Journal of Bacteriology and Parasitology

Dar et al., J Bacteriol Parasitol 2016, 7:3
DOI: 10.4172/2155-9597.1000273

Research Article Open Access

# Characterization of *Aeromonas sobria* Isolated from Fish Rohu (*Labeo rohita*) Collected from Polluted Pond

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**Received date:** April 07, 2016; **Accepted date:** April 29, 2016; **Published date:** May 05, 2016



**Abstract**

One of the major threats to fish aquaculture sector is the infection by *Aeromonas* Spp. The current study assesses the phenotypic characteristics and biochemical characterization of the *A. sobria* strains from the fish cases with septicaemia in order to understand the frequency and occurrence of this infection in the state of Jammu and Kashmir. Clinically the infected fish Rohu (*Labeo rohita*), one of the Indian Major carps (IMC), was observed for symptoms like loss of escape reflex and skin darkness associated with skin haemorrhages. We isolated 30 colonies of *A. sobria* strain from 10 cultured *Labeo rohita* collected from a controlled fish pond in District Poonch of the state. The pond was affected by mismanagement practices, elevated pollution levels and anthropogenic activities. Microscopic examination revealed that the strain was rod-shaped and gram negative. The revealed percent probability identification of *A. sobria* from the biochemical characterization in Vitek system was 93% with GN card. This study could give us clues for understanding *A. sobria* harbouring in fish species and shall help in better understanding of the threat prevalent to the fish species of the region by this infection.

**Keywords:** *Labeo rohita*; Indian Major Carp (IMC); *Aeromonas sobria*; Characterization; Vitek-2 system

## Introduction

*Aeromonas* species can cause infections not only in humans but in fish as well, isolated frequently from surface waters, estuarine water, fresh water, food products, sewage, diseased or healthy fish, human and animal excreta, are ubiquitous in aquatic ecosystems [1-5]. Infections of these types are perhaps the most widespread due to bacterial diseases diagnosed in cultured warm water fish [6,7]. *Aeromonas* species are of no interest in food because at ambient temperature, they are known as active spoilers of fish and meat [8,9]. These species are known to be opportunistic pathogens for fish and generally the incidence rate of this disease is linked to stress conditions such as overcrowding (because of Polyculture), poor water quality, or rough handling and can cause major epidemic outbreaks [2,10,11] and are straight nonspore-forming rods, Gram-negative, normally facultative anaerobic, cytochrome oxidase positive, chemoorganotrophic and usually characterized by being capable to grow at 0% NaCl but not at 6% NaCl [2]. Taxonomically, the genus *Aeromonas* belongs to the class *Gammaproteobacteria*, order Aeromonadales and family Aeromonadaceae [12].

*Aeromonas septicaemia* is a critical contagious disease of cold-blooded animals and humans [13,14] and is frequently caused by the motile *Aeromonas*, particularly *A. hydrphila*, *A. caviae* and *A. sobria*. *Aeromonas* species are facultative anaerobic Gram-negative bacteria and are psychrophilic and mesophilic in nature [13,15]. The release of two important virulence factors namely extracellular hemolysin and aerolysin potentially contribute to the occurrence of septicaemia [6,7]. To identify these bacteria, numerous biochemical schemes have been proposed [16] and some papers reported that the Vitek GN card could be useful in identification of bacteria within the genus *Aeromonas* [17].

The objectives of this study were to focus on the isolation and identification of the strains of *Aeromonas* from the cases of septicaemia in fishes of Jammu and Kashmir State for the first time and develop some understanding regarding the distribution of this infection in fishes of this part of the world.

## Material and Methods

### Sampling of Rohu (*Labeo rohita*) fish

The Rohu (*Labeo rohita*) specimens obtained from the fish pond at District Poonch of Jammu and Kashmir state were collected in the month of December 2013, with a cast net and identified by the help of various taxonomic keys [18,19]. Help was also sought from from the local experts in the field. It is one of the most extensively cultured Indian Major Carps (IMC). Out of 20 fishes collected, 3 were found to be infected with *A. sobria*. Therefore, the prevalence rate was found to be (15%). Body weights of the collected specimens ranged from 50 ± 10 g.

### Assessment of morphological/clinical pathological symptoms

Collected specimens were carefully examined for symptoms of diseases with special focus towards the lesions on the skin [20]. Rohu (*Labeo rohita*) was assessed for bacterial infection by observing the following symptoms: pale gills indicative of anaemia, exophthalmia, abdominal distension, skin blisters, shallow ulcers, haemorrhages and

intramuscular cavities filled with blood-tinged caseous or necrotic material [17]. The surface of the skin was showing red rashes on the body along with ulceration. External surface skin swabs from the samples were inoculated onto the nutrient agar (NA) medium for culturing bacteria [21].

## Isolation and identification of *A. sobria*

Isolation of fish pathogenic bacteria was carried out by culture dependent approach and for this purpose spread plating technique was used. The surface of the fish was swabbed for bacteria isolation, and the inoculums were spread over nutrient-rich medium i.e. nutrient agar medium [17,22], with incubation at 25°C-30°C for 2-3 days [23-25]. The purified stocks of the bacterial strains were obtained and stored for further morphological and biochemical identification.

## Morphological characterization

The bacterial films were prepared from each purified isolate and thereafter Gram's staining was carried [26]. The slides were examined under the bright field microscope with oil immersion lens.

## Biochemical characterization

The characterization of bacteria is carried phenotypically and a wealth of knowledge is available on the phenotypic characteristics of the microbes. Though in recent times, emphasis towards molecular based approaches has increased and phenotypic approach has declined. But, nevertheless, in polyphasic studies whereby many facts of the biology of an organism are studied, phenotypic data has a role [27]. Biochemical identification and characterization of the isolated A. sobria was carried out using the VITEK 2 system which is based on 47 biochemical and physiological test reactions (Table 1). The VITEK 2 compact system is a fully automated system that performs bacterial identification by biochemical analysis using colorimetry. VITEK 2 system automatically performs all of the steps required for identification of bacteria. This system allows kinetic analysis by reading each test every 15 min. The optical system combines multichannel fluorimeter and photometer readings to record fluorescence, turbidity, and colorimetric signals [12].

| APPA | Alla-phe-pro-ARYLAMIDASE | ADO | ADONITOL | PyrA | L-Pyrrolydonyl-ARYLAMIDASE |
|---|---|---|---|---|---|
| IARL | L-ARABITOL | dCEL | D-CELLOBIOSE | BGAL | BETA-GALACTOSIDASE |
| H2S | H2S PRODUCTION | BNAG | BETA-N-ACETYL-GLUCOSAMINIDASE | AGLTp | Glutamyl Arylamidase pNA |
| dGLU | D-GLUCOSE | GGT | GAMA-GLUTAMYL-TRANSFERASE | OFF | FERMENTATION/GLUCOSE |
| BGLU | BETA-GLUCOSIDASE | DMAL | D-MALTOSE | dMAN | D-MANNITOL |
| dMNE | D-MANNOSE | BXYL | BETA-XYLOSIDASE | BALap | BETA-Alanine arylamidase pNA |
| ProA | L-Proline ARYLAMIDASE | LIP | LIPASE | PLE | PALATINOSE |
| TyrA | Tyrosine ARYLAMIDASE | URE | UREASE | dSOR | D-SORBITOL |
| SAC | SACCHAROSE/SUCROSE | dTAG | D-TAGATOSE | dTRE | D-TREHALOSE |
| CIT | CITRATE(SODIUM) | MNT | MALONATE | 5KG | 5-KETO-D-GLUCONATE |
| ILATk | L-LACTATE alkalinisation | AGLU | ALPHA-GLUCOSIDASE | SUCT | SUCCINATE alkalinisation |
| NAGA | Beta-N-ACETYL-GALACTOSAMANIDASE | AGAL | ALPHA-GALACTOSIDASE | PHOS | PHOSPHATE |
| GLyA | Glycine ARYLAMIDASE | ODC | ORNITHINE DECARBOXYLASE | LDC | LYSINE DECARBOXYLASE |
| IHISa | L-HISTIDINE assimilation | CMT | COUMARATE | BGUR | BETA-GLUCORONIDASE |
| O129R | 0/129 RESISTANCE | GGAA | Glu-Gly-Arg-ARYLAMIDASE | IMLTa | L-MALATE assimilation |
| ELLM | ELLMAN | ILATa | L-LACTATE assimilation | | |

**Table 1:** Details of biochemical Tests carried in Vitek-2 System.

## Results

The clinical examination of diseased fish reveals the presence of red spots on the body. Ulceration was also spotted on the body of fish. Isolation of bacteria was achieved by swabbing the surface of the fish and then followed by inoculation of bacterial strain on nutrient-rich medium, such as nutrient agar medium (NA) with incubation at 25°C-30°C for 2-3 days. Different types of colonies were obtained during the study period. Some colonies were circular in shape and some irregular, few colonies were Rhizoid and filamentous. A total of 30 colonies of the *A. sobria* strain were completely counted on nutrient agar media plates. The colonies of *A. sobria* were creamy in color and morphologically they were circular in appearance, entire in margin and were having flat elevation. Creamy colonies were selected and restreaked three times onto fresh media to obtain pure isolates. The Strain was observed under microscope for cell shape and it was found to be rod in shape and it gave Grams negative reaction upon Gram

staining. In this study, the isolated bacterium was found to be gram negative, and the results of the isolate from the database of Vitek system on GN card showed that the percent probability of identification of *A. sobria* was 93%. Biochemical identification results and characterization observations made on this strain in VITEK- 2 system with 47 tests are indicated in Table 2.

| Well | Mnemonic | Reaction | Well | Mnemonic | Reaction | Well | Mnemonic | Reaction |
|---|---|---|---|---|---|---|---|---|
| 2 | APPA | + | 3 | ADO | _ | 4 | PyrA | (+) |
| 5 | lARL | _ | 7 | dCEL | + | 9 | BGAL | + |
| 10 | H2S | _ | 11 | BNAG | + | 12 | AGLTp | _ |
| 13 | dGLU | + | 14 | GGT | _ | 15 | OFF | + |
| 17 | BGLU | _ | 18 | Dmal | + | 19 | dMAN | + |
| 20 | dMNE | + | 21 | BXYL | _ | 22 | BALap | _ |
| 23 | ProA | + | 26 | LIP | _ | 27 | PLE | _ |
| 29 | TyrA | + | 31 | URE | (-) | 32 | dSOR | _ |
| 33 | SAC | + | 34 | Dtag | _ | 35 | dTRE | + |
| 36 | CIT | + | 37 | MNT | _ | 39 | 5KG | _ |
| 40 | lLATk | _ | 41 | AGLU | _ | 42 | SUCT | + |
| 43 | NAGA | + | 44 | AGAL | + | 45 | PHOS | _ |
| 46 | GLyA | _ | 47 | ODC | _ | 48 | LDC | _ |
| 53 | lHISa | _ | 56 | CMT | + | 57 | BGUR | _ |
| 58 | O129R | + | 59 | GGAA | (-) | 61 | IMLTa | + |
| 62 | ELLM | (-) | 64 | ILATa | _ | | | |

**Table 2:** Biochemical Details of *Aeromonas sobria* on test substrates on GN Card.

## Discussion

Aquaculture sector is under a persistent threat due to fish pathogen *Aeromonas* spp. Biochemical studies like the ones discussed here are important in the search for alternative and more effective methods of control of these fish pathogens. Mismanagement practices, elevated pollution levels and anthropogenic activities often trigger the *Aeromonas* infections in fish in aquaculture. The fish pond explored during the present study was affected by all these parameters. Fishes in aquaculture are prone to a variety of diseases due to inapt farm management systems, so vulnerability of fish to pathogenic infections is enhanced [28] and diversity of mobile *Aeromonas* Spp. has been reported in the aquatic environment and fish [29-31]. The exposure of the fish pond to human and other activities might have induced some pollution activities into the aquatic environment of the pond and scattering of pathogenic *A. sobria* into the aquatic environment by excreta material can pollute not only fish fauna but also other fauna harvested from these waters and once these bacteria are in the aquatic environment, plasmid exchange between the bacteria is readily facilitated and can result in a higher frequency of multiple antibiotic resistant strains and the development of fish disease in the fish [32]. Usage of medicated feeds in agriculture sector and their application to the rapidly developing fish and shellfish farming [33] can result in the production of virulent and resistant bacterial pathogens in the natural environment and thus potentially into the human food chain, which may have also prompted for the breakout of *Aeromonas* infection in the water body studied during the present study. *Aeromonas* species causes septicaemia with widespread skin lesions and affecting internal organs such as liver, spleen and muscles. Infected fish obtained revealed the presence of red rashes on the body. Similar observations were recorded by [17,20] who mentioned that the infection created by *Aeromonas* Spp could be the cause of skin ulcers in fishes. Characteristic colonies of *A. sobria* on Nutrient Agar medium were indicated by creamy colonies and the results obtained agree with the findings of some authors who mentioned that the color of bacterial colony could be a diagnostic factor to ascertain its genus level [17,34]. The morphological results and biochemical tests of a total of 30 strains of *A. sobria* were carried out which established the species level of the isolated bacterial species. Similar results were reported by various authors, who mentioned that *A. sobria* was most commonly isolated Spp. from apparently healthy fishes and suggest that the morphological and biochemical characteristic results could be diagnostic tools to identify the bacterial species [35,36]. During this study, the isolated bacterium was found to be gram negative, and showed 93% probability using the Vitek System. *Edwardsiella tarda* indicated 98% probability in Vitek System 2 test which is in consonance with the present study [37]. Similarly, the results from the Vitek database indicated that the percent probabilities of identification of *A. veronii* were 95 to 99%, however the percent probabilities of identification of *A. hydrophila*, *A. caviae* and *A. sorbia* were only 69 to 83% [38] and *A. sobria* was 93% [17].

## Conclusion

The study revealed the presence of *A. sobria* in Rohu *Labeo rohita*, from aquaculture farm at Poonch J & K, India. The revealed percent probability identification of *A. sobria* from the biochemical characterization in Vitek system was 93% with GN card. The study highlights the diversity of *A. sobria* that could potentially be associated with skin surfaces of the fish and trigger infections. The results obtained highlight the need to promote responsible fish ownership, good husbandry practices and prudent use of antimicrobials in the fish industry so as to control this type of infection.

## Acknowledgements

The authors would like to thank Department of Science and Technology (DST), Govt. of India and Govt. of Jammu and Kashmir Department of Fisheries for financial assistance. The authors are also thankful to Dr Qadris Haematology Centre, Srinagar for identification of bacterial strains. This work coincides with partial fulfillment of PhD dissertation of principal author (GHD).

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