophila* isolates.

DISCUSSION

Fish and fish products are of primary significance for men because of their nutritional and health values. They are very prone to contamination and could transmit microbial pathogens, which normally inhabit the aquatic environment (Seethalakshmi *et al.*, 2010). *A. hydrophila* produces virulence factors as haemolysin, aerolysin, proteases, lipases, DNA-ases with important role in the pathogenesis of human and fish diseases (Castro-Escarpuli *et al.*, 2003). Two haemolytic toxins (haemolysin and aerolysin) are described in *A. hydrophila*. Haemolytic proteins are isolated from microbial pathogens, and β-haemolysins are among the essential virulence factors (Pandey *et al.*, 2010). In our study, we isolated 45 *Aeromonas* spp. strains, 80% of which with β-haemolytic activity. The tested 20 β-haemolytic isolates were identified as *A. hydrophila*. A high percentage of haemolytic *A. hydrophila* strains in fish products was also reported by other researchers. Thayumanavan *et al.* (2003) found out that 78.4% of 255 *A. hydrophila* isolates produced haemolysin, whereas Radu et al. (2003) established haemolytic activity in more than 90% *A. veronii* bv *sobria*, *A. hydrophila* and *A. caviae* isolates. Tsai & Chen (1996) isolated 16 *A. hydrophila* strains from fish fillets, 14 of which produced haemolysin and cytotoxin. Yucel *et al.* (2005) confirmed haemolytic activity in more than 80% of *A. veronii* bv *sobria* and *A. hydrophila* isolates, and Erdem *et al.* (2010) – in 89% of *A. hydrophila* strains. Unlike us, Wang & Silva (1999) established haemolytic activity only in 5.8% of *A. hydrophila* strains isolated from channel catfish fillets.

Several authors demonstrated that all investigated *A. hydrophila* strains were resistant to ampicillin (Vila *et al.*, 2002; Castro-Escarpuli *et al.*, 2003; Radu *et al.*, 2003; Hatha *et al.*, 2005; Kaskhedikar & Chhabra, 2010). The results from the present study were comparable, and established MIC of ampicillin was over 32 μg/mL. This is attributed to the fact that aeromonads form three classes of β-lactamases which determine their resistance to a broad spectrum to β-lactams (Chen *et al.*, 2012). In contrast, Vaseeharan *et al.* (2005) determined MIC for ampicillin in the range from 89.5 to 126.7 μg/mL. MIC of amoxicillin/clavulanic acid against *A. hydrophila* was 8/4–32/16 μg/mL, which is the same (8/4 μg/mL) as that reported by Aravena-Roman *et al.* (2012).

The European Committee on Antimicrobial Susceptibility Testing has set clinical breakpoints for gentamicin

Table 2. MICs of antimicrobial drugs for *A. hydrophila* (n=20)

Amtimicrobial drugs	Number of isolates with MIC (µg/mL) of:															Panel MIC range (µg/mL)	Number of isolates with MIC (µg/mL) beyond the panel range
	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256		
Cefoxitin						1	1	0	0	0	9	3				0.5–32	6 (>32)
Azithromycin				0	0	1	1	0	12	1	2					0.12–16	3 (>16)
Chloramphenicol								16	3	0	1	0				2–32	0
Tetracycline									13	1	5	0				4–32	1 (>32)
Ceftriaxone					5	4	3	4	1	2	0	1	0			0.25–64	0
Ciprofloxacin	11	2	2	2	1	1	1	0	0							0.015–4	0
Gentamicin					0	0	2	15	3	0	0					0.25–16	0
Nalidixic acid						12	0	0	1	3	0	0				0.5–32	4 (>32)
Ceftiofur				0	0	0	2	1	1	14						0.12–8	2 (>8)
Sulfisoxazole											0	0	1	2	6	16–256	11 (>256)
Kanamycin										14	6	0	0			8–64	0
Ampicillin							0	0	0	0	0	0				1–32	20 (>32)
Streptomycin												20	0			32–64	0

Table 3. MICs of antimicrobial drug combinations for *A. hydrophila* (n=20)

	Number of isolates with MIC (µg/mL) of:											Panel MIC range (µg/mL)	Number of isolates with MIC (µg/mL) beyond the panel range
	1/0.5	2/1	4/2	8/4	16/8	32/16	0.12/ 2.38	0.25/ 4.75	0.5/ 9.5	1/19	2/38	4/76	
Amoxicillin/ Clavulanic acid	0	0	0	2	11	6							1/0.5–32/16	1 (>32/16)
Trimethoprim/ Sulfamethoxazole							1	11	4	4	0	0	0.12/2.38–4/76	0

(S≤4 mg/L; R>4 mg/L), doripenem (S≤1 mg/L; R>4 mg/L), ofloxacin (S≤0.5 mg/L; R>1 mg/L) and tobramycin (S≤2 mg/L; R>4 mg/L) against *A. hydrophila* (EUCAST, 2014). All strains in our study were sensitive to gentamicin at concentrations of ≤4 μg/mL. Other researchers (Wang & Silva, 1999; Vila *et al.*, 2002; Hatha *et al.*, 2005; Ottaviani *et al.*, 2006; Awan *et al.*, 2009) have also confirmed that *A. hydrophila* was sensitive to gentamicin. Unlike us, Thayumanavan *et al.* (2003) showed a resistance to gentamicin (3.6%). The MIC of gentamicin according to Dibua & Okpokwasili (2006) is 50 μg/mL, of kanamycin – 45 μg/mL and of streptomycin –25 μg/mL vs. *A. hydrophila*. The MIC values obtained in this study for gentamicin (≤1–4 μg/mL) and kanamycin (≤8–16 μg/mL) were lower, and the results for streptomycin – comparable (≤ 32 μg/mL). A previous study of ours (Stratev *et al.*, 2013) provided similar evidence as 6 out of 8 β-haemolytic *A. hydrophila* isolates were found to be sensitive to gentamicin at a concentration ≤ 4 μg/mL.

Quinolones are broad-spectrum antimicrobial drugs, widely used in human and veterinary medicine. They, as well as cotrimoxazole, are recommended as first-choice therapeutics for treatment of *Aeromonas*-induced infections. Less than 25% of aeromonads from the environment and less than 5% of clinical isolates are resistant to quinolones (Goni-Urriza *et al.*, 2000). Our results support this statement with MICs of ciprofloxacin for all tested strains ≤0.015–1 μg/mL and trimethoprim/sulfamethoxazole ≤0.12/2.38–1/19 μg/mL, whereas the MIC of nalidixic acid varied from less than 0.5 up to 8 μg/mL. Goni-Urriza *et al.* (2000) established higher MIC values of ciprofloxacin (2 μg/mL) and nalidixic acid (16 μg/mL), while Overman (1980) reported the MIC of trimethoprim/sulfamethoxazole of ≤0.5–9.5 μg/mL against *A. hydrophila*.

The resistance to first- and second-generation cephalosporins is different, but more than 90% of *Aeromonas* spp. isolates are sensitive to the third generation of these antimicrobial drugs (Jones & Wilcox, 1995). Panigua *et al.* (2006) established MIC of ceftriaxone of 3.9 μg/mL, and Goni-Urriza *et al.* (2000) – 16 μg/mL of cefoxitin. Our results were in agreement with these data, with MIC values for cefoxitin of ≤0.5–16 μg/mL and for ceftriaxone of ≤0.25-32 μg/mL.

Dibua & Okpokwasili (2006) indicated that MIC of chloramphenicol vs. *A. hydrophila* was 15 μg/mL, and that of tetracycline – 80 μg/mL. Jones *et al.* (1988) reported that the MIC of azithromycin against *A. hydrophila* was 4 μg/mL. These values are comparable to the results of our study, where most of *A. hydrophila* strains had MICs of 4 μg/mL, MICs of chloramphenicol varied within 2–16 μg/mL, and MICs of tetracycline – from 4 to 16 μg/mL.

In conclusion, cooled and frozen fish products were often contaminated with *A. hydrophila*. Most strains belonging to this species possessed β-haemolytic activity, posing a risk from infection as *A. hydrophila* produces haemolysins during cold storage of fish products. The MICs of cefoxitin, azithromycin, chloramphenicol, tetracycline, ceftriaxone, amoxicillin/clavulanic acid, ciprofloxacin, gentamicin, nalidixic acid, ceftiofur, sulfisoxazole, trimethoprim/, kanamycin and streptomycin against β-haemolytic *A. hydrophila* isolates were determined. Tested strains were resistant to ampicillin concentrations in the plate: >32 μg/mL.

REFERENCES

Aravena-Roman, M., T. J. J. Inglis, B. Henderson, T. V. Riley & B. J. Changa, 2012. Antimicrobial susceptibilities of *Aeromonas* strains isolated from clinical and environmental sources to 26 antimicrobial agents. *Antimicrobial Agents and Chemotherapy*, **56**, 1110–1112.

Awan, M., A. Maqbool, A. Bari & K. Krovacek, 2009. Antibiotic susceptibility profile of *Aeromonas* spp. isolates from food in Abu Dhabi, United Arab Emirates. *New Microbiologica*, **32**, 17–23.

Burke, V., J. Robinson, H. M. Atkinson & M. Gracey, 1982. Biochemical characteristics of enterotoxigenic *Aeromonas* spp. *Journal of Clinical Microbiology*, **15**, 48–52.

Castro-Escarpulli, G., M. J. Figueras, G. Aguilera-Arreola, L. Soler, E. Fernandez-Rendon, G. O. Aparicio, J. Guarro & M. R. Chacon, 2003. Characterisation of *Aeromonas* spp. isolated from frozen fish intended for human consumption in Mexico. *International Journal of Food Microbiology*, **84**, 41–49.

Chen P.-L., W.-C. Ko, C.-J. Wu, 2012. Complexity of β-lactamases among clinical *Aeromonas* isolates and its clinical implications. *Journal of Microbiology, Immunology and Infection*, **45**, 398–403.

Dibua, U. E. & G. Okpokwasili, 2006. Production of some virulence factors under different growth conditions and antibiotic susceptibility pattern of *Aeromonas hydrophila*. *Animal Research International*, **3**, 439–447.

Erdem, B., E. Kariptas & T. Kaya, 2010. Siderophore, hemolytic, protease, and pyrazinamidase activities and antibiotic resistance in motile *Aeromonas* isolated from fish. *Turkish Journal of Biology*, **34**, 453–462.

EUCAST, 2014. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters version 4.0, valid from 2014-01-01. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_4.0.pdf (24 November 2014 date last accessed).

Evangelista-Barreto, N., R. Vieira, F. Carvalho, R. Torres, E. Santanna, D. Rodrigues & C. Reis, 2006. *Aeromonas* spp. isolated from oysters (*Crassostrea rhizophorea*) from a natural oyster bed, Ceara, Brazil. *Revista do Instituto de Medicina Tropical de Sao Paulo*, **48**, 129–133.

Goni-Urriza, M., L. Pineau, M. Capdepuy, C. Roques, P. Caumette & C. Quentin, 2000. Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers. *Journal of Antimicrobisl Chemotherapy*, **46**, 297–301.

Hatha, M., A. A. Vivekanandhan, G. J. Joice & Christol, 2005. Antibiotic resistance pattern of motile aeromonads from farm raised fresh water fish. *International Journal of Food Microbiology*, **98**, 131–134.

Janda, J. M. & S. L. Abbott, 1998. Evolving concepts regarding the genus *Aeromonas*: An expanding panorama of species, disease presentations and unanswered questions. *Clinical Infectious Diseases*, **27**, 332–344.

Jones, B. L. & M. H. Wilcox, 1995. *Aeromonas* infections and their treatment. *Journal of Antimicrobial Chemotherapy*, **35**, 453–461.

Jones, K., D. Felmingham & G. Ridgway, 1988. *In vitro* activity of azithromycin (CP-62,993), a novel macrolide, against enteric pathogens. *Drugs under Experimental and Clinical Research,* **14**, 613–615.

Kaskhedikar, M. & D. Chhabra, 2010. Multiple drug resistance in *Aeromonas hydrophila* isolates of fish. *Veterinary World*, **3**, 76–77.

Kirov, S. M., 2001. *Aeromonas* and *Plesiomonas* species. In: *Food Microbiology: Fundamental and Frontiers*, 2nd edn., ed. M. P. Doyle, L. R. Beuchat, T. J. Montville, ASM Press, pp. 301–328.

Ko, W. C., S. R. Chiang, H. C. Lee, H. J. Tang, Y. Y. Wang & Y. C. Chuang, 2003. *In vitro* and *in vivo* activities of fluoroquinolones against *Aeromonas hydrophila*. *Antimicrobial Agents and Chemotherapy*, **47**, 2217–2222.

Ottaviani, D., S. Santarelli, S. Bacchiocchi, L. Masini, C. Ghittino & I. Bacchiocchi, 2006. Occurrence and characterization of *Aeromonas* spp. in mussels from the Adriatic Sea. *Food Microbiology*, **23**, 418–422.

Overman, T. L., 1980. Antimicrobial susceptibility of *Aeromonas hydrophila*. *Antimicrobial agents and chemotherapy*, **17**, 612–614.

Palu, A. P., L. M. Gomes, M. A. L. Miguel, I. T. Balassiano, M. L. P. Queiroz, A. C. Freitas-Almeida & S. S. de Oliveira, 2006. Antimicrobial resistance in food and clinical *Aeromonas* isolates. *Food Microbiology*, **23**, 504–509.

Pandey, A., M. Naik & S. K. Dubey, 2010. Hemolysin, protease, and EPS producing pathogenic *Aeromonas hydrophila* strain An4 shows antibacterial activity against marine bacterial fish pathogens. *Journal of Marine Biology*, doi:10.1155/2010/563205.

Paniagua, G. L., E. Monroy, M. P. E. Negrete & O. G. S. Vaca, 2006. Antibiotic and heavy metal resistance of *Aeromonas hydrophila* isolated from charal (*Chirostoma humboldtianum*, Valenciannes, 1835). *Hidrobiologica*, **16**, 75–80.

Parker, J. & J. Shaw, 2010. *Aeromonas* spp. clinical microbiology and disease. *Journal of Infection*, **20**, 1–10.

Popovic, N. T., R. Coz-Rakovac & I. Strunjak-Perovic, 2007. Commercial phenotypic tests (API 20E) in diagnosis of fish bacteria: A review. *Veterinarni Medicina*, **52**, 49–53.

Radu, S., N. Ahmad, F. H. Ling & A. Reezal, 2003. Prevalence and resistance to antibiotics for *Aeromonas* species from retail fish in Malaysia. *International Journal of Food Microbiology*, **81**, 261–266.

Seethalakshmi, I., S. K. Jayaraman, M. S. Manoharan & S. Valsalam, 2010. Virulence and cytotoxicity of seafood borne *Aeromonas hydrophila*. *Brazilian Journal of Microbiology*, **41**, 978–983.

Sharma, I. & A. Kumar, 2011. Occurrence of enterotoxigenic *Aeromonas* species in foods of animal origin in North East India. *European Review for Medical and Pharmacological Sciences,* **15**, 883–887.

Singh, D. V. & S. C. Sanyal, 1992. Production of haemolysis and its correlation with enterotoxicity in *Aeromonas* spp. *Journal of Medical Microbiology*, **37**, 262–267.

Stratev, D., I. Vashin & H. Daskalov, 2013. Antimicrobial resistance of β-haemolytic *Aeromonas hydrophila* strains isolated from rainbow trouts (*Oncorhynchus mykiss*). *Bulgarian Journal of Veterinary Medicine*, **16**, 289–296.

Thayumanavan, T., G. Vivekanandhan, K. Savithamani, R. Subashkumar & P. Lakshmanaperumalsamy, 2003. Incidence of haemolysin-positive and drug-resistant *Aeromonas hydrophila* in freshly caught finfish and prawn collected from major commercial fishes of coastal South India. *FEMS Immunology and Medical Microbiology*, **36**, 41–45.

Tsai, G.-J. & T.-H. Chen, 1996. Incidence and toxigenicity of *Aeromonas* hydrophila in seafood. *International Journal of Food Microbiology*, **30**, 121–131.

Vaseeharan, B., P. Ramasamy, T. Murugan & J. C. Chen, 2005. *In vitro* susceptibility of antibiotics against *Vibrio* spp. and *Aeromonas* spp. isolated from *Penaeus monodon* hatcheries and ponds. *International Journal of Antimicrobial Agents*, **26**, 285–291.

Vila, J., F. Marco, L. Soler, M. Chacon & M. Figueras, 2002. *In vitro* antimicrobial susceptibility of clinical isolates of *Aeromonas caviae*, *Aeromonas hydrophila* and *Aeromonas veronii* biotype *sobria*. *Journal of Antimicrobial Chemotherapy*, **49**, 697–702.

Wang, C. & J. Silva, 1999. Prevalence and characteristics of *Aeromonas* species iso-

lated from processed channel catfish. *Journal of Food Protection*, **62**, 30–34.

Wilcox, M. H., A. M. Cook, K. J. Thickett, A. Eley & R. C. Spencer, 1992. Phenotypic methods for speciating clinical *Aeromonas* isolates. *Journal of Clinical Pathology*, **45**, 1079–1083.

Yucel, N., B. Aslim & Y. Beyatli, 2005. Prevalence and resistance to antibiotics for *Aeromonas* species isolated from retail fish in Turkey. *Journal of Food Quality*, **28**, 313–324.

Paper received 25.06.2014; accepted for publication 02.10.2014

Correspondence:

Dr. Deyan Stratev
Department of Food Hygiene and Control,
Veterinary Legislation and Management,
Faculty of Veterinary Medicine,
6000 Stara Zagora, Bulgaria
e-mail: deyan.stratev@trakia-uni.bg