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BACTERIAL SPECIES ISOLATED FROM *CLARIAS GARIEPINUS* CULTURED IN EGYPT AND THE RELATED PATHOLOGICAL ALTERATIONS

BY

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ABSTRACT

This study was carried out on 200 *catfishes (Clarias gariepinus*) collected from farms at Al-Dakahlia governorate from September, 2013 to March, 2014. Fishes were selected on the basis of the characteristic clinical abnormalities such as hemorrhage, redness, erosions ulcerations and blisters . Bacteriological examination was carried out under complete aseptic conditions and the affected organs were preserved in formal saline solution for histopathological examination. The experiment revealed the isolation of 360 Bacterial isolates. The isolated strains were classified as follow: *photobacterium damselae* 30%, *Aeromonas. Hydrophila* 20%, *Aeromonas. Salmonicida* 10%, *Aeromonas. Sobria* 10%, Vibrio. Cholera 25% and 5% for *Citrobacter* and *Salmonella* species. Particular concern was given to monitor the pathologic alterations of these pathogens in gonads of both sexes. Another experiment on eggs was conducted to investigate the evidence of vertical transmission of *Aeromonas. Salmonicida.*

Keywords: Bacterial infection, Clarias gariepinus, Aeromonas, Vibrio.

INTRODUCTION

Bacterial diseases are responsible for heavy mortality in both wild and cultured fish. These microorganisms are essentially opportunist pathogens which invade the tissues of a fish host rendered susceptible to infection by stress factors or other disease processes. The most significant group of microorganisms in this respect is the motile aeromonads. (Allen et al., 1983 ; Austin & Austin 2007) although it is likely that, as in the higher vertebrates, other Gram - negative bacteria also play a significant role, but are not as yet properly studied (Austin 2006), Cited by (Ronald, 2012). Bacteria are intimately associated with all life stages of marine organisms. In aquaculture, high population densities and suboptimal rearing conditions often make conditions ideal for opportunistic pathogens, with high mortalities being the result. Knowledge of the different ecological relations between bacteria and the different cultivated species is essential if we are to ensure increased survival. Studies of the pathogenic or mutualistic bacteria of early life stages have been hampered by the lack of adequate protocols for challenge experiments and methods for isolation and detection of bacteria associated with eggs and larvae (Øivind, 1999). Hence the objectives of this study were to survey the diversity of most common bacterial infections in Clarias gariepinus and the possibility of vertical transmission.

MATERIAL AND METHODS

2.1. Sample collection and isolation of bacteria:

A total of 200 adult brooders catfishes of 350±20 g were examined for lesions and clinical signs prior to autopsy. Postmortem examination was carried out after euthanasia under sterile conditions, the ventral abdominal surface was sterilized by cotton moistened with absolute alcohol then blunt dissection started from the vent to expose the abdominal viscera. Samples were collected from liver, spleen, anterior

kidney and gonads. Trypticase Soy Agar (TSA), Thiosulphate Citrate Bile salt Sucrose Agar (TCBS)and Rimler Schott's media (RS) were used for bacterial culture. Samples were incubated at a temperature ranged from 25 to 28°C for 24-48 hours with daily monitoring of bacterial growth.

Organs which showed characteristic lesions were preserved in formal saline solution 10% (To prepare one liter of this solution; 8.5 gram Sodium Chloride was thoroughly mixed with 900 ml of tap water, then 100 ml of strong formalin 38% was added). The solution was used for fixation of organs for histopathology. The organs were placed in plastic jars and covered with the solution with periodic change once per hour until the solution become clear.

2.2. Identification and biochemical characterization of isolates:

Identification of the isolates was carried out by determining their morphology, culture and biochemical characteristic according to the criteria of Bergey's Manual of Determinative Bacteriology. In addition to, API-20E and API-20NE commercial kits system.

2.3. Isolation of Aeromonas. Salmonicida from eggs:

1.3.1. Sample collection:

Eggs were selected on a random basis from Al-Manzala hatchery.

2.3.2. Experimental design:

Eggs were homogenized in a sterile mortar and a representative samples were taken for bacteriological examination.

2.3.3. Aeromonas. Salmonicida Reisolation:

Samples were cultured on plain Nutrient Agar to show the characteristic brown pigmentation of Aeromonas. Salmonicida.

RESULTS

Bacteriogical examination revealed the isolation of 360 bacterial isolates from 200 catfishes belonging to 7 species having the following

percentages as follow: photobacterium damselae 30%, Aeromonas. Hydrophila 20%, Aeromonas. Salmonicida 10%, Aeromonas. Sobria 10%, Vibrio. Cholera 25% and 5% for Citrobacter and Salmonella species. These bacteria were isolated from different organs of catfishes with the following incidences: Ovary 33%, Liver 22%, Kidney 22%, Spleen 14%

and Testis 9%. Aeromonas. Salmonicida was also reisolated from the vitelline fluid of the homogenized eggs.

Histopathology revealed the pathologic changes which were associated with the isolated pathogens such as the systemic reactions represented by leukocytic infiltration in different organs. Gonads were of particular concern and ovaries illustrated variable degrees of degeneration and/or regression of ovarian follicles while testes showed necrosis of sperms manufacturing cells. Interstitial nephritis was recorded in kidney.

DISCUSSION

The phenotypic strain identification based on the three species (A. hydrophila, A. cuviae, A. sohria) concept may be misleading because many commercial test kits do not always recognize all Aeromonas species correctly (Carnahan et al., 1991; Altwegg et al., 1990). A rather low incubation temperature of 28-30°C is needed because many Aeromonas species do not grow at temperatures above 35°C or even if they do grow their biochemical characteristics are not typical (Altwegg et al., 1987; Hanninen and Siitonen, 1995). The results of the biochemical characterization of the isolates were interpreted and found in agreement with those reported by (Nieto et al.,1984) and (Toranzo et al.,1986). The successful isolation and identification of A. hydrophila from extra-intestinal organs of naturally infected fish is in agreement with the results of (Janda.,1991) and (Ali,1996) who reported that A. hydrophila isolates recovered from sterile extra-intestinal organs are considered to have

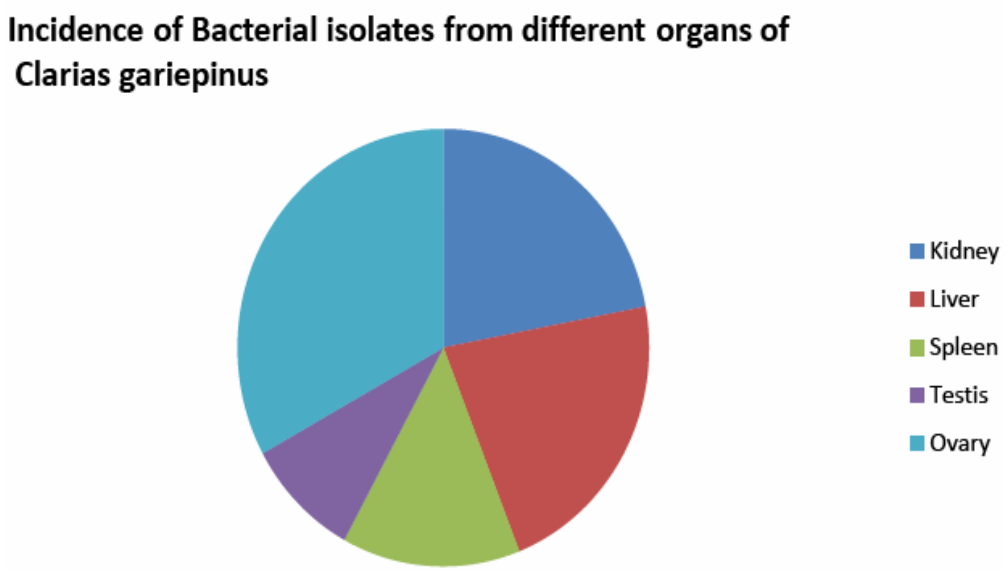

Figure.1: A pie chart demonstrating the incidence of bacterial isolates from different organs of Clarias gariepinus. Ovary 33%, Liver 22%, Kidney 22%, Spleen 14% and Testis 9%.

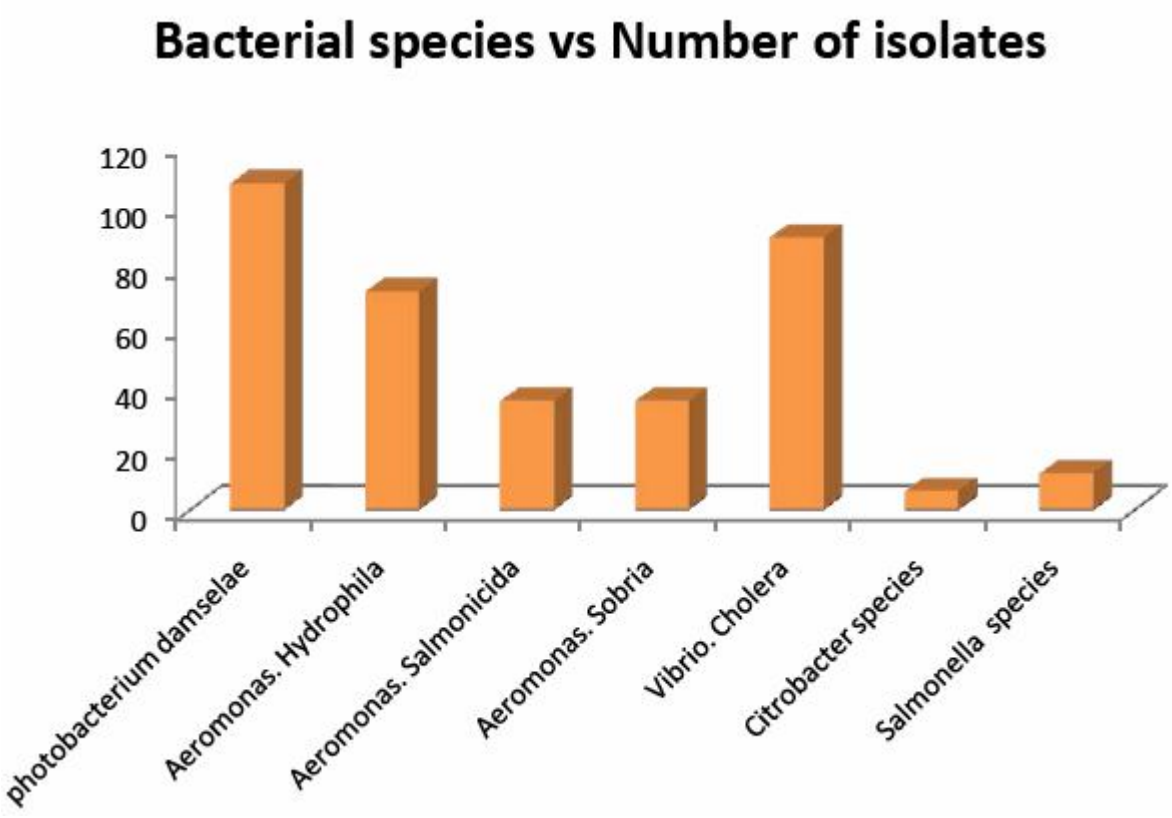

Figure.2. A chart illustrating the number of isolates of each bacterial species.

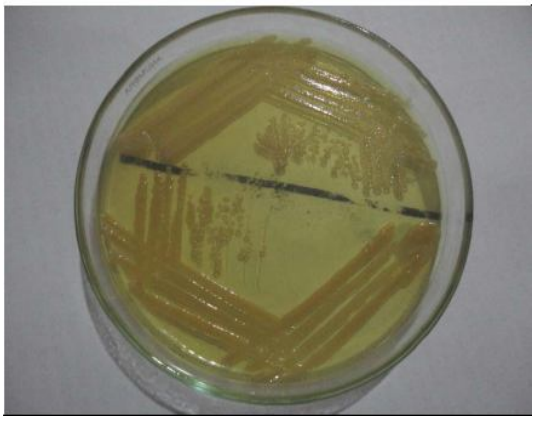

Fig.3. Trypticase Soy Agar plate 24 hours old culture showing Aeromonas. Hydrophila above and A. salmonicida (brown pigment) below

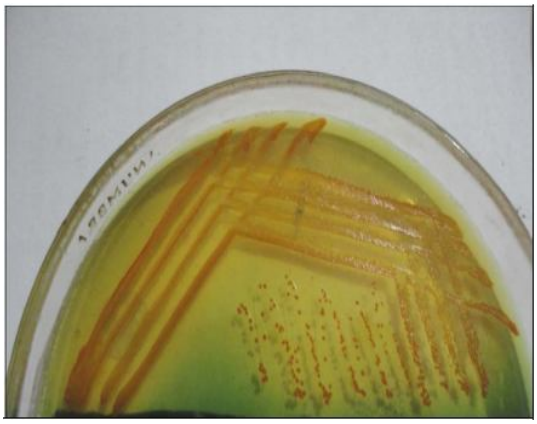

Fig.4. Aeromonas. Hydrophila on Rimler Schotts Media appear yellow to orange.

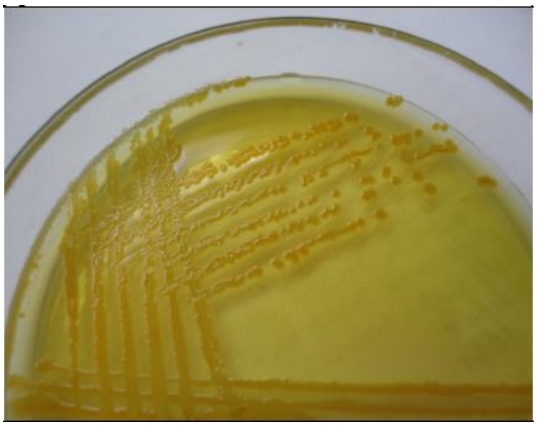

Fig.5. Vibrio. Cholera appear yellow on TCBS and change the colour of the medium from green to yellow.

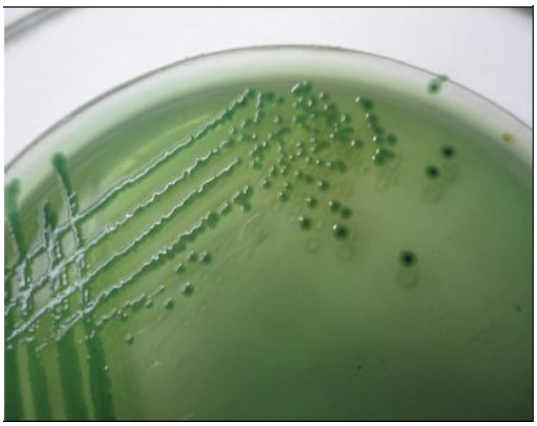

Fig.6. Photobacterium damselae on TCBS appear green button like colonies.

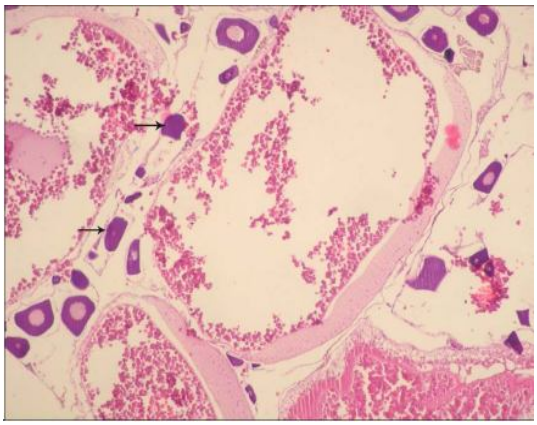

Fig.7. Ovary, Showing degeneration of mature follicle with regression of immature follicle (arrows).

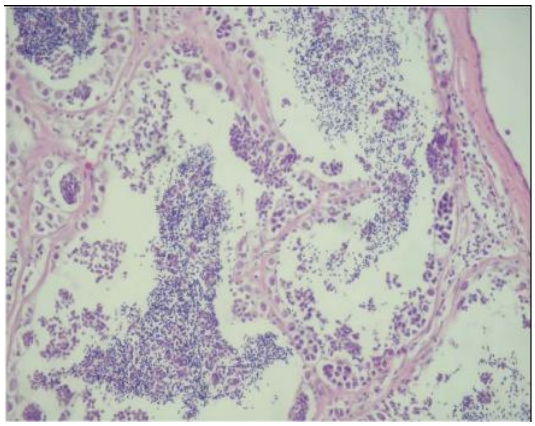

Fig.8. Testis, Showing necrosis of spermatocytes and spermatogonia.

originated from invasive disease and the acute Motile Aeromonas Septicemia may result in localization of colonies identified as A. hydrophila within the hematopoetic tissue. The results of this study match the findings of (Faisal et al.,1989) who reported that outbreaks of Motile Aeromonas Septicemia occurred mainly during winter in cultured fish, while in wild Nile fish mortality was observed in late spring and summer.

Isolation of A. Salmonicida from eggs does not necessarily confirm the vertical transmission agree with (Mackie et al.,1930) noted the presence of A. salmonicida within the ovaries and testes of infected fish, but failed to experimentally induce intra-ovum infections and vertically transmit furunculosis between parent and offspring. Consequently, Mackie et al (1933) concluded that A. salmonicida contaminated the surface of the eggs and recommended that such eggs should be disinfected to minimize or prevent further contagion (Mackie et al., 1933). (McCarthy, 1977) indicated that vertical transmission was not a significant route of infection because A. salmonicida cells from infected parents were unlikely to survive in the eyed-egg stage. Similarly, (Bullock and Stuckey, 1987) were unable to document vertical transmission of furunculosis among the progeny of parental stocks that had either survived furunsulosis expizootics or had been experimentally injected with A. salmonicida prior to spawn.

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