Isolation and Identification of *Pseudomonas aruginosa* from Goldfish (*carassius auratus*) and studing the antibiotic sensitivity in Al-Kufa city

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Abstract

This study was conducted to isolate and diagnose bacterial agents and studying the antibiotics sensitivity in gold fish (*carassius auratus*). 43 goldfish were tested from the fish market in Al-Kufa city . The results of macroscopic examination showed hemorrhagic skin lesion , brown to red spotted skin of a varying degree were found as well as observed darkens color and the presence of black color on the fin. Also, noted the accumulation of fluid in the abdominal (ascitis) with no damage to internal organs observed. *Pseudomonas aruginosa* was isolated and diagnosed from the skin and the fins, Biochemical tests were conducted to confirm the diagnosis. The antibiotic sensitivity was tested by using seven antibiotics clindamycin (2 mg), ceftriaxone (30 mg), amikacin (30 mg), Sevbaudoxim (10 mg), ampicillin (10 mg), cefotaxime (30 mg), and gentamicin (10 mg)). The results of the sensitivity test were showed that amikacin, clindamycin and ceftriaxone was influential on the growth of bacteria with a diameters Sense (30 ,25 and 20 mm) respectively, while remained antibiotics, the bacteria were resistant to them.

عزل وتشخيص جراثيم الزوائف الزنجارية من الاسماك الذهبية ودراسة حساسيتها للمضادات الحياتية في مدينة الكوفة

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الخلاصة:

أجريت هذه الدراسة لعزل وتشخيص المسببات البكتيرية و دراسة حساسيتها للمضادات الحياتية من الأسماك الذهبية (القشري).تم فحص 43 سمكة ذهبية من سوق السمك في مدينة الكوفة، اظهرت نتائج الفحص العياني وجود افات جلدية نزفية ولوحظت بقع حمراء مائله للبني على الجلد بدرجات متفاوتة وكذلك لوحظ تغمق اللون و وجود اللون الأسود على الزعنفة، كذلك لوحظ تجمع السوائل في منطقة البطن مع عدم وجود اضرار في الاحشاء الداخلية. تم عزل وتشخيص جراثيم *Pseudomonas aruginosa* من الجلد والزعانف وتم اجراء الفحوصات الكيموحيوية لتأكيد التشخيص. كذلك تم فحص مدى حساسيتها للمضادات الحياتية حيث استخدم سبعة مضادات حياتية (الكليندااميسين (2ملغ)، سيفترياكسون(30 ملغ) ،الأميكاسين (30 ملغ، سيفبودوكسيم (10ملغ)، الأمبيسلين (10ملغ)،سيفوتاكسيم (30ملغ)، وجنتاميسين (10ملغ).اظهرت نتائج فحص الحساسية ان الاميكاسين ،الكليندامايسين و سيفترياكسون كانت مؤثرة على نمو البكتريا وباقطار تحسس (25,30 و 20 mm) على التوالي بينما بقيت المضادات الاخرى فكانت البكتريا مقاومة لها.

INTRODUCTION

Pseudomonas aruginosa are an ubiquitous group of environmental Gram-negative motile(have one flagella) (Stead., 1992). Bacterial pathogens in aquatic environments that can cause infections in fish, where a growing number of pathogenic bacteria causing the infection and death of fish (especially Koi fish freshwater aquaculture (Tor Monsen *et al*.,2008). Several species of *Pseudomonas* have been reported cause disease in a number of fish species, include goldfish like *Carassius auratus* (Bullock .,1965),and are associated with septicemia in aquatic animals (Roberts .,1978).The lesions and hemorrhages were noted at the base of the fins or on the skin due to bacterial infection isolated bacteria were Gram negative, rod shaped and motile positive for oxidase, catalase, pyocyanin production, citrate utilization, gelatin liquefaction test .The bacteria were unable to ferment glucose. The biochemical properties and the characteristic blue-green appearance of culture due to the mixture of pyocyanin (blue) were indicative of *Pseudomonas aeruginosa.* (Somerita *et al*.,2012).These bacteria have been considered as

 ISSN 2072-3875

opportunistic pathogens, causing diseases when the host is subjected to stress (Somsiri and Soontornvit ., 2002).The lesions ,hemorrhages and ulceration were recorded at the base of the fins or on the skin due to bacterial infection (*p. aruginosa*) (Hossain., 2008). This study aimed to determine the phenotypic and biochemical of the disease causing bacterial which isolated and determine effective antibiotic treatments.

MATRERIAL AND METHOD:

1-Sampling of fish:

A total of 43 live fish were collected from fish farms located at NAJAF market. Afterward, the infected fishes were acclimatized in water of glass aquarium in the laboratory(University of Kufa, college of Veterinary medicine) conditions approximately at 25 Ċ.

2- isolation of Bacteria and culture:

Fish mucus, caudal fin and skin region were carefully scraped from the dorsal body using a sterilized cotton swab, ventral skin mucus was not collected to avoid intestinal and sperm contamination the sample were cultured in broth and agar incubated at 37Ċ for 24 hour for growth and multiplication. Then the bacteria spread over the surface of MaCconkey agar plate , Nutrient agar and pseudomonas agar.The pseudomonas agar was used for isolation of pure cultures of Pseudomonas species, the isolated bacteria were selected and characterized based on their biochemical properties (Holt and Krieg ., 1984). Dominant isolates were purified and identified using conventional biochemical tests described by (West and Colwell., 1984). Api 20E test was done to sure the diagnosis.

Antibiotic sensitivity test:

Kirby-Bauer disk diffusion method was used to determine the antibiotic-resistant characteristics of the isolated organisms (Bauer *et al*.,1996).Study of resistance pattern of *Psudomonas aeruginosa* isolates were cultured on the muller hinton agar plate by using cotton swabs ,and then 7 types of antibiotic were placed on the surface of agar by using sterile forceps and incubated at 37Ċ for 18 h. These antibiotic were Cefpodoxime(10 mg), Clindamycin(2mg), Ampicillin(10mg), Cefotaxime(30mg), Gentamycin(10mg), Ceftriaxone(30mg), Amikacin(30mg). The diameter of inhibition zones (mm) were recorded for all of the plates and then compared with the standard (National Committee., 1997).

Result and discussion

1-Clinical signs:

The clinical signs were Showed lethargy and loss of appetite , gross lesion include black color on skin, fin is dark in color, the end of fin is damage and necrosis(fig 1) , ascites, internally the intestine was distended with clear, viscous fluid, hemorrhaging is common in the viscera and around the intestines, with enlargement of kidney , liver, and spleen and this agree with (Somerita *et al*.,2012).The results also agreed with (Eissa *et al*.,2010) who stated ulceration and necrotic lesions of skin , fin and gill rot , accumulation abdomen fluid , haemorrhagic septicemia and fish mortality. The lesions and hemorrhages were noted at the base of the fins or on the skin due to bacterial infection. Fluid accumulation in the abdomen is a common feature; the liver and spleen were distended and fluid filled.

2- Organismic Identity:

P. aeruginosa Colony on the nutrient agar was blue green fluorscent (fig 2), on MacConkeys agar the colonies were blue green pigment because the *Pseudomonas aeruginosa* was produce lactose non fermatation and this agree with(Tor Monsen *et al*.,2008),(fig3). Also these results agreed with (Akinyemi.,2012) who isolated *P.aureginosa* from the gill, skin.Also our results agreed with those of (Hossain *et al*.,2006; Musa *et al*.,2009). Biochemical test delivered a preliminary identification of the *Pseudomonas aeruginosa* strain. *Pseudomonas aeruginosa* isolates were identified as Gram negative, motile, oxidase-positive will show a Blue color due to produce the enzyme cytochrome- c oxidase, and ability to reduce oxygen and this consent with (West *et al*.,1985). Simmons citrate-negative will show green mean no. growth because unable to utilize citrate as the

 ISSN 2072-3875

sole carbon and energy source and this assent with (Collins and Lawson2000) and urease negative because there is no rise in pH due to accumulation of ammonia and this approved with (Christensen.,1946). *P.aureginosa* were parallel to previous studies which identified the same organisms from other fish species (Austin and Austin 2007). *P. aeruginosa* are opportunistic Gram negative pathogens, naturally occurring in aquatic environment and as a part of normal gut flora of healthy fish, it cause outbreak when the normal environmental conditions changed (Angelini and Seigneur.,1988).

3- Antibiotic susceptibility

The antibacterial activity of seven drugs type were evaluated bacterial isolates *P. aeruginosa* (fig 3) Which exhibited more sensitive with tested antibacterial drugs in Clindamycin(2mg), Ceftriaxone(30mg) and Amikacin(30mg) the inhibition zones of other antibacterial drugs were (30,25,20 mm) respectively and the *Pseudomonas aeruginosa* was resistant with Cefpodoxime(10 mg), Ampicillin(10mg), Cefotaxime(30mg) and Gentamycin(10mg),(Table 1) The variation in antibiotic sensitivity test of isolated pathogen was due to *P. aeruginosa* is intrinsically resistant to several antibiotics because of the low permeability of its outer-membrane, the constitutive expression of various efflux pumps, and the production of antibiotic-inactivating enzymes (e.g.,cephalosporinases) (Hancock.,1998).Also these variation may be related to the large size and the versatility of its genome, and to its distribution in aquatic habitats, which could constitute a reservoir for bacteria carrying other resistance genes (Vaisvila *et al*.,2001).Also variation may be depend on culture circumstances and highlight (Dominic *et al*.,2005). This result agreed with (*Saraswathi et al.*,2013). (Algama *et al*.,2012) who showed the *P. aeruginosa* sensitive to Gentamycin these results disagreement with our results, These isolates were high resistance against Kanamysin, Cefpodoxime, Cephotaxime. Also, (Eissa *et al.*,2010**)** who experimented the antibiogram sensitivity ,he observed *P.* highly sensitive to Amikicin and sensitive to Gentamicin. We concluded that *P. aeruginosa* isolates were identified the most common bacteria isolated from gold fish, *P. aeruginosa* isolates were sensitive to Clindamycin, Ceftriaxone and Amikacin.

Table (1) Inhibition zones of seven antibiotic drugs against *Pseudomonas aeruginosa* in vitro.

Types and concentration of antibiotic	Inhibition zone (mm)
Clindamycin(2mg)	25±1.9
Ceftriaxone(30mg)	20±2.1
Amikacin(30mg)	30±2.30
Cefpodoxime(10 mg)	-
Ampicillin(10mg)	-
Cefotaxime(30mg)	-
Gentamycin(10mg)	-

ISSN 2072-3875

Fig 1 . Gross lesion include black color on skin and fin

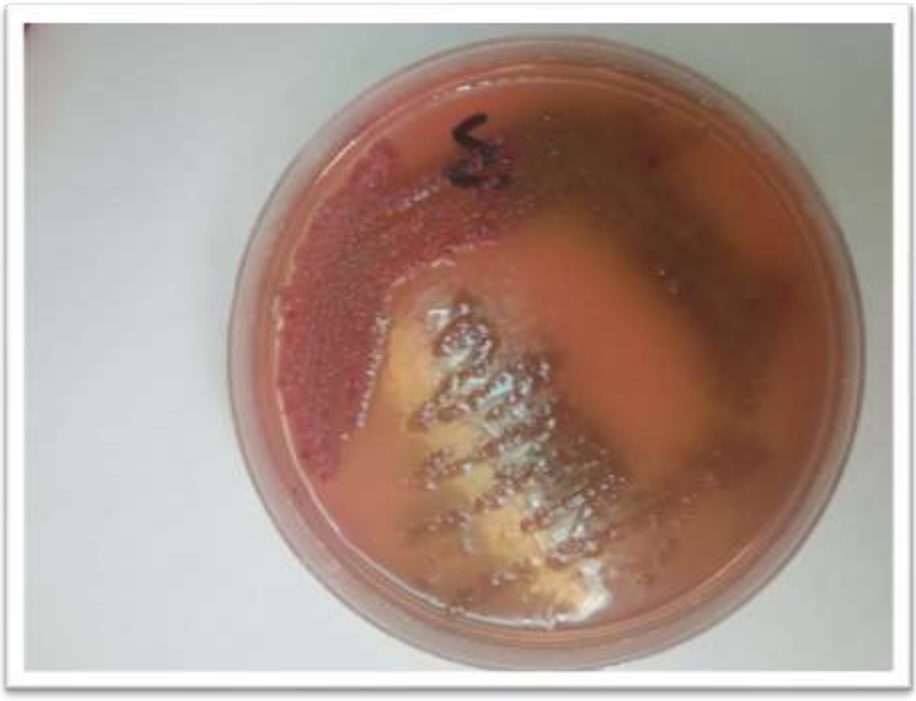

Fig 2: *P. aeruginosa* Colony on MacConkey agar

ISSN 2072-3875

Fig 3: Inhibition zones of seven antibiotic drugs against *Pseudomonas aeruginosa*

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 ISSN 2072-3875

 ISSN 2072-3875