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Short Communication

Identification, Characterization and Antibiotic Sensitivity of *Aeromonas hydrophila*, a Causative Agent of Epizootic Ulcerative Syndrome in Wild and Farmed Fish from Potohar, Pakistan

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SA, IK, WAK, MNI, AM and SU collected the samples and analyzed the data. SA, HA and SA wrote the article. QA helped in preparation of the article.

Key words
***Aeromonas hydrophila*, epizootic ulcerative syndrome, antibiotic sensitivity, multi-drug resistance.**

A B S T R AC T

A total of 82 samples of fish (80 from fish farm; 2 from river) infected with epizootic ulcerative syndrome (EUS) were collected. Identification and characterization of isolates was done by biochemical tests, fermentation of sugars and analytical profile index (API 20NE). A total of 60 bacterial isolates were confirmed as *Aeromonas hydrophila*, a causative agent of EUS in cultured fish species of Potohar region, Pakistan. None of bacterial species was recovered from disease wild (river) fish. All isolates were sensitive to chloramphenicol (100%) and partly sensitive to norfloxacin (55.0%), streptomycin (90.0%), gentamycin (60.0%), erythromycin (33.3%) and tetracycline (66.7%). Moreover, all isolates showed resistance (100%) to amoxicillin, penicillin and novobiocin. It is concluded that *A. hydrophila* was the causative agent of EUS in fish farms of Potohar region, Pakistan and strains of *A. hydrophila* have developed multi-drug resistance against antibiotics.

Freshwater fish culture is one of the fast growing sectors among aquaculture practices for the production of food to fulfill the requirement of uncontrolled human population worldwide (Govind *et al.*, 2012; Mert and Bulut, 2014). In addition to managemental issues, fish diseases, specifically bacterial infections are responsible for heavy losses in fish farms. Infestation of fish by pathogenic bacteria lead to develop responses like septicemia, focal dermomyonecrosis leading to ulceration, chronic proliferate response and resistance against body surface mucus/serum of host. The diseases caused by pathogenic bacteria include epizootic ulcerative syndrome (EUS) tail rot, fin rot, gill rot, hemorrhagic septicemia, scale oedema, furunculosis, bacterial kidney diseases and columnaris disease (Robert, 1989; Thune *et al.*, 1993; Chowdhury and Baqui, 1997).

EUS is a seasonal epizootic in all kinds of estuarine and freshwater fish (Sosa *et al.*, 2007). Among the etiological agents of bacterial EUS, the motile *Aeromonas* group, especially *Aeromonas hydrophila*, is of considerable importance causing primary infection in wounds or the secondary problem following stress from temperature change, handling, or poor water quality (Inglis *et al.*, 1993; Salyers and Whitt, 1994). *A. hydrophila* is a heterotrophic, gram-negative, rods with rounded ends (bacilli to cocobacilli shape), motile, does not form endospores (Markov *et al.*, 2007), mainly found in areas with a warm climate. This bacterium can be found in fresh, salt, marine, estuarine, chlorinated, and un-chlorinated waters. *A. hydrophila* can survive in both aerobic and anaerobic environments.

In Pakistan, a single study described incidence of EUS in freshwater fishes (Rab *et al.*, 2001), with no evidence of causative agent and its detailed characterization till date. Therefore, the present study was planned to illustrate the characteristics of *A. hydrophila* from EUS infected fish and antibiotic sensitivity against different antibiotics.

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Materials and methods

The infected fish having external symptoms like ulcer formation and fin rot were collected from fish farms and a river in Potohar region of Pakistan. A total of 82 (80 from fish farm; 2 from river) samples (alive and dead) were collected and transported to the National Veterinary Laboratories, Islamabad, Pakistan.

For isolation and characterization of *A. hydrophila*, methodology described by Lee *et al*. (2000) was followed with certain modifications. Fish was dissected under sterile laboratory conditions (Safety cabinet). For this purpose, body surface of fish was cleaned with 70% alcohol. The infected region (muscle, kidney, spleen and liver) was collected with sterile scissor, transferred to nutrient broth and incubated at 37°C for 48 h. After incubation, samples were streaked on tryptic soya agar and incubated at 37°C for 24 h. Suspected colonies were selected for further analysis and re-inoculated on tryptic soya agar for purification. For characterization of pure isolates upto species level, biochemical tests *viz*., Gram staining, shape, oxidation, catalase, motility, voges-proskauer, indole production, nitrate reduction, urease, citrate utilization, growth in 5% NaCl, growth on DNase agar, MacConkey agar, blood agar, ampicillin (12.5 µl/litre) blood agar and fermentation of sugars (L-arabinose, glucose, lactose, maltose, mannose, raffinose, salicin, D-sorbitol, starch, mannitol, rhamnose and sucrose) were performed according to standards procedures (Holt, 1986). The haemolytic activity was studied by formation of zone of alpha and beta haemolysis around the colonies on blood agar plates containing 5% human blood. Further, isolated bacteria were characterized by API 20NE (Biomerieux, France) and results were compared with analytical profile index according to manufacture instructions.

The antibiotic sensitivity of isolates was determined against nine antibiotics (norfloxacin 10 µg, streptomycin 10 µg, gentamycin 10 µg, erythromycin 10 µg, chloramphenicol 30 µg, amoxicillin 10 µg, penicillin 10 µg, novobiocin 30 µg, tetracycline 30 µg) by standard method of Kirby and Bauer on Muller Hinton agar (Bauer *et al*., 1966; NCCLS, 2002).

Results and discussion

In present study, a total of 60 isolates were recovered from farmed fish affected with EUS. None of bacterial species was recovered from diseases wild (river) fish. On the basis of biochemical reactions, fermentation of sugars, and API 20NE, all the isolates were identified as *A. hydrophila*. Out of 16 biochemical reactions, 14 reactions showed uniform results for isolates of *A. hydrophila*, while, out of 12 sugars used for fermentation of *A. hydrophila* isolates, 8 showed uniform results for all isolates.

The isolates were bacilli, Gram negative which could grow on Dnase agar, MacConkey agar, blood agar, oxidation, catalase, otility, indole production, ß-haemolysis, citrate utilization and Voges-proskauer. The above identified *A. hydrophila* could ferment L-arabinose, glucose, maltose, mannose, raffinose, D-sorbitol, starch, mannitol, rhamnose and sucrose. These isolates also showed positive indole production, glucose aicidification, arginine dihydrolase, esculin hydrolysis, gelatin hydrolysis, 4-nitrophenyl-ßD-galactopyranoside, Glucose assimilation, arabinose assimilation, mannose assimilation, mannitol assimilation, N-acetyl-glucoseamine, maltose glucoseamine, gluconate glucoseamine, caprate glucoseamine, adipate glucoseamine, malate glucoseamine and cytochrome oxidase.

A. hydrophila isolates were sensitive to chloramphenicol 30 µg (100%), streptomycin 10 µg (90%), tetracycline 30 µg (66.7%), gentamycin 10 µg (60%), norfloxacin 10 µg (55%) and erythromycin 10 µg (33.3%), while, these isolates were resistant to amoxicillin 10 µg (100%), penicillin 10 µg (100%) and novobiocin 30 µg (100%).

Our study confirms the previous study that EUS affected fish have ulcer and eroded fin. No haemorrhage and raised scales were, however, observed in the present study.

On the basis of results of present study, *A. hydrophila* is causative agent of EUS (Dugenci and Candan, 2003; Awan *et al*., 2009). *A. hydrophila*, has also been isolated from fish affected with EUS in Bangladesh, Bulgaria, India, Nepal, Serbia, Southern Asia and Tanzania (Rahman *et al*., 2004; Yesmin *et al*., 2004; Dahail *et al*., 2008; Orozova *et al*., 2010; Stojanov *et al*., 2010; Shayo *et al*., 2012; Joseph *et al*., 2013). In present study, all isolates of *A. hydrophila* were completely resistant to amoxicillin, penicillin and novobiocin. Resistance of *A. hydrophila* isolates against amoxicillin and penicillin has been reported from fish affected with EUS in India (Saha and Pal, 2002). Amoxicillin resistance was also reported in *A. hydrophila* isolates from catfish of Coimbatore, India (Jayavignesh *et al*., 2011). *A. hydrophila* isolated from *Piaractus mesopotamicus* and *Oreochromis niloticus* was found resistant against novobiocin from Brazil (Belem-Costa and Cyrino, 2006). About 40% isolates of *A. hydrophila* were found resistant to gentamicin in present study. In previous studies, *A. hydrophila* isolated from fish in Brazil and India were found sensitive to gentamicin (Belem-Costa and Cyrino, 2006; Kaskhedikar and Chhabra, 2010). In present study, 76.7% *A. hydrophila* isolates were found resistant to erythromycin. Previously,

resistance of *A. hydrophila* isolates of diseased catfish was found resistant to erythromycin (Jayavignesh *et al*., 2011).

In present study, about 45% of *A. hydrophila* isolates were resistant to norfloxacin. Similar finding has been reported from Iran, where 50% *A. hydrophila* isolated from muscles of different fish species were found resistant to said antibiotic (Ansari *et al*., 2011). However, a recent study conducted in China reported susceptibility of all isolates of *A. hydrophila* from grass carp *Ctenopharyngodon idellus* to norfloxacin (Zheng *et al*., 2012).

References

Abowei, J.F.N. and Briyai, O.F., 2011. *Asian J. med. Sci*., **3:** 206-217.

Ansari, M., Rahimi, E. and Raissy, M., 2011. *Afr. J. Microbiol. Res*., **5:** 5772-5775.

Awan, M.B., Maqbool, A., Bari, A. and Krovacek, K., 2009. *New Microbiol.*, **32:** 17-23.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C. and Turck, M., 1966. *Am. J. clin. Pathol.*, **36:** 493-496.

Belem-Costa, A. and Cyrino, J.E.P, 2006. *Sci. Agric. (Piracicaba, Braz.).* **63:** 281-284.

Chowdhury, M.B.R. and Baqui, M.A., 1997. *Fish health section*, Asian Fisheries Society, Manila, pp. 101-105

Dahail, S.P., Shrestha, M.K., Pradhan, S.K. and Jha, D.K., 2008. *Fish health section*, Asian Fisheries Society, Manila, Philippines, pp. 169-178

Dugenci, S.K. and Candan, A., 2003. *Turk. J. Vet. Anim. Sci*., **27:** 1071-1075.

Govind, P., Shrivastav, A.B. and Sharma, M., 2012. *Int. Res. J. Pharm*., **3:** 120-123.

Holt, J.G., 1986. *Bergey's manual of systematic bacteriology*. vol. 1**,** Williams and Wilkins, Baltimore.

Inglis, V., Roberts, R.J. and Bromage, N.R., 1993. *Bacterial diseases of fish.* Blackwell Science Ltd, U.K.

Jayavignesh, V., Kannan, K.S. and Bhat, A.D., 2011. *Arch. appl. Sci. Res*., **3:** 85-93.

Joseph, A.V., Sasidharan, R.S., Nair, H.P. and Bhat, S.G., 2013. *Vet. World*, **6:** 300-306.

Kaskhedikar, M. and Chhabra, D., 2010. *Vet. World*, **3:** 76-77.

Lee, S., Sookyung, K., Yoojung, O. and Yeonhee, L., 2000. *J. Microbiol*., **38:** 1-7.

Markov, G., Geno, K., Veselin, L. and Mincho, K., 2007. *Wounds*, 19: 223-226.

Mert, R. and Bulut, S.., 2014. *Pakistan J. Zool.*, **46**: 337-346.

NCCLS, 2002. *Methods for dilution antimicrobial susceptibility for bacteria that grow aerobically*. Wayne.

Orozova, P., Chikova, V. and Najdenski, H., 2010. *Bul. J. agric. Sci*., **16:** 376-386.

Rab, A., Afzal, M., Akhtar, N., Barlas, A. and Qayyum, M., 2001. *Bangladesh J. Fish. Res*., **5:** 45-52.

Rahman, M.M., Somsiri, T., Tajima, K. and Ezura, Y., 2004. *Pak. J. biol. Sci*., **7:** 258-268.

Robert, R.J., 1989. The pathophysiology and systematic pathology of teleosts. In: *Fish pathology* (ed. R.J. Robert), Bailliere Tindall, London, pp. 56-134.

Saha, D. and Pal, J., 2002. *Lett. appl. Microbiol*., **34:** 311-316.

Salyers, A.A. and Whitt, D.D., 1994. *Bacterial pathogenesis.* ASM Press, Washington DC, U.S.A.

Shayo, S.D., Mwita, C.J. and Hosea, K., 2012. doi:10.4172/scientificreports. **1:** 115.

Sosa, E.R., Jan, H., Landsberg, Christy, M.S. and Ann, B.F., 2007. *J. aquat. Anim. Hlth.*, **19:**14-26.

Stojanov, I., Nada, P., Dragica, S., Ratajac, R., Jasna, P.R., Pusic, I. and Kapetanov, M., 2010. *Lucr. Stiinlif. Med. Vet.*, **1:** 132-136.

Thune, R.L., Stanley, L.A. and Cooper, R.K., 1993. *Annu. Rev. Fish Dis.*, **3:** 17-68.

Yesmin, S., Rahman, M.M., Afzal, M., Hussain, Khan, A.R., Farzana, P. and Hossain, M.A., 2004. *Pak. J. biol. Sci*., **7:** 409-411.

Zheng, W., Cao, H. and Yang, X., 2012. *Afr. J. microbiol. Res*., **6:** 4512-4520.