27. Bijma, J., Faber, W. W. and Hemleben, Ch., Temperature and salinity limits for growth and survival of some planktonic foraminifers in laboratory cultures. *J. Foramin. Res.*, 1990, **20**, 95–116.
28. Ravelo, A. C., Fairbanks, R. G. and Philander, S. G. H., Reconstructing tropical Atlantic hydrography using planktonic foraminifera and an ocean model. *Paleoceanography*, 1990, **5**, 409–431.
29. Duplessy, J. C., Moyes, J. and Pujol, C., Deep water formation in the North Atlantic Ocean during the last ice age. *Nature*, 1980, **286**, 479–482.
30. Ivanova, E., Schiebel, R., Singh, A. D., Schmiedl, G., Niebler, H. and Hemleben, C., Primary production in the Arabian Sea during the last 135,000 years. *Palaeogeogr., Palaeoclimatol., Palaeoecol.*, 2003, **197**, 61–82.
31. Oberhänsli, H., Bénier, C., Meinecke, G., Schmidt, H., Schneider, R. and Wefer, G., Planktonic foraminifers as tracers of ocean currents in the eastern South Atlantic. *Paleoceanography*, 1992, **7**, 607–632.
32. Emeis, K.-C., Anderson, D. M., Doose, H., Kroon, D. and Schulz-Bull, D., Sea-surface temperatures and the history of monsoon upwelling in the northwest Arabian Sea during the last 500,000 years. *Quaternary Res.*, 1995, **43**, 355–361.
33. Budziak, D., Schneider, R. R., Rostek, F., Muller, P., Bard, E. and Wefer, G., Late Quaternary insolation forcing on total organic carbon and C37 alkenone variations in the Arabian Sea. *Paleoceanography*, 2000, **15**, 307–321.
34. Rostek, F., Ruhland, G., Bassinot, F. C., Beaufort, L., Müller, P. J. and Bard, E., Fluctuations of the Indian monsoon regime during the last 170,000 years: evidence from sea surface temperature, and salinity and organic carbon records. In *Global Precipitations and Climate Change* (eds Besbois, M. and Desalmand, F.), NATO ASI Series 126, Springer, Heidelberg, 1994, pp. 27–51.
35. Rao, K. K., Jayalakshmy, K. V., Kumaran, S., Balasubramanian, T. and Kutti, M. K., Planktonic foraminifera in waters of the Coromandel coast, Bay of Bengal. *Indian J. Mar. Sci.*, 1989, **18**, 1–7.
36. Wiggert, J. D., Jones, B. H., Dickey, T. D., Brink, K. H., Weller, R. A., Marra, J. and Codispoti, L. A., The northeast monsoon's impact on mixing, phytoplankton biomass and nutrient cycling in the Arabian Sea. *Deep-Sea Res. II*, 2000, **47**, 1353–1385.
37. Weller, R. A. *et al.*, Moored observations of upper-ocean response to the monsoons in the Arabian Sea during 1994–1995. *Deep-Sea Res. II*, 2000, **49**, 2195–2230.
38. Veldhuis, M. J. W., Kraay, G. W., Van Bleijswijk, J. D. L. and Baars, M. A., Seasonal and spatial variability in phytoplankton biomass, productivity and growth in the northwestern Indian Ocean: the southwest and northeast monsoon, 1992–1993. *Deep-Sea Res. I*, 1997, **44**, 425–449.
39. Hansell, D. A. and Peltzer, E. T., Spatial and temporal variations of total organic carbon in the Arabian Sea. *Deep-Sea Res. II*, 1998, **45**, 2171–2193.
40. Dickey, T. *et al.*, Seasonal variability of bio-optical and physical properties in the Arabian Sea: October 1994–October 1995. *Deep-Sea Res. II*, 1998, **445**, 2001–2025.
41. Schott, F. A., Xie, S.-P. and McCreary Jr, J. P., Indian Ocean circulation and climate variability. *Rev. Geophys.*, 2009, **47**, RG1002.

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Emerging new multi-drug resistant bacterial pathogen, *Acinetobacter baumannii* associated with snakehead *Channa striatus* eye infection

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Although *Acinetobacter baumannii* acts as a severe human pathogen, there are only few studies to date that report it as a pathogen for fish. In the present study, one virulent bacterial strain was isolated from diseased *Channa striatus*, from a farm at the Central Institute of Freshwater Aquaculture, Bhubaneswar, Orissa, which showed symptoms like cloudy eyes, pop eye (exophthalmia), opaque lenses and mild ulceration on the whole body irrespective of sex and size of fish. Based on morphology, different biochemical tests and sequence analysis of 16S rDNA segment as well as phylogenetic study, the causative bacterium (called ChE) was identified as *A. baumannii*. The pathogenicity was further confirmed by artificial infectivity study (LD_{50} dose of $10^{8.37}$ CFU/fish). In the drug sensitivity study, this isolate was highly resistant to many antibiotics. The isolate was also highly resistant to all three tested heavy metals (Cu^{+2}, Cr^{+6}, Hg^{+2}), thus proving its virulent nature.

Keywords: *Acinetobacter baumannii*, *Channa striatus*, drug resistant, pathogen.

THE genus *Acinetobacter* belonging to the family Moraxellaceae, within the gamma subdivision of proteobacteria is ubiquitous in nature as it is found frequently in soil, water and dry environments[1–5]. It is a group of Gram-negative, strictly aerobic, non-motile coccobacilli. Currently, *Acinetobacter* sp. is emerging as a serious human nosocomial pathogen being involved in several infections, e.g. bacteremia, urinary tract infection, secondary meningitis and ventilator-associated pneumonia[2]. This species, especially *Acinetobacter baumannii* is treated as the most clinically important microorganism due to its remarkable ability to develop resistance to many antibiotics[6–11]. There are some reports on the incidence of infection in fish, which suggest that it could also be treated as severe fish pathogen. The most probable first report was from China and the bacterial strain isolated from mandarin fish (*Siniperca chutasi*) was confirmed as *A. baumannii* in terms of its biochemical characteristics[12]. Another report was also from China and the species isolated from diseased channel catfish proved as the virulent pathogen for this fish[13]. In the earlier reports, the methods of identification

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were based on biochemical tests, polymerase chain reaction (PCR)-based assay and infectivity study. The present communication deals with isolation, identification, pathogenicity trial, antibiogram and heavy metals resistance study of *A. baumannii* isolated from diseased snakehead fish (*Channa striatus*) obtained from culture tanks.

Upon the report of high mortality due to disease outbreak from a hatchery at the Central Institute of Freshwater Aquaculture, Bhubaneswar, India, few disease samples of snakehead fish with swollen eyes were brought to the laboratory for diagnosis. The disease was seen mainly on juvenile fish of size 50–100 g being monocultured in 50 m^2 cement tanks.

The diseased fish were examined both gross and microscopically for the presence of any parasite or bacteria. Samples from kidney, blood, eye swabs and eye fluid were aseptically inoculated into tryptic soya broth (TSB) (Becton Dickinson, USA) and nutrient broth. After incubation at 30°C for 24 h, the bacteria isolated from eye swab and eye fluid of fish were subcultured under similar conditions to obtain the pure isolate for further identification. The samples were also examined for involvement of any other type of pathogen (fungal or viral) following routine mycological techniques and intramuscular injection of infected tissue filtrate (passing through 0.22 µm filters) to healthy *Channa* to rule out the possibility of involvement of viral pathogens.

Pure culture colonies of the above isolate were characterized using morphology, cultural and biochemical characteristics (Table 1)[14,15]. Chromosomal DNA of the isolated strain was isolated by phenol–chloroform extraction method. The 16S rDNA sequence was amplified using 16S universal primers. Each PCR reaction consisted of 40.70 µl dH_2O, 5 µl 10× PCR buffer (Genei, India), 1 µl 10 mM dNTPs (Chromus Biotech), 1 µl (10 p mol) of each forward and reverse primer, followed by 0.3 µl (1.5 U) *Taq* DNA polymerase (Genei, India) and 1 µg genomic DNA. The amplification profile was 95°C for 3 min followed by 45 cycles of denaturation for 30 s at 95°C, at appropriate annealing temperature for 1 min and extension at 72°C for 1 min, followed by a final extension for 10 min at 72°C. The generated PCR products (8 µl) were then analysed by electrophoresis on 1% agarose gel. The PCR products were purified using PCR product purification kit (Genei, India) and subcloned into pGEMT vector (Promega) according to the manufacturer's instructions. Plasmid DNAs were prepared by the alkaline lysis method[16] and cleaved with restriction enzymes to check for positive clones. Three positive clones from the above were further purified by phenol–chloroform extraction method before sequencing. Sequencing was done using the cycle sequencing kit (Bigdye Terminator V.3.1, ABI, USA) with T7 universal primer (New England Biolab) in the 310 Genetic Analyser, ABI, USA. The obtained sequence of 1492 bp was then compared to sequences available in GenBank using the NCBI–BLAST program. Phylogenetic analysis was performed using the neighbour-joining algorithm with MEGA software (version 4.1) and the resulting tree was displayed with Tree View software (version 1.6.6). All published *Acinetobacter* genomic sequences, obtained from GenBank were used to confirm the different relationships between the present (ChE) isolate and others. Bootstrapping was performed to assess the confidence values of the clusters formed. Identification to the genomic

Table 1. Comparison of biochemical characteristics of ChE isolated from *Channa striatus* in the present study with published results of *Acinetobacter baumannii*

Biochemical test	ChE	*A. baumannii**	Biochemical test	ChE	*A. baumannii**
Cytochrome oxidase	–	–	Fermentation/oxidation		
Catalase	+	+	Mannitol	+	–
Phenylalanine deamination	–	–	Sorbitol	–	–
Arginine dihydrolase	–	–	Salicin	+	na
Lysine decarboxylase	–	–	Arabinose	+	+
Ornithine decarboxylase	–	–	Maltose	+	–
Citrate utilization	+	–	Mannose	+	na
H_2S production	–	–	Fructose	+	na
Urease	–	–	Adonitol	+	na
Tryptophan deaminase	na	–	Sucrose	–	na
Indole production	–	–	Xylose	+	–
Gelatinase	–	–	Cellobiose	+	na
Growth on 6.5% NaCl	–	na	Inulin	+	na
Motility	–	na	Dulcitol	+	na
VP test	–	na	Raffinose	–	–
Esculin hydrolysis	–	na	Inositol	+	–
Nitrate reduction	+	na	Trehalose	+	na
			Galactose	+	na

+, Positive; –, negative; na, not applicable. This test was not done for the reference strain.
*Phenotypic characteristics of *A. baumannii* observed earlier as described in Gu *et al.*[12] and Xia *et al.*[13].

species level was defined as a 16S rDNA sequence similarity above 99% with the query sequence[17]. Only 16S rDNA sequences of approximately 1500 bp were selected for comparison. Five 16S rDNA sequences, FJ 867355, EU 760622, EU 760624, FJ 867354 and EU 883588 belonging to *A. baumannii* were included in the test.

The partial sequence (1492 bp) of 16S rDNA of *A. baumannii* isolate has been deposited in the GenBank database under accession number FR750378.

Apparently healthy *Channa* juveniles (50 ± 10 g) were obtained from the Aquaculture Production and Environment Division of the Central Institute of Freshwater Aquaculture, Bhubaneswar. The fish, which had no history of disease or abnormality were stocked in 500 l ferro-cement tanks with aerated freshwater in a wet laboratory and acclimatized for 15 days before starting the experiment. They were fed with a standard diet in two divided doses daily during the experiment. Water in the tanks was exchanged partially daily to remove waste feed and faecal matter. Mean lethal dose (LD_{50}) of this isolate was estimated according to Reed and Muench[18]. Six groups (groups I–VI) with six fish in each were challenged with a series of dilutions of isolate ChE (Table 2). The bacterial suspensions prepared in phosphate buffered saline (0.15 M, pH 7.4) were injected to each fish intraperitoneally with 0.1 ml of different dilutions of bacteria. The final concentration of the bacteria injected to each dose group of fish was 10^5–10^8 CFU/ml. A control fish group was injected with 0.1 ml phosphate buffered saline. Mortality in any group was observed till 10 days and the cause of mortality was reconfirmed by reisolating the bacteria from the kidney, eye swab and blood.

Drug sensitivity to various antibiotics (HiMedia, Mumbai) was assayed by Kirby–Bauer disc diffusion method on Mueller–Hinton agar (Himedia, Mumbai) plates as described by Bauer *et al.*[19]. The concentrations of tested antibiotics are given in Table 3. The minimal inhibitory concentration (MIC) of this isolate to heavy metals was determined by the plate dilution method[20]. Three different heavy metals (Cu^{+2}, Cr^{+6}, Hg^{+2} using $CuSO_4$, $K_2Cr_2O_7$, $HgCl_2$), each in 10 graded concentrations ranging from 12.5 to > 3200 μg/ml were added to Muller–Hinton agar (Difco, Detroit MI, USA). For the purpose of defining metal resistance, the isolates were considered to be resistant when growing at a concentration of 100 μg/ml Cr^{+6}, 600 μg/ml Cu^{+2} (ref. 21) and 200 μg/ml Hg^{+2} (ref. 22). The operational definition of tolerance in our study was based on positive bacterial growth when the concentration of heavy metals was above the stated concentration for resistance.

The diseased fish showed grossly cloudy eyes, pop eye (exophthalmia), opaque lenses and mild skin ulcer on the whole body irrespective of size and sex. The isolated bacteria were aerobic, Gram-negative and most of the biochemical characteristics were in accordance with published characters of *A. baumannii* (Table 1). The obtained 1492 bp of DNA fragment, coding the 16S rRNA (GenBank accession no. FR 750378) after comparison with the sequences of 16S rRNA available in GenBank showed 99.9% homology with that of *A. baumannii* (GenBank accession no. FJ 867355.1) having only one base difference between the two sequences (Figure 1). Similar homology (≥ 99%) was also observed with the other four *A. baumannii* strains. The phylogenetic tree constructed using the above five *A. baumannii* strains and six other *Acinetobacter* sequences, *A. junii* (AB 101444), *A. venetianus* (AJ 295007), *A. radioresistens* (GU 145275), *A. guillouiae* (HM 536960), *A. bouvetii* (HQ 180181) and *A. haemolyticus* (EU 352764) showed that the ChE strain and the earlier deposited *A. baumannii* strains were in the same cluster (Figure 2). This confirms

Table 2. Experimental infection of isolate ChE from *C. striatus*

Group	Dose of bacteria received (cfu/ml)	Cumulative mortality (%)
I (Control)	PBS injected	0
II	10^6	0
III	5×10^6	11
IV	10^7	32
V	5×10^7	87.5
VI	10^8	100

Table 3. Drug sensitivity of ChE isolate to various antibiotics

Antibiotic	Dose/disc	Response
Amikacin	30 mcg	Sensitive
Amoxyclav	30 mcg	Highly resistant
Ampicillin	10 mcg	Resistant
Ampicillin/sulbactam	10/10 mcg	Highly resistant
Aztreonam	30 mcg	Sensitive
Bacitracin	10 units	Highly resistant
Cefotaxime	30 mcg	Sensitive
Ceftazidime	30 mcg	Intermediate
Cefuroxime	30 mcg	Highly resistant
Cephalothin	30 mcg	Highly resistant
Cephoxitin	30 mcg	Highly resistant
Chloramhenicol	30 mcg	Resistant
Ciprofloxacin	5 mcg	Sensitive
Co-trimoxazole	25 mcg	Resistant
Doxycycline hydrochloride	30 mcg	Resistant
Gentamicin	10 mcg	Sensitive
Imipenem	10 mcg	Highly resistant
Levofloxacin	5 mcg	Sensitive
Nalidixic acid	30 mcg	Highly resistant
Neomycin	30 mcg	Sensitive
Netillin	30 mcg	Sensitive
Nitrofurantoin	300 mcg	Highly resistant
Ofloxacin	5 mcg	Intermediate
Penicillin G	10 units	Highly resistant
Piperacillin	100 mcg	Resistant
Polymixin B	300 mcg	Sensitive
Tetracycline	30 mcg	Sensitive
Tobramycin	10 mcg	Sensitive

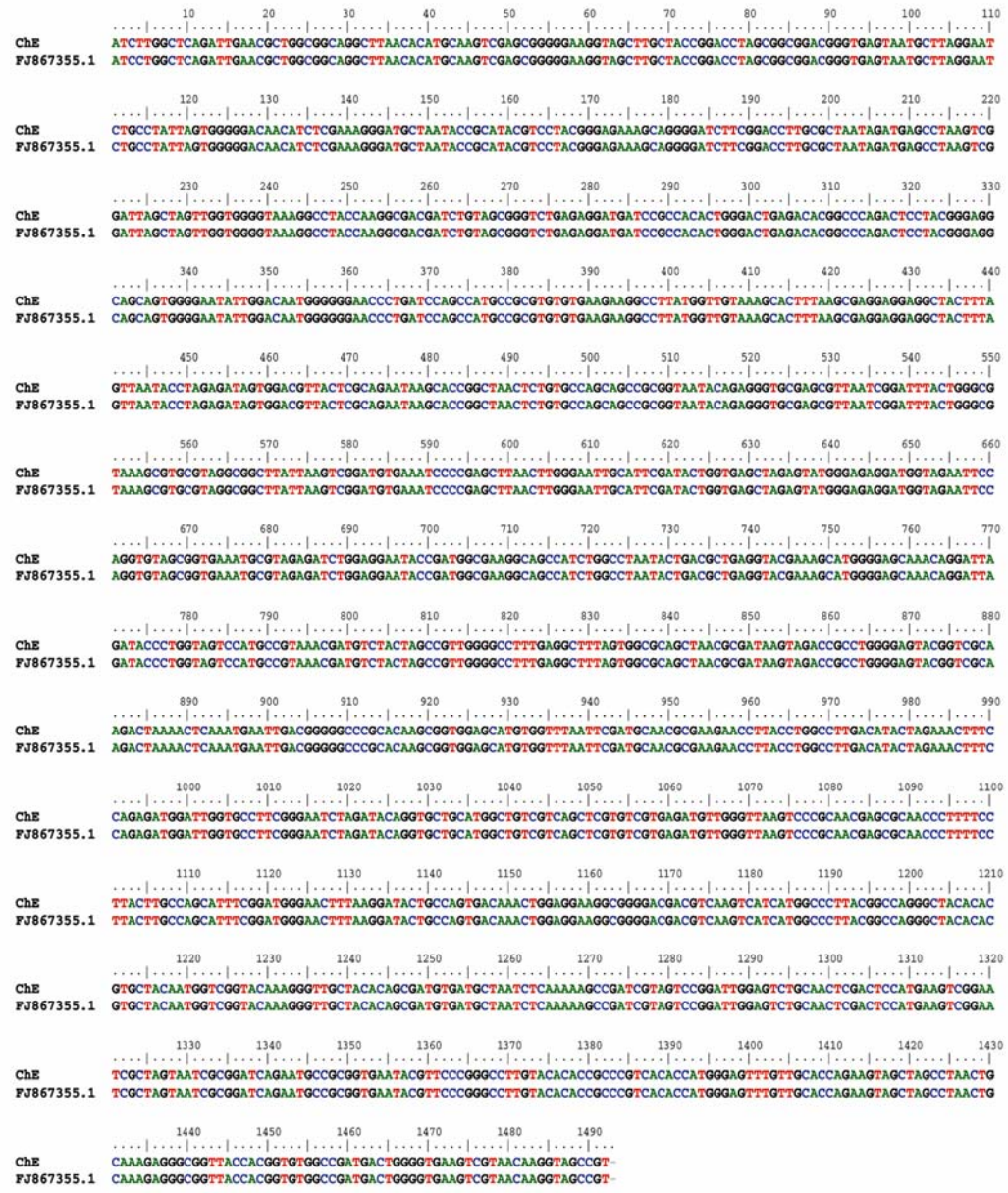

Figure 1. Comparison of 16S rDNA sequence of ChE (GenBank accession no. FR750378) with that of *Acinetobacter baumannii* (GenBank accession no. FJ867355.1).

the close relationship between the query sequence and *A. baumannii*.

Antibiotic susceptibility test indicated that the isolate was sensitive to amikacin, aztreonam, cephotaxime, ciprofloxacin, gentamicin, levofloxacin, neomycin, netillin, polymixin B, tetracycline and tobramycin. The isolate was intermediate in sensitivity to ceftazidime and ofloxacin. However, it was found to be moderately resistant to ampicillin, chloramphenicol, co-trimoxazole, doxycycline hydrochloride, piperacillin and highly resistant to amoxyclav, ampicillin/sulbactam, bacitracin, cefuroxime, cephalothin, cephoxitin, imipenem, nalidixic acid, nitrofurantoin and penicillin G (Table 3). The samples were found to be negative under virological and mycological examination. In the present study, the MIC values for Cu^{+2}, Cr^{+6} and Hg^{+2} of ChE were found to be 800, 2400 and 400 µg/ml respectively. This isolate was highly resistant to all the three heavy metals.

All fish that died of experimental infection with ChE isolate showed similar external signs to those collected from the farm outbreak. Besides, no mortality or gross lesions were noticed in fish after injecting the infected-tissue filtrate. Though fish challenged with 10^6 CFU/fish did not show any mortality till the end of the experiment, a dose of 5×10^6 CFU/fish caused 10% mortality within 5 days post-challenge. All the fish in the group injected with 10^8 CFU/fish died within 2 days post challenge. The LD_{50} dose of ChE isolate was determined to be

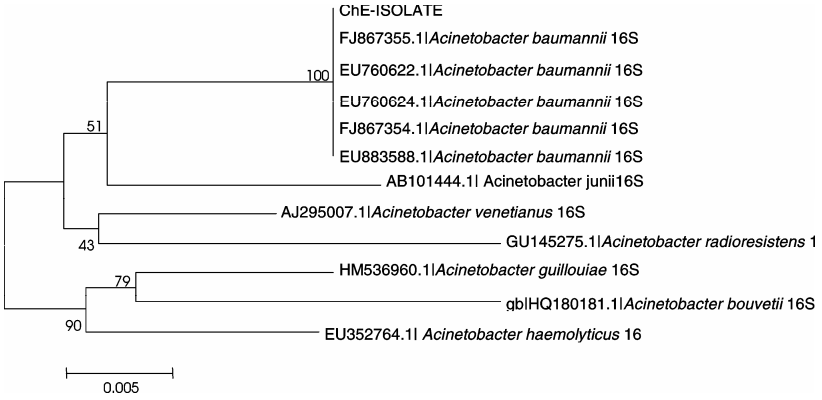

Figure 2. Identification of the *Acinetobacter* spp. *Channa* isolate using 16S rDNA sequences. The 16S rDNA sequences were aligned and used to construct the neighbour-joining phylogenetic tree. Scale bar indicates the genetic distance and the numbers shown next to each node indicate the bootstrap values from 1000 replicons.

$10^{8.37}$ CFU/fish (Table 2). The bacteria isolated from the infected fish (including dead and surviving fish) were identified as *A. baumannii* by the above identification procedures.

C. striatus, commonly known as striped murrel is a native freshwater fish of tropical Africa and Asia[23]. Murrels are highly priced all over India for their good keeping quality, unique flavour, and nutritive, recuperative and medicinal properties[24]. Although *A. baumannii* has emerged as a severe human pathogen, it also causes disease in case of fish as reported in channel catfish in China[13]. External symptoms like exophthalmia and ulceration were also noticed in the present study in diseased *C. striatus* as reported in channel catfish in the earlier study. The present ChE isolate from diseased *C. striatus* showed many phenotypic characteristics common with the previously reported *A. baumannii*[12,13]. The ChE isolate was a non-motile, oxidase-negative, catalase-positive, Gram-negative coccobacilli, having biochemical characteristics similar to those previously reported in *A. baumannii* (Table 1). Presently, bacterial species identification using the 16S rDNA-based method is the most widely accepted, as large public-domain sequence databases are available in GenBank for comparison[25,26]. Also, 16S rRNA gene sequencing has a substantially higher percentage accuracy compared to the conventional methods[27]. The current amplicon of 1492 bp of 16S sequence (GenBank accession no. FR 750378) showed 99–100% similarity with *A. baumannii* 16S partial sequence (GenBank accession nos FJ 867355, EU 760622, EU 760624, FJ 867354, EU 883588). From the above results, it may be concluded that the ChE isolate is *A. baumannii*.

Experimental infection study further confirmed the pathogenicity of *A. baumannii* to *C. striatus*. The LD_{50} of $10^{8.37}$ CFU/fish determined suggested that this isolate was a moderately virulent strain of *A. baumannii*, capable of causing disease in *C. striatus*.

Recent increase in the use of antibiotics and other synthetic chemicals in fish farms to prevent or control fish diseases has resulted in increasing antibiotic resistance among pathogenic bacteria. Drug sensitivity tests are often done to check the development of antibiotic resistance[28]. Frequent hospital outbreaks of infection caused by the selection of multiresistant *A. baumannii* strains have been reported worldwide[29–32]. *A. baumannii* is resistant to a wide range of antibiotics, including broad spectrum β-lactams, carbapenems, aminoglycosides and fluroquinolones[2,5,11,13,33,34]. The isolate in the present study was also highly resistant to many antibiotics like amoxyclav, ampicillin, bacitracin, cefuroxime, cephalothin, cephoxitin, impipenem, nalidixic acid, nitrofurantoin and penicillin G, which confirmed its multi-resistant nature. So instead of using antibiotics, research should focus more on other preventive management practices like use of vaccines[35] to prevent *A. baumannii* infection in fish. However, better management practices in fish culture with proper stocking density and optimum water quality should be followed to reduce stressful conditions in fish. Heavy-metal resistance in bacteria has been shown to be associated with single or multi-drug resistance[36–39]. There is also a strong correlation between heavy-metal resistance and pathogenicity[36,40]. In the present study, the isolate was highly resistant to all three heavy metals (Cu^{+2}, Cr^{+6}, Hg^{+2}). From this result, it was further confirmed that heavy metal resistance might be associated with pathogenicity of the isolate. The increasing presence of antibiotic and heavy-metal resistant *Acinetobacter* may become a potential fish health hazard, which was also seen by Matyar *et al.*[41] in the case of *Aeromonas* and *Pseudomonas*.

The involvement of *A. baumannii* in disease of *Channa* species poses a potential threat to freshwater fish farming as an important emerging pathogen. Further, the resistance to many antibiotics and heavy metals shown by this isolate poses serious concern.

1. Baumann, P., Isolation of *Acinetobacter* from soil and water. *J. Bacteriol.*, 1968, **96**, 39–42.
2. Bergogne-Berezin, E. and Towner, K. J., *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical and epidemiological features. *Clin. Microbiol. Rev.*, 1996, **9**, 148–165.

3. Joly-Guillou, M. L., Clinical impact and pathogenicity of *Acinetobacter*. *Clin. Microbiol. Infect*., 2005, **11**, 868–873.
4. Thomson, J. M. and Bonomo, R. A., The threat of antibiotic resistance in Gram-negative pathogenic bacteria: beta-lactams in peril. *Curr. Opin. Microbiol*., 2005, **8**, 518–524.
5. Poirel, L. and Nordmann, P., Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin. Microbiol. Infect.*, 2006, **12**, 826–836.
6. Corbella, X. *et al*., Emergence and rapid spread of carbapenem resistance during a large and sustained hospital outbreak of multiresistant *Acinetobacter baumannii*. *J. Clin. Microbiol.*, 2000, **38**, 4086–4095.
7. Manikal, V. M., Landman, D., Saurina, G., Oydna, E., Lal, H. and Quale, J., Endemic carbapenem-resistant *Acinetobacter* species in Brooklyn, New York: citywide prevalence, inter institutional spread, and relation to antibiotic usage. *Clin. Infect. Dis.*, 2000, **31**, 101–106.
8. Rodriguez-Bano, J. *et al*., Clinical features and epidemiology of *Acinetobacter baumannii* colonization and infection in Spanish hospitals. *Infect. Control Hosp. Epidemiol.*, 2004, **25**, 819–824.
9. Turton, J. F., Kaufmann, M. E., Warner, M., Coelho, J., Dijkshoorn, L., Van Der Reijden, T. and Pitt, T. L., A prevalent, multiresistant clone of *Acinetobacter baumannii* in Southeast England. *J. Hosp. Infect.*, 2004, **58**, 170–179.
10. Zarrilli, R., Crispino, M., Bagattini, M., Barretta, E., Di Popolo, A., Triassi, M. and Villari, P., Molecular epidemiology of sequential outbreaks of *Acinetobacter baumannii* in an intensive care unit shows the emergence of carbapenem resistance. *J. Clin. Microbiol.*, 2004, **42**, 946–953.
11. Naas, T. *et al*., VEB-1 extended-spectrum beta lactamase producing *Acinetobacter baumannii*. *Fr. Emerg. Infect. Dis*., 2006, **12**, 1214–1222.
12. Gu, T. Z., Lu, C. P. and Chen, H. Q., *Acinetobacter baumannii*, a novel pathogen of acute epidemic in mandarin fish (*Siniperca chuatsi*). *Microbiology*, 1997, **2**, 104–106 (in Chinese, with English abstract).
13. Xia, L., Xiong, D., Gu, Z., Xu, Z., Chen, C., Xie, J. and Xu, P., Recovery of *Acinetobacter baumannii* from diseased channel catfish (*Ictalurus punctatus*) in China. *Aquaculture*, 2008, **284**, 285–288.
14. Holt, J. G. N. R., Krieg, P. H. A., Sneath, J. T., Staley, J. T. and Williams, S. T., Group 5. Facultatively anaerobic Gram-negative rods – Genus *Aeromonas*. In *Bergey's Manual of Determinative Bacteriology* (Williams & Wilkins Co, Baltimore), 1993, 9th edn.
15. Dong, X. Z. and Cai, M. Y., *Manual of Familiar Bacterium Identification*, Science Press, Beijing, 2001, pp. 364–398 (in Chinese).
16. Sambrook, J., Fritsch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Press, Cold Spring Harbor, NY, 1989, 2nd edn.
17. Drancourt, M., Bollet, C. and Carlioz, A., 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. *J. Clin. Microbiol.*, 2000, **38**, 3623–3630.
18. Reed, L. J. and Muench, H., A simple method of estimating fifty per cent endpoints. *Am. J. Hyg.*, 1938, **27**, 493–497.
19. Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Twick, M., Antibiotic susceptibility testing by a standardized single disk method. *Am. J. Clin. Pathol.*, 1966, **45**, 493–496.
20. Summers, A. O. and Silver, S., Mercury resistance in a plasmid-bearing strain of *Escherichia coli*. *J. Bacteriol.*, 1972, **112**, 1228–1236.
21. Allen, D. A., Austin, B. and Colwell, R. R., Antibiotic resistance patterns of metal-tolerant bacteria isolated from an estuary. *Antimicrob. Agents Chemother.*, 1977, **12**, 545–547.
22. Hall, B. M., Distribution of mercury resistance among *Staphylococcus aureus* isolated from a hospital community. *J. Hyg.*, 1970, **68**, 111–119.
23. Ng, P. K. L. and Lim, K. K. P., Snakeheads (pisces; Channidae): Natural history, biology and economic importance. In *Essays in Zoology* (eds Ming, C. L. and Ng, P. K. L.), Papers commemorating the 40th anniversary of the Department of Zoology, National University of Singapore, Singapore, 1990, pp. 127–152.
24. Chakrabarty, N. M., *Murrels and Murrel Culture*, Narendra Publishing House, New Delhi, vol. viii, p. 112.
25. Maidak, B. L., Olsen, G. J. and Larsen, N., The Ribosomal Database Project (RDP). *Nucleic Acids Res.*, 1996, **24**, 82–85.
26. Van de Peer, Y., Jansen, J. and De Rijk, P., Database on the structure of small ribosomal subunit RNA. *Nucleic Acids Res.*, 1997, **25**, 111–116.
27. Morgan, M. C., Boyette, M., Goforth, C., Katharine, V. S. and Shermalyn, R., Comparison of the Biolog OmniLog Identification System and 16S ribosomal RNA gene sequencing for accuracy in identification of atypical bacteria of clinical origin. *Greece J. Microb. Meth.*, 2009, **79**, 336–343.
28. Leano, E. M., Inglis, V. B. M. and MacRac, I. H., Antibiotics resistance of *Vibrio* spp. and *Aeromonas* spp. isolated from fish and shrimp tissues and rearing water in Panay Island, Philippines. *UPV J. Natl. Sci.*, 1999, **3**, 25–28.
29. Marqué, S. *et al.*, Regional occurrence of plasmid-mediated carbapenem hydrolyzing oxacillinase OXA-58 in *Acinetobacter* spp. in Europe. *J. Clin. Microbiol*., 2005, **43**, 4885–4888.
30. Agodi, A. *et al.*, Alert-surveillance of ICU-acquired *Acinetobacter* infections in a Sicilian hospital. *Clin. Microbiol. Infect*., 2006, **12**, 241–247.
31. Pournaras, S. *et al*., Outbreak of multiple clones of imipenem-resistant *Acinetobacter baumannii* isolates expressing OXA-58 carbapenemase in an intensive care unit. *J. Antimicrobiol. Chemother*., 2006, **57**, 557–561.
32. Van Den Broek, P. J. *et al.*, Epidemiology of multiple *Acinetobacter* outbreaks in The Netherlands during the period 1999–2001. *Clin. Microbiol. Infect.*, 2006, **12**, 837–843.
33. Nemec, A., Dolzani, L., Brisse, S., Van Den Broek, P. and Dijkshorn, L., Diversity of aminoglycoside-resistance genes and their association with class 1 integrons among strains of Pan-European *Acinetobacter baumannii* clones. *J. Med. Microbiol.*, 2004, **53**, 1233–1240.
34. Van Looveren, M. and Goossens, H., ARPAC Steering Group. Antimicrobial resistance of *Acinetobacter* spp. in Europe. *Clin. Microbiol. Infect.*, 2004, **10**, 684–704.
35. Harikrishnan, R. and Balasundaram, C., Modern trends in *Aeromonas hydrophila* disease management with fish. *Rev. Fish. Sci.*, 2005, **13**, 281–320.
36. Moore, B., A new screen test and selective medium for the rapid detection of epidemic strains of *Staphylococcus aureus*. *Lancet*, 1960, **2**(7148), 453–458.
37. Novick, R. P. and Roth, C., Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. *J. Bacteriol.*, 1968, **95**, 1335–1342.
38. Hall, B. M., Mercury resistance of *Staphylococcus aureus*. *J. Hyg.*, 1970, **68**, 121–129.
39. Kondo, I., Ishikawa, T. and Nakahara, H., Mercury and cadmium resistances mediated by the penicillinase plasmid in *Staphylococcus aureus*. *J. Bacteriol.*, 1974, **117**, 1–7.
40. Groves, D. J. and Young, F. E., Epidemiology of antibiotic and heavy metal resistance in bacteria: resistance patterns in *Staphylococci* isolated from populations not known to be exposed to heavy metals. *Antimicrob. Agents Chemother.*, 1975, **7**, 614–621.
41. Matyar, F., Akkan, T. and Uçak, Y., Buse Eraslan *Aeromonas* and *Pseudomonas*: antibiotic and heavy metal resistance species from Iskenderun Bay, Turkey (northeast Mediterranean Sea). *Environ. Monit. Assess*, 2010, **167**, 309–320.

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