Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(12):284-288

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Drug's resistant activity of *Proteus vulgaris* isolated from gut of *Mystus seenghala* of northern Punjab region

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ABSTRACT

Fish are permanently exposed to different external hazards because of their intimate contact with aquatic environments. The bacterial infection is an important and limiting factor of intensive fish production because they have shown tight connection with various body functions of host i.e., metabolism, immunity, energy utilization and health maintenance. Proteus vulgaris is an opportunistic pathogen of fish because it is the most common bacterial species of inland water and sediments. It causes gastrointestinal and non-gastrointestinal disease i.e. hemolytic syndrome, kidney disease, ulcerative disease, urinary tract infection (UTI) etc.in various teleost. Proteus vulgaris acknowledge their presence in many catfish, which were collected from fish market of and around Jalandhar district during spring season. Confirmation came after biochemical test performed in university lab and specific test through BD Phoenix machine done at ICAR Research Complex (Meghalaya). It has been found that isolated colonies were susceptible to azithromycin, kanamycin, chloramphenicol and ciprofloxacin. Against neomycin and gentamycin 72% colonies were susceptible and rest 28% shows partial growth. About 86% of colonies were susceptible to ofloxacin. But important finding of this work is that, colonies are resistant against drugs like amoxicillin, penicillin, ampicillin and methicillin. But with ofloxacin, gentamycin and neomycin more study is to be carried out.

Keywords: *Proteus vulgaris*, *Mystus seenghala*, multi-drug resistance (MDR).

INTRODUCTION

A large number of diseases occur in all aquatic animals including fish. Fishes have very close relationship with their environment. Pathogenic bacteria have the potential to proliferate or maintain them in the aquatic environment. The pathogenic bacteria are constantly taken up by the fishes through feeding and osmoregulation process [1]. All species of fish infected with disease causing by bacteria, protozoan, fungi, worms, helminthes etc. The bacterial infection is an important and limiting factor in intensive fish production[2]. The first requirement of a pathogenic bacterium is to penetrate the primary barriers so that they can establish the infection. In fish, the main route of infection is though skin, gills and gastrointestinal tract. In the last two decades, the infection and cellular damage (specific attack on tight junction and desmosomes) caused by pathogenic bacteria has increased [3].

The most common fresh water diseases are dropsy, ick (ich), tail and fin rot, gill diseases, fungal infection, white spot disease, pop eye, cloudy eye, swim bladder diseases, water quality induced diseases, anorexia, tuberculosis,

glugea, hexamita, marine velvet diseases etc. [4]. The dominant bacteria of pond water, pond sediment and fish are *Proteus vulgaris*,*Pseudomonas* species, *Bacillus* species and*Micrococcus* species more over [2].

The fish pathogenic bacteria become resistant to a number of antibiotics due to frequent use of wide range of drugs in aquaculture [5].

Proteus vulgaris is a gram negative, rod shaped, chemoheterotrophic bacteria containing flagella belongs to the family *Enterobacteriaceae*. The size of bacteria varies from 0.4~0.6 µm by 1.2~2.5 µm [6].

Proteus species are differentiated from most other genera because they have the ability to swarm across an agar plate. These organisms are widely distributed in the environment including polluted water, soil and manure [7].

Proteus vulgaris is opportunistic pathogen of fish because it is most common bacterial species of inland water and sediments [6]. It causes gastrointestinal and non-gastrointestinal disease i.e. hemolytic syndrome, kidney disease, ulcerative disease [5, 8], blotch diseases, red spot, tail rot and spottiness of skin [9].

Proteus vulgaris are commonly associated with complicated urinary tract infection (UTI), causing infection such as urolithiasis, cystitis and a variety of noscomial infection including respiratory tract, eye, skin, burns and wounds [7]. The *Proteus* isolates are highly susceptible to cefotaxime, amikacin and gentamycin. However they are resistance to ampicillin, netilline, cefuroxime and pefloxacin [10]

EXPERIMENTAL SECTION

Sample collection and isolation:

Collect the fish sample from Phagwara

↓

Dissect the gut of catfish

↓

The isolation of the microbe was done by pouring the sample into the peptone water

↓

Kept on shaker for 30 minutes

↓

Streak it on the S.A. agar plates

↓

Incubate the Petri plates for 24 hrs. at 37°C

↓

After 24 hrs. of incubation colony appeared on the Petri plates

Ingredients	gms/l
Casein enzyme hydrolysate	10.00
Sodium chloride	5.00
Starch soluble	1.00
Phenol red	0.018
Agar	15.00
Final pH	7.2 - 7.6

The fresh gut and gill sample of the catfish was collected from fish market of and around Jalandhar district and isolation of the microbe was done by pouring the sample into the peptone water and the mixture was kept on shaker for 30minutes. The starch ampicillin agar medium was used to culture the *Proteus vulgaris.*We modified protocol by adding Novobiocin and Sodium deoxycholate, which inhibits the growth of gram-positive bacteria.

Identification Method:

The most important task in the bacteriology is the identification of pathogen so that appropriate treatment can be instituted [11]. Identification can be performed by two methods i.e., biochemical and molecular. The biochemical tests, which were performed according to instructions given by the Himedia laboratoryare gram staining, hemolysis test, oxidase test, catalase test, urease test, mannitol fermentation test, glucose fermentation, H_2S production,

motility test, citrate utilization test, nitrate reduction, indole test and methyl red test for the identification of *Proteus vulgaris* [12].

For molecular identification specific tests were performed through BD Phoenix machine done at ICAR Research Complex (Meghalaya) (Fig. 2).

Multi-drug treatment:
Mix the ingredients of Muller Hinton Agar in distilled water and sterilization was done by autoclaving the mixture. Allowed the media to cool and pour into sterilized Petri plates. Then using sterile spreader, streak the colony of *Proteus vulgaris* onto the MHA plates, with the help of applicator place the antibiotic discs onto the surface of *P. vulgaris*-Mueller Hinton agar and then press gently with forceps to ensure firm contact of the antibiotic disc with the agar surface and incubate for 24 hours. Various drugs were used to determine the MDR of the *Proteus vulgaris* is chloramphenicol, kanamycin, ciprofloxacin, ofloxacin, cefotaxime, amoxicillin, penicillin, methicillin, neomycin, gentamycin, ampicillin, and azithromycin [13].

RESULT AND DISCUSSION

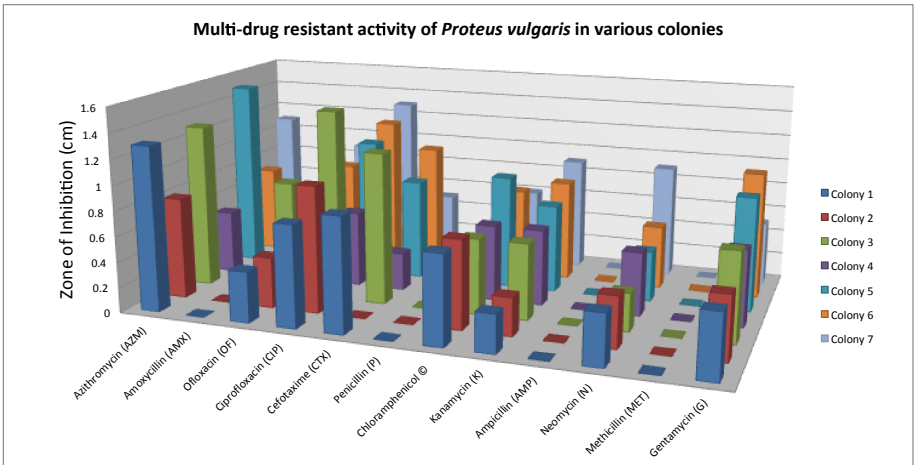
For biochemical analysis, the bacterial isolates were taken from gut and gill sample (A, B, C, D) of the fish which gives the accompanying results as shown in table 1. By which it is to be confirmed that the isolated bacteria belongs to *Proteus vulgaris*. The bacteria give the positive result to oxidase test which means it oxidize the substrate N, N, N, N tetramethyl-p-phenyleneamine dihydrochloride into indophenols, H_2S test, glucose test, motility test, indole test, catalase test and haemolysis test which means it produce haemolysin enzyme that lysis the RBCs.

After confirmation it has been found that sample D (*Mystus* gut) contains *P. vulgaris*. So drug susceptibility of bacterial colony of sample D was checked and following results were obtained (shown in table 2).
We randomly select 7 colonies of *Proteus vulgaris* and check their multidrug resistant activity.

Table 1: Biochemical characterization of isolates

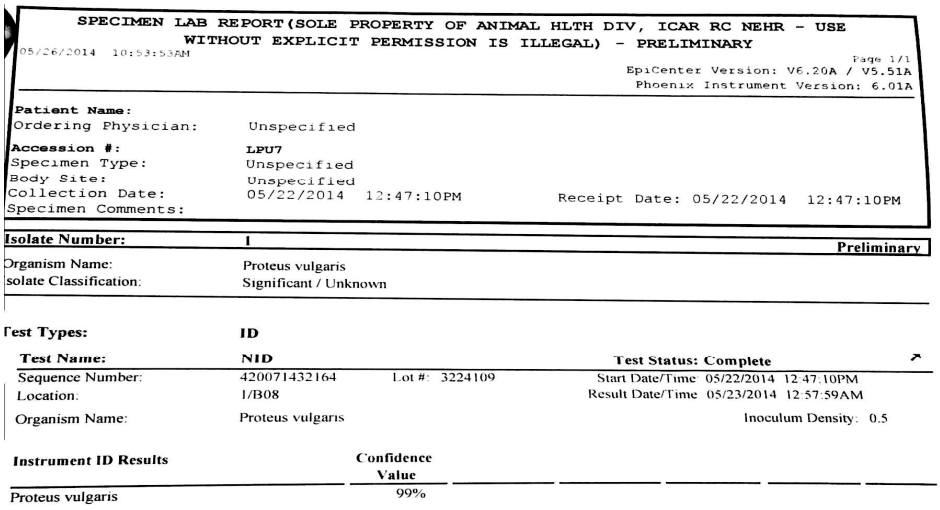
Tests	Sample A (Gut of *W.attu)*	Sample B (Gill of *W. attu*)	Sample C (Gill of *Mystus*)	Sample D (Gut of *Mystus)*
Gram staining	-ve	-ve	-ve	**-ve**
Oxidase	+ve	+ve	+ve	**+ve**
Haemolysis	+ve	+ve	+ve	**+ve**
TSIA(H_2S)	-ve	-ve	+ve	**+ve**
TSIA(glucose)	-ve	+ve	+ve	**+ve**
Motility	+ve	+ve	+ve	**+ve**
Citrate	+ve	+ve	+ve	**+ve**
MR-VP (Methyl red)	-ve	-ve	-ve	**+ve**
(Voges-proskauer)	-ve	-ve	-ve	**-ve**
Nitrate	-ve	+ve	-ve	**+ve**
Mannitol	-ve	-ve	-ve	**-ve**
Urease	-ve	-ve	-ve	**-ve**
Indole	+ve	+ve	+ve	**+ve**
Catalase	+ve	+ve	+ve	**+ve**

Table 2: Multi-drug resistant activity of *Proteus vulgaris* in various colonies



	Azithromycin (AZM)	Amoxycillin (AMX)	Ofloxacin (OF)	Ciprofloxacin (CIP)	Cefotaxime (CTX)	Penicillin (P)	Chloramphenicol ©	Kanamycin (K)	Ampicillin (AMP)	Neomycin (N)	Methicillin (MET)	Gentamycin (G)
Colony 1	1.3	0	0.4	0.8	0.9	0	0.7	0.3	0	0.4	0	0.5
Colony 2	0.8	0	0.4	1	0	0	0.7	0.3	0	0.4	0	0.5
Colony 3	1.3	0	0.9	1.5	1.2	0	0.6	0.6	0	0.3	0	0.7
Colony 4	0.5	0	0.3	0.6	0.3	0	0.6	0.6	0	0.5	0	0.6
Colony 5	1.5	0	0.7	1.1	0.8	0	0.9	0.7	0	0.4	0	0.9
Colony 6	0.7	0	0.8	1.2	1	0	0.7	0.8	0	0.5	0	1
Colony 7	1.1	0	0.9	1.3	0.5	0	0.6	0.9	0	0.9	0	0.5

Drug susceptibility of *P. vulgaris* tested by disc diffusion method with antibiotics on Mueller Hinton Agar in which clear zone shows that the *P. vulgaris* is susceptible to antibiotic and where there is no clear zone, that means *P. vulgaris* is resistant to that antibiotic.



SPECIMEN LAB REPORT(SOLE PROPERTY OF ANIMAL HLTH DIV, ICAR RC NEHR - USE WITHOUT EXPLICIT PERMISSION IS ILLEGAL) - PRELIMINARY

05/26/2014 10:53:53AM Page 1/1

EpiCenter Version: V6.20A / V5.51A
Phoenix Instrument Version: 6.01A

Patient Name:
Ordering Physician: Unspecified

Accession #: LPU7
Specimen Type: Unspecified
Body Site: Unspecified
Collection Date: 05/22/2014 12:47:10PM Receipt Date: 05/22/2014 12:47:10PM
Specimen Comments:

Isolate Number: 1 **Preliminary**

Organism Name: Proteus vulgaris
Isolate Classification: Significant / Unknown

Test Types: ID

Test Name: NID **Test Status: Complete**
Sequence Number: 420071432164 Lot #: 3224109 Start Date/Time 05/22/2014 12:47:10PM
Location: 1/B08 Result Date/Time 05/23/2014 12:57:59AM
Organism Name: Proteus vulgaris Inoculum Density: 0.5

Instrument ID Results	Confidence Value
Proteus vulgaris	99%

Fig (2): Confirmation report from ICAR (Meghalaya)

CONCLUSION

Proteus vulgaris presence has been found after isolation of microbes from common edible fishes (carp and catfish) from different fish markets of Phagwara and Jalandhar district (Punjab).Our biochemical tests also support the biochemical characteristics of this species given by several microbiologists and molecular identification was also performed for final confirmation. *Proteus vulgaris* is most common microbe of fish gill and intestine out of reported

species.

It has been found that all (seven colonies) isolated belongs to the *Proteus vulgaris.* These isolated colonies were susceptible to azithromycin, kanamycin, chloramphenicol and ciprofloxacin. About 72% colonies were susceptible to neomycin and gentamycin and rest 28% shows partial growth. About 86% colonies were susceptible to ofloxacin and rest 14% shows partial growth. About 43% colonies were susceptible, 43% were resistant and rest 14% show partial growth to cefotaxime.

But important finding of this work is that, colonies are resistant against drugs like amoxicillin, penicillin, ampicillin and methicillin.

Acknowledgement
It gives us immense pleasure to express our sincere gratitude and heartfelt thanks Lovely Professional University Punjab to provide lab facility. We are also thankful to ICAR Research Complex (Meghalaya).

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