DE GRUYTER OPEN

Bull Vet Inst Pulawy 58, 193-199, 2014
DOI: 10.2478/bvip-2014-0029

Acinetobacter johnsonii and *Acinetobacter lwoffii* - the emerging fish pathogens

Alicja Kozińska[1], Ewa Paździor[1], Agnieszka Pękala[1], Wiktor Niemczuk[2]

[1]Department of Fish Diseases,
National Veterinary Research Institute, 24-100 Pulawy, Poland
[2]Department of Epizootiology and Clinic of Bird and Exotic Animals, Faculty of Veterinary Medicine,
University of Environmental and Life Sciences, 50-366 Wroclaw, Poland
koala@piwet.pulawy.pl

Received: October 30, 2013 *Accepted: June 2, 2014*

Abstract

The aim of this study was to characterise *Acinetobacter* sp. isolated from fish. Eight isolates obtained from diseased rainbow trout and common carp cultured in Poland were analysed. The isolates were identified using API 20 NE system as *Acinetobacter* sp. Afterwards, they were identified by sequencing 16S rDNA gene fragment. The bacteria were identified as *A. johnsonii* (two isolates), *A. lwoffii* (two isolates), *A. junii/johnsonii* (one isolate), *A. calcoaceticus* (one isolate), and *Acinetobacter* sp. (two isolates). The drug resistance of isolates was examined. The majority of the isolates were resistant to ampicilin, amoxicillin, and cephalothin and all demonstrated sensitivity to fluoroquinolones, except of one isolate. Two isolates were selected for the experimental infection of trout and carp to confirm their pathogenicity. Experimentally infected fish showed disease symptoms similar to those observed in fish naturally infected with these bacteria. This is the first report concerning pathogenicity of *A. johnsonii* for rainbow trout and *A. lwoffii* for common carp. These bacteria were regarded as emerging opportunistic pathogens of fish farmed in Poland. *Acinetobacter* strains are commonly known as microorganisms transmitting the antibiotic resistance genes. Therefore, they might have a great impact on the resistance transfer in aquaculture.

Key words: rainbow trout, common carp, *Acinetobacter*, emerging pathogens.

Introduction

Bacteria from the genus *Acinetobacter* are Gram-negative, non-motile, non-fermentative rods. The cells may be spherical especially in the stationary phase of growth. Most strains of these organisms grow at temperature from 20ºC to 37ºC. Colonies are generally non-pigmented, pale yellow, or grayish-white. The bacteria are strictly aerobic, catalase positive, and oxidase negative (10, 19). The members of *Acinetobacter* are widely distributed in the nature. They occur commonly in soil, marine fish, and water (2, 8, 15), freshwater fish (13), and vegetables (4, 12).

The bacteria were regarded as saprophitic microorganisms a long time ago. However, nowadays the organisms, especially *A. baumanii* and *A. calcoaceticus*, are well documented as emerging, opportunistic pathogens in hospitalised humans (9, 17, 18, 24). Extreme drug resistance of these species to many chemiotherapeutics has been observed during the last years (5, 20, 22). Therefore, few drugs are available to treat infection with these bacteria.

There is still not much information on the role of *Acinetobacter* in fish pathology. Only in the last decade an association of *Acinetobacter* sp. with pathological conditions of various fish species in some countries, such as Croatia (8), China (29), Turkey (30), and India (25) was considered. Two cases of *Acinetobacter* sp. infection have been also reported in Poland (23, 31). However, there were only two reports concerning pathogenicity of *A. baumanii* confirmed by the experimental infection of fish (25, 29). There was also very few data regarding antibiotic susceptibility of *Acinetobacter* sp. isolated from fish (16, 25, 30).

For the last years, *Acinetobacter* sp. was isolated relatively frequently from the damaged tissues of common carp *Cyprinus carpio* (L) and rainbow trout *Oncorhynchus mykiss* (Walbaum), which are the two main fish species cultured in Poland. These bacteria were often predominant in damaged fish tissues. The aim

of the study was to identify and characterize *Acinetobacter* species isolated from diseased common carp and rainbow trout, and to examine their pathogenicity by experimental infection of fish. The results may be substantial for control of *Acinetobacter* sp. infections and evaluation of risk associated with these microorganisms in fish farms.

Material and Methods

Fish. Diseased rainbow trout, weighing from 80 to 100 g, originating from one farm, and common carp, weighing from 50 to 80 g, from two various farms in Poland were investigated. The scale losses, ragged fins, exophthalmia, and bloody gills were symptoms of the disease in rainbow trouts (Fig. 1). Carps showed haemorrhages on the skin and bloodshot of the gills. Internally, the inflammation of the intestine in both carps and trouts was observed. Mortality rate among the fish was about 5%-20%, especially in trout populations. The disease was noted in various seasons, but the most often in May and September.



Fig. 1. Rainbow trout naturally infected with *Acinetobacter* sp.

Isolation of bacteria. The body surface and gills were carefully washed, at first under the stream of tap water and then using sterile phosphate buffered saline (PBS) in order to remove saprophitic bacterial flora. Samples from skin, gills, and eyes were collected. Then, the fish were disinfected with 70% ethanol and after opening the body cavity, samples from the kidneys and damaged intestine were taken. The samples were diluted 1:1 in PBS and homogenised. Afterwards, 50 µL of the homogenates were inoculated onto tripticase soya agar (TSA, bioMèrieux, France) and nutrient agar (Biomed, Poland) supplemented with 5% horse blood (BA). After 48 h incubation at 27°C ± 1°C, the material from a few randomly chosen dominant colonies was inoculated onto TSA and incubated for 24 h. Pure cultures of three selected isolates (Pt404, Pt405, Pt425) from rainbow trout and of five isolates from carp (K629, K692, K712 K715, and SC8) were stored at 4ºC ± 1ºC for further examination.

Phenotypic characterisation of the isolates. At the beginning, morphology of colonies was studied and recorded. Overnight cultures were automatically Gram-stained using PREVI-Color Gram (bioMèrieux) for cell morphology determination. Then, the bacteria were tested for production of catalase with 2% hydrogen peroxide and cytochrome oxidase using oxidase reagent (bioMérieux). Motility was tested in drop of PBS under light microscope at 400 × magnification. For further identification, API 20E and API 20NE systems (bioMérieux) were used. Moreover, drug resistance of the isolates was assayed by disc diffusion method according to recommendation of Clinical and Laboratory Standards Institute (CLSI) (7). The following chemotherapeutics were used for drug-resistance assays: ampicilin, amoxicilin, cephalotin, cefuroxime sodium, enrofloxacin, flumquine, norfloxacin, gentamycin, oxytetracycline, and sulphamethoxazole/terimethoprim. The strains were evaluated as sensitive, medium resistant, or resistant if inhibitory zones were ≥2.0 mm, 1.5-2.0 mm or <1.5 mm respectively.

Molecular identification of the isolates. The tests were conducted by sequencing of 16S rDNA gene fragment. Genomic DNA was extracted from bacterial suspension using the DNeasy Blood & Tissue Kit (Qiagen) according to the manufacturer's instruction. For PCR, the universal primers U8f (5' AGAGT TTGATCMTGGCTCAG 3') and U1492 (5' TACGGY TACCTTGTTACGACTT 3') (29) were used. The reaction mixture of 50 µL consisted of: 1 µL of genomic DNA; 2.0 U of *JumpStart Taq DNA* polymerase; 5 µL of 10 X PCR buffer containing 15 mM $MgCl_2$; 0.05 mM dNTPs, and 0.2 µM of each primer. Amplification was conducted under conditions described previously (6): initial denaturation at 93°C for 3 min followed by 35 cycles of 1 min at 94°C, 1 min at 56°C, 2 min at 72°C and final elongation at 72°C for 10 min. Amplified products were analysed by electrophoresis in a 2.0% agarose gel containing ethidium bromide (0.5 mg/mL), and detected by UV transillumination. PCR products (about 1460 bp) were purified and sequenced by the 3730xl DNA Analyzer by Genomed S.A. The 16S rRNA gene sequence of eight isolates and the representatives of the other species from GenBank database were analysed using MEGA 5.05 software. Similarity between the sequences of the tested isolates and sequences available at GenBank was defined with the use of MEGA 5.05 software.

Experimental infection. Healthy rainbow trout weighing 80-100 g and common carp weighing 100-120 g were used. The fish were adapted to laboratory conditions for 14 d. They were maintained in 300 L glass tanks with circulated and aerated water before and during experiments. Water temperature was 20°C ± 1°C for carp and 12°C ± 1°C for trout. The fish were fed pellets (Aller Aqua, Poland) suitable for given fish species. Two strains Pt405 and K629 isolated from trout and carp, respectively, were selected for the challenge. Bacteria were grown in tripticase soya broth (TSB) for 24 h at 27°C ± 1°C with gentle rotation. The cultures were diluted in sterile PBS to the final concentration of 5×10^6 bacterial cells mL^{-1}. The fish were anaesthetised by bath for 2-5 min in solution of MS-222 (Sigma), at the concentration from 75 to 150 µg L^{-1} (lower doses for

trouts and higher for carps). Twenty carps and 20 trouts were infected intramuscularly with 0.2 mL of suspensions of the strain Pt405 or K629 respectively. The same number of both carps and trouts were injected with sterile PBS as the controls. Each group of fish was placed into separate aquariums. Symptoms of the disease were recorded daily for four weeks. Freshly dead fish and survivals were used for post-mortem and bacteriological examination. Local Ethic Commission in Lublin approved the procedure concerning experiments on fish.

Results

Isolation and phenotypic characteristics of bacteria. Abundant growth of bacteria was obtained in samples collected from skin, gills, and intestine of carps and trouts, and trout eyes. Significantly fewer bacteria grew from the kidney samples. White or white-greyish, smooth colonies with diameter of 1-2 mm on BA were predominant in trout and carp specimens. The bacteria formed non-pigmented colonies on TSA medium. Pure cultures of the isolates comprised Gram-negative cocci, forming chains or non-regular clusters after 24 h incubation. They were oxidase-negative except isolate K692, catalase-positive, and non-motile. Each of the isolates was non-reactive when tested with API 20 E system. The results of biochemical and assimilation tests in API 20 NE system are presented in Table 1. All isolates were negative for all biochemical reactions except the isolate K692 (positve for gelatin hydrolysis), and were unable to assimilate the most substrates. All isolates revealed positive reaction for capric acid assimilation. Different results depending on isolate were noted for malate, trisodium citrate, and phenylacetic acid assimilation.

Table 1. Characteristics of *Acinetobacter* isolates tested using API 20 NE system

Characteristics	Pt404	K629	Pt405	Pt425	K712	K715	K692	Sc8
Reduction of nitrates to nitrites (NO_3)	-	-	-	-	-	-	-	-
Indole production (IND)	-	-	-	-	-	-	-	-
Glucose fermentation (GLU)	-	-	-	-	-	-	-	-
Arginine dihydrolase (ADH)	-	-	-	-	-	-	-	-
Urease (URE)	-	-	-	-	-	-	-	-
Aesculin hydrolysis (ESC)	-	-	-	-	-	-	-	-
Gelatin hydrolysis (GEL)	-	-	-	-	-	-	+	-
β-galactosidase (PNG)	-	-	-	-	-	-	-	-
Assimilation of:								
- glucose (GLU)	-	-	-	-	-	+	-	-
- arabinose (ARA)	-	-	-	-	-	-	-	-
- mannose (MNE)	-	-	-	-	-	-	-	-
- mannitol (MAN)	-	-	-	-	-	-	-	-
- N-acetyl-glucosamine (NAG)	-	-	-	-	-	-	-	-
- maltose (MAL)	-	-	-	-	-	-	-	-
- potassium gluconate (GNT)	-	-	-	-	-	-	-	-
- capric acid (CAP)	+	+	+	+	+	+	+	+
- adipic acid (ADI)	-	-	-	-	-	+	-	-
- malate (MLT)	+	-	+	+	+	+	+	+
- trisodium citrate (CIT)	-	-	+	+	+	+	+	+
- phenylacetic acid (PAC)	+	+	-	-	-	+	-	-
Cytochrome oxidase (OX)	-	-	-	-	-	-	+	-

+ positive; - negative reactions

The results of the drug resistance are presented in Table 2. The majority of the isolates tested showed resistance to β-lactams (ampicilin and amoxicillin) and cephalothin; however, all were sensitive to fluoroquinolones (flumequine, enrofloxacin, norfloxacin) and gentamycin, except the isolate Pt405. This isolate showed resistance to all drugs except cefuroxime sodium, whereas the isolates K692 and Sc8 were sensitive to most drugs.

Molecular identification. The similarities of 16S rDNA gene sequences between eight isolates and the sequences of species from GenBank database are presented in Table 3. On the basis of similarity percentage (99.1 %-100%), the isolates Pt425 and Pt405 were identified as *A. johnsonii*, the isolates K629 and Pt404 as *A. lwoffii*, and the isolate K715 as *A. calcoaceticus*. The isolate K712 showed similarity (99.6 %) to *A. junii* and *A. johnsonii*. The isolates K692 and Sc8 were classified as *Acinetobacter* sp. with 98.8% similarity to *A. haemolyticus* and *A. johnsonii* respectively.

The 16S rDNA partial sequences of the tested *Acinetobacter* strains have been deposited in the GenBank database with the following accession numbers: Pt425-KC758136, Pt405-KC758141, K629-KC758137, Pt404-KC758140, K715-KC758143, K712-KC758142, K692-KC758138, and Sc8-KC758139.

Challenge. There was no mortality during the acclimatisation period prior to challenge. No mortality and disease symptoms occurred in the control groups during the observation period. The first lesions in infected trouts were observed after 7 d post challenge. The fish showed exophthalmia, haemorrhages in the eyes, scale loses (Fig. 2) and gills congestion. The first mortalities appeared 7 d later. Internal organs and the intestine were also congested. Cumulative mortality of the fish in the infected group was 30%. Experimentally infected carps showed haemorrhages on the skin and mild congested region around the anus (Fig. 3). Internal damages were similar to these observed in rainbow trout. Cumulative mortality of the fish in the infected group was 20%. The bacteria used for experimental infection were isolated in pure culture from skin, gills, kidneys, and intestine of moribund trouts and carps, and from trout eyes.

Table 2. Drug resistance of the *Acinetobacter* isolates tested

Chemotherapeutic (μg)	Pt404	K629	Pt405	Pt425	K712	K715	K692	Sc8
Ampicylin (10)	R*	S	R*	R*	R*	R*	S	S
Amoxycylin (30)	R	R*	R	R*	R*	R*	S	S
Cephalotin (30)	R	R*	R	R*	R*	R*	S	R
Cefuroxime sodium (30)	M	R	S	M	S	R*	S	S
Enrofloxacin (5)	S	S	R*	S	S	S	S	S
Flumequine (30)	S	S	R*	S	S	S	R	S
Norfloxacin (10)	S	S	R*	S	S	M	S	S
Gentamycin (10)	S	S	M	S	S	S	S	S
Oxytetracycline (30)	M	S	R	M	R	R	S	M
Sulphamethoxasole/Trimethoprim (25)	S	R	R	R	M	S	S	S

R – resistant, S – sensitive, M – medium resistant, * no growth inhibition zone was observed

Table 3. 16S rRNA gene sequence similarity between Polish isolates and strains available in GenBank

	GenBank strains				
Polish isolates	*A. lwoffii* (accession no. FJ860882.1)	*A. haemolyticus* (accession no. GU991530.1)	*A. junii* (accession no. EF669479.1)	*A. johnsonii* (accession no. EU977715.1)	*A. calcoaceticus* (accession no. HE651903.1)
K692-A3-12	97.9%	98.8%	98.4%	98.5%	96.6%
Pt405-A7-11	97.2%	98.5%	99.9%	100.00%	96.1%
K712-A9-12	97.0%	98.2%	99.6%	99.6%	96.4%
Pt425-A1-12	97.2%	98.0%	99.0%	99.1%	96.2%
Sc8-A4-12	96.5%	98.1%	98.8%	98.8%	95.8%
K715-A10-12	96.7%	97.2%	96.5%	96.5%	100.00%
K629-A2-11	99.8%	97.5%	97.3%	97.4%	96.2%
Pt404-A6-11	99.8%	97.3%	97.2%	97.3%	96.6%



Fig. 2. Rainbow trouts experimentally infected with *A. johnsonii* strain Pt405: A-exophthalmia and haemorrhages in the eye, B-scale loses (arrow)



Fig. 3. Common carp experimentally infected with *A. lwofii* strain K629: haemorrhages on the body flank and mild congested region around the anus

Discussion

It is obvious that *Acinetobacter* sp. are often isolated from healthy or diseased fish as the component of mixed bacterial flora because most bacteria prevailing in water environment colonise skin, gills, and digestive tract of the aquatic animals. These microorganisms may also penetrate into internal organs after any damage to these tissues. Bacteria from the genus *Acinetobacter* are usually perceived as normal saprophytic microorganisms. However, the role of *A. baumanii* for channel catfish, *Ictalurus punctatus*, and snakehead, *Channa striatus*, as the fish pathogen has been well documented in the last years (25, 29). *A. lwoffii* was isolated from altered skin and gill of sharptooth catfish, *Clarias gariepinus*, living in the Nile River in Egypt (11).

As it was found in the study, various *Acinetobacter* sp. were isolated from diseased fish cultured in Poland and it is the first report concerning pathogenicity of *A. johnsonii* for rainbow trout and *A. lwoffii* for common carp. These species were isolated more frequently from rainbow trout and common carp than other *Acinetobacter* sp. In previous reports concerning the *Acinetobacter* sp. infection in carp (23, 31), the bacteria were identified only by phenotypic tests and their pathogenicity was not confirmed by experimental infection. In this study, tentative identification of particular isolates as *A. johnsonii* and *A. lwoffii* was confirmed by sequencing of the 16S

rDNA gene fragment. The pathogenicity of two selected strains belonging to *A. johnsonii* (strain Pt405) and *A. lwoffii* (strain K629) was proved by experimental infections of rainbow trout and common carp respectively. The disease symptoms were similar to those observed in naturally infected fish. It is interesting that some of these symptoms, especially exophthalmia and haemorrhages or ulceration on the body surface, were similar to those described in fish infected with *A. baumanii* (25, 29). This fact indicates that various *Acinetobacter* sp. have probably some similar virulence factors. However, in our experiments the fish showed congested internal organs, whereas the pale liver was observed in channel catfish infected with *A. baumanii* (29). It should be emphasised that significant numbers of *A. lwoffii* and *A. johnsonii* were also recovered from human specimens often associated with bacteriaemia (28). This fact indicates that fish may transmit these pathogens to humans.

The results of the drug resistance of the strains tested may be only estimated because there is still a complete lack of data concerning interpretation of zone diameters for bacteria isolated from fish (7). In this study, no zone inhibition, especially by ampicillin, amoxicillin, and cephalothin, for most strains was observed. Previously, *A. baumanii* has also been reported as the most commonly resistant species to these antibiotics (26). Ampicillin and second generation cephalosporins were previously used to treat *Acinetobacter* infections (10). This indicates that the resistance to these antibiotics is not natural but it has been acquired. Bergognee-Bérézin and Towner (3) reported that *A. lwoffii* and *A. johnsonii* are generally more susceptible to antibiotics than *A. baumanii*. Another study showed that *A. lwoffii* strains were susceptible to greatest number of antibiotics (28). In opposition to this data, multi-drug resistance of this species was reported by Tega *et al.* (27) and by Ahmadi *et al.* (1). Our study demonstrated that strains Pt404 and K629, identified as *A. lwoffii*, were resistant to cephalosporins and amoxicillin but they responded to ampicilin and sulphamethoxasole/trimethoprim in different ways. The differences in drug resistance were also found among the strains Pt405, Pt425 identified as *A. johnsonii*. Pt405 showed multi-drug resistance like *A. baumanii* isolated from channel catfish (29). The most of the *Acinetobacter* strains tested in the study showed sensitivity to quinolones; however, the use of these drugs can affect the levels of antimicrobial resistance of natural aquatic microflora and, therefore, they should be considered carefully (16).

According to Okamura and Feist (21), the emerging disease is the one, which appeared in fish population for the first time, or which existed previously but now is rapidly increasing and spreading to other geographic areas. Therefore, *A. johnsonii* and *A. lwoffii* were regarded as emerging opportunistic pathogens for fish farmed in Poland. This may be associated with environmental conditions. These bacteria are able to survive in various environments containing small amounts of nutritious components and show a low sensitivity to adverse physical and chemical conditions (14). The use of antibiotics for treatment or prevention of bacterial fish diseases, and developing the multi-drug resistance of these microorganisms may also influence the pathogenicity of *Acinetobacter* sp. for fish. These bacteria are commonly known as microorganisms transmitting the antibiotic resistance genes. Therefore, they may have a great impact on the resistance transfer in aquaculture.

In further studies the pathogenicity and drug resistance of other *Acinetobacter* sp. isolated from fish cultured in Poland will be investigated.

References

1. Ahmadi H., Boroumand M.A., Anvari M.S., Karimi A., Moshtaghi N.: Left-sided endocarditis associated with multi–drug resistance Acinetobacter lwoffii. J Teh Univ Heart Cent 2009, 3, 189–192.
2. Baumann P.: Isolation of Acinetobacter from soil and water. J Bacteriol 1968, 96, 39–42.
3. Bergogne-Bérézin E., Towner K.J.: Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev 1996, 9, 148–165.
4. Berlau J., Aucken H., Houang M., Epitt T.L.: Isolation of Acinetobacter spp. including A. baumannie from vegetables: Implications for hospital-acquired infections. J Hosp Infect 1999, 42, 201–204.
5. Blossom D., Srinivasan A.: Drug-resistant Acinetobacter baumannii-calcoaceticus complex: an emerging nosocomial pathogen with few treatment options. Infect Dis Clin Pract 2008, 16, 1–3.
6. Borrell N., Acinas S.G., Figueras M.J., Martínez-Murcia A.J.: Identification of Aeromonas clinical isolates by restriction fragment length polymorphism of PCR-amplified 16S rRNA genes. J Clin Microbiol 1997, 35, 1671–1674.
7. Clinical and Laboratory Standards Institute: Methods for antimicrobial disk susceptibility testing of bacteria isolated from aquatic animals; approved guideline. CLSI document M42–A, 2006, vol. 26, no. 23 (ISBN 1–56238–611–5), USA.
8. Čož-Rakovac R., Strunjak-Perović I., Popović N.T., Hacmanjek M., Šimpraga B., Teskeredžić E.: Health status of wild and cultured sea bass in the northern Adriatic Sea. Vet Med-Czech 2002, 47, 222–226.
9. Dijkshoorn L., Nemec A., Seifert H.: An increasing threat in hospitals: multi-drug resistant Acinetobacter baumannii. Nat Rev Microbiol 2007, 5, 939–951.
10. Doughari H.J., Ndakidemi P.A., Human I.S., Benade S.: The ecology, biology and pathogenesis of Acinetobacter spp.: an overview. Microb Environ 2011, 26, 101–112.
11. El-Sayyad H.I., Zaki V.H., El-Shebly A.M., El-Badry D.A.: Studies on the effects of bacterial diseases on skin and gill structure of Clarias gariepinus in Dakahlia province, Egypt. Ann Biol Res 2010, 1, 106–118.
12. Gennari M., Stegagno F.: Isolation and characterization of Acinetobacter calcoacetisus from raw, washed and frozen vegetables. Arch Vet Ital 1986, 37, 131–137.
13. González C.J., Santos J.A., García-López M.L., Otero A.: Psychrobacters and related bacteria in freshwater fish. J Food Prot 2000, 63, 315–321.
14. Gospodarek E., Ziółkowski G.: Antibiotic-resistant strains of Acinetobacter baumannii occurring in Poland. Epidemiol Rev 2000, 54, 88–96.

15. Grawiński E., Podolska M., Kozińska A., Pękala A.: Bacteria pathogenic for fish and humans isolated from Baltic cod. Życie Wet 2009, 84, 409–416.
16. Guardabassi L., Dalsgaard A., Raffatellu M., Olsen J.E.: Increase in the prevalence of oxolinic acid resistant Acinetobacter spp. observed in a stream receiving the effluent from a freshwater trout farm following the treatment with oxolinic acid-medicated feed. Aquaculture 2000, 188, 205–218.
17. Jakoniuk P., Wieczorek P., Sacha P., Zalewska M., Leszczyńska K.: In vitro sensitivity to cefoperazone/sulbactam of multiresistant strains of Acinetobacter spp. Zakażenia 2007, 2, 50–54.
18. Joly-Guillou M.L.: Clinical impact and pathogenicity of Actinetobacter. Eur Soc Clin Microbiol Infect Dis 2005, 11, 868–873.
19. Juni E.: Genus II. Acinetobacter. In: *Bergey's Manual of Systematic Bacteriology*, edited by D.J. Brenner, N.R. Krieg, J.T. Staley, Springer, Michigan State University, USA, 2005 vol. 2 (part B), pp. 425–437.
20. Nowak J., Pacholczyk A., Petroniec V., Dziedzicka B., Zwolska Z.: The results of molecular epidemiological investigations in patients infected with strains of the genus Acinetobacter. Pneumonol Alergol Pol 2010, 78, 386–391.
21. Okamura B., Feist S.W.: Emerging diseases in freshwater systems. Freshwater Biol 2011, 56, 627–637.
22. Park Y.K., Peck K.R., Cheong H.S., Chung D.R., Song J.H., Ko K.S.: Extreme drug resistance in Acinetobacter baumannii infections in intensive care units, South Korea. EID J 2009, 15, 1325–1327.
23. Pękala A.: New risks of bacterial infections in carp. In: *Health care in the fishing economy*, edited by J. Żelazny, Nat Vet Res Inst Pulawy, 2007, pp. 59–64.
24. Peleg A.Y., Seifert H., Paterson D.L.: Acinetobacter baumannii: emergence of a successful pathogen. Clin Microbiol Rev 2008, 21, 538–582.
25. Rauta P.R., Kumar K., Sahoo P.K.: Emerging new multi–drug resistant bacterial pathogen, Acinetobacter baumannii associated with snakehead Channa striatus eye infection. Curr Sci 2011, 101, 548–553.
26. Savov E., Chankova D., Vatcheva R., Dinev N.: In vitro investigation of the susceptibility of Acinetobacter baumannii strains isolated from clinical specimens to ampicillin/sulbactam aloe and in combination with amikacin. Int J Antimicrob Agents 2002, 20, 390–392.
27. Tega L., Raieta K., Ottaviani D., Russo G.L., Blanco G., Carraturo A.: Catheter-related bacteremia and multidrug-resistant Acinetobacter lwoffii. EID J 2007, 13, 355–356.
28. Turton J.F., Shah J., Ozongwu C., Pike R.: Incidence of Acinetobacter species other than A. baumannii among clinical isolates of Acinetobacter: Evidence for emerging species. J Clin Microbiol 2010, 48, 1445–1449.
29. Xia L., Xiong D., Gu Z., Xu Z., Chen C., Xie J., Xu P.: Recovery of Acinetobacter baumannii from diseased channel catfish (Ictalurus punctatus) in China. Aquaculture 2008, 284, 285–288.
30. Yonar M.E., Karahan M., Kan N., Yonar S., Sağlam N: A study of Acinetobacter sp. infection in some cultured rainbow trout (Oncorhynchus mykiss) in Kahramanmaras. J Fisheries Sci 2010, 4, 287–293.
31. Zacharow I., Snoch-Bajek J., Niemczuk W.: Microbiological evaluation of farmed carp from particular ponds in province Dolnośląskie. Acta Sci Pol Med Vet 2010, 9, 33–38.