

ISOLATION AND IDENTIFICATION OF STAPHYLOCOCCUS BACTERIA FROM FISH OF FRESH WATER AND ITS ANTIBIOTICS SENSITIVITY IN MOSUL CITY

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ABSTRACT

Bacteriological examination was performed on 60 local healthy fish of fresh water include 30 Carp fish (*Cyprinus carpio*) and 30 Cat fish (*Silurus glanis*) with different weights from local retail fish markets at Mosul city, during the period from sept. 2011 - Sept. 2012. Swabs from skin and parts of muscles, livers, intestines incubated in brain heart infusion broth for 24 hours at 37 ° C (aerobic culture), a loopful from incubated broth were streaked on blood agar, milk agar, mannitol salt agar incubated plates at 37 ° C for 24 h, selected colonies were submitted to gram staining, morphological characteristics biochemical tests for Staphylococcus. The percentage of Staphylococcus isolation was 100% for all examined samples of fish. A total of 130 isolates from both two species of examined fish (62) isolates from *Cyprinus carpio* and (68) isolates from *Silurus glanis* , a five species of Staphylococcus *S. saprophyticus* (29% , 29.4%), *S. epidermidis* (21% , 22%), *S. hyicus* (17.75% , 17.7%), *S. aureus* (17.75% , 19.1), *S. intermedius* (14.5% , 11.8%) , were identified with different numbers and percentages for *Cyprinus carpio* and *Silurus glanis* respectively . While percentages of Staphylococcus isolates from skins (35.5% , 36.8%), muscles (17.7% , 20.6%), livers (25.8% , 25%), intestines (21% , 17.60%) from both species *Cyprinus carpio* and *Silurus glanis* respectively. Antibiotic sensitivity test result for six antibiotics (Ampicillin, Gentamicin, Chloramphenicol, Polymixin, CO-Trimaxazol , Ciprofloxacin) were variable most species of Staphylococcus isolates were resistant to Ampicillin but sensitive to Ciprofloxacin.

INTRODUCTION

Fish meat has excellent nutritional value being rich in proteins, vitamins and unsaturated fatty acid, it is also one of the most important feed stuffs as they are the cheapest source of animal protein during the last years (1 , 2). Fish become contaminated due to its adverse environment such as sewage, contaminated water, harvesting area and from contamination by workers, utensils , equipment, unhygienic handling resulting in presence of great number of bacteria in the fish (3,4,5), many investigations concerning bacterial flora of fish has been increasingly carried out, some of this investigations prove that the flesh and internal organs of healthy freshly fish are microbiological sterile although some investigations have recorded the presence of bacteria in fish muscles (6,7).

Bacterial disease are responsible of heavy mortality in both wild and cultured fish, most of the causative bacteria are naturally occurring saprophytes and they are essentially opportunistic pathogens which invade the tissue of a fish host rendered or other disease processes a few bacterial species appear to be obligatory parasite of fish and they may be survive for varying length of time in the environment (7,8). More than 92 bacterial genera have been implicated as pathogens of fresh water and marine fish, most of them have been identified and classified and some of these bacteria are potential pathogens such as *Aeromonas*, *Pseudomonas*, *Mycobacterium*, *Edwardsiella*, *Streptococcus*, *Staphylococcus*, *Closteridium*(9,10,11). *Staphylococcus* species are one of the most important food borne opportunistic bacteria which isolated from fish samples and some of *Staphylococcus* species are Potential pathogens and the high population of these bacteria indicates the general quality of fish and the degree of the spoilage it might have undergone (1,12,13,14). The distribution of multidrug resistant of *Staphylococcus* species increased in last years and act as etiological infection agent responsible for food poisoning and representing a risk to health when they are enterotoxogenic strains and responsible for significant levels of morbidity and mortality in human (1,15,16,17). The aim of this study is isolation and identification of *Staphylococcus* species from two local fish species include *Cyprinus*

carpio and *Silurus glanis* from skin, muscle, liver, intestine and to investigate the presence of antibiotic resistance of Staphylococcus isolates.

MATERIALS AND METHODS

The microbiological quality of two species of fresh water fish which were collected from local retail fish markets at Mosul city during the period from Sept. 2010- Sept. 2011. A total of 60 local healthy fish , include 30 Carp fish (*Cyprinus carpio*) and 30 Cat fish (*Silurus glanis*) with different weight from 500gm- 2500 gm. The samples transported immediately to the laboratory of Microbiology – Veterinary Medicine / Mosul University , under sterile conditions, cotton swaps from fish surfaces was placed in tube of 10 ml of brain heart infusion broth, then the fish was aseptically dissected and parts of muscles, intestines, livers incubated in tube of 10 ml of brain heart infusion broth for 24 h in 37 ° C (aerobic culture). For microbial analysis, a loopful from incubated broth were streaked on blood agar, milk agar, mannitol salt agar (HIMEDIA), incubated plates at 37 ° C for 24 h, selected colonies were submitted to Gram staining and those with morphological characteristics of Staphylococcus were submitted to biochemical test (18,19,20) . Antibiotic sensitivity test : Strains identified as Staphylococcus were inoculated in nutrient broth for 4h at 37 ° C using sterile swaps for spread the broth on Mueller-Hinton agar, standard antibiotic discs of Ampicillin- 10 µg (AM), Gentamicine-30 µg (GM), Chloramphenicol-30 µg (CL), Polymixin-300 IU(POL), CO-Trimaxazol-25µg (CO-T), Ciprofloxacin 5µg (CIP) (BIOANALYS), were placed on top of the plates and incubated at 37 ° C for 24h. (NCCLS methods). The diameter of the inhibition zone were measured with a ruler (21,22)

RESULT

From the two species of fresh water fish which include (30) Carp fish (*Cyprinus carpio*) and (30) Cat fish (*Silurus glanis*) which examined. The percentage of Staphylococcus isolation was 100% for all examined samples for both species of fish. As a total of 130 isolates from both *Cyprinus carpio* (62 isolates) and *Silurus glanis* (68 isolates). A five species of Staphylococcus were identified with different

numbers and percentage according to morphological characteristics , Gram stain and biochemical test (table 1).

Table (1): Biochemical reactions and other characteristics of Staphylococcus isolates from fish.

Species	Pigmentation Production	Haemolysis	Coagulase	Uearse	Oxidase	Catalase	MR	Glucose	Lactose	Mannitol	Sucrose	Motility
<i>S.saprophyticus</i>	-	-	-	+	-	+	+	+	+	+	+	-
<i>S.epidermidis</i>	-	D	-	+	-	+	+	+	+	+	+	-
<i>S.hyicus</i>	-	-	D	D	-	+	+	+	+	+	+	-
<i>S.intermedius</i>	-	+	+	+	-	+	+	+	+	+	+	-
<i>S.aureus</i>	+	+	+	D	-	+	+	+	+	+	+	-

-:negative , + : positive , D: different

Isolated species were included: *S. saprophyticus* (29%, 29.4%), *S. epidermidis* (21%, 22%) in *Cyprinus carpio* and *Silurus glanis* , *S. hyicus* (17.75%, 17.7%), *S. aureus* (17.75%, 19.1%), *S. intermedius* (14.5%, 11.8%) from total isolates (tables 2 , 3) .

Table (2): Number and percentage of Staphylococcus species isolated from skin, muscles, intestine, livers of *Cyprinus carpio*.

Species	Number and percentage of isolates					
	30/skin	30/muscle	30/intestine	30/liver	Total	%
<i>S. saprophyticus</i>	8	3	3	4	18	29
<i>S. epidermidis</i>	6	2	2	3	13	21
<i>S. hyicus</i>	3	2	3	3	11	17.75
<i>S. aureus</i>	3	2	2	4	11	17.75
<i>S. intermedius</i>	2	2	3	2	9	14.5
Total	22	11	13	16	62	
%	35.5	17.7	21	25.8		100

Table (3): Number and percentage of Staphylococcus species isolated from skin, muscle, intestine, livers of *Silurus glanis*.

Species	Number and percentage of isolates					
	30/skin	30/muscle	30/intestine	30/liver	Total	%
<i>S. saprophyticus</i>	10	4	2	4	20	29.4
<i>S. epidermidis</i>	5	3	3	4	15	22
<i>S. hyicus</i>	3	3	2	4	12	17.7
<i>S. aureus</i>	5	3	3	2	13	19.1
<i>S. intermedius</i>	2	1	2	3	8	11.8
Total	25	14	12	17	68	
%	36.8	20.6	17.60	25		100

Results of Antibiotic Sensitivity test were varied. Most species of Staphylococcus were resistance to Ampicillin and sensitive to Ciprofloxacin while varied in there sensitivity to other antibiotics (table 4).

Table (4): Antibiotic sensitivity test for Staphylococcus species isolates

Antibiotic	Number of Isolate	Poly		CN		CO-T		AM		Cip		CL	
		S	R	S	R	S	R	S	R	S	R	S	R
<i>S.saprophyticus</i>	38	0	24	14	8	11	9	0	24	22	4	14	10
<i>S.epidermidis</i>	28	0	24	13	7	10	5	4	18	20	3	11	8
<i>S.hyicus</i>	23	6	15	12	8	12	8	5	13	18	2	13	7
<i>S.aureus</i>	23	0	16	10	9	13	9	1	21	15	0	10	8
<i>S.intermedius</i>	17	9	0	7	4	9	6	0	13	13	1	12	2

S: sensitive, R: resistant.

Note: Number of other tested isolates were intermediat

DISCUSSION

Fish and fishery products could be the major source and acts as a vehicles for many important species of food poisoning bacteria, which include in addition to Salmonella, Staphylococcus, *Cl. botulinum*, the so-called nonspecific group of microorganisms such as *E. coli*, *Proteus spp.*, *St. fecalis*, *Cl. perefiringnes* and *B.*

cereus (3 , 4). In this study five species of Staphylococci were isolated from both *Cyprinus carpio* and *Silurus glanis* fish. The isolated species from skins, muscles, livers, intestins showed different percentages from both two species of fish. The dominant species were *S. saprophyticus* (29%,29.4%), *S. epidermidis* (21%,22%), *S. hyicus* (17.75%,17.7%), *S. aureus* (17.75%,19.1), *S. intermedius* (14.5%,11.8%). These results agree with other studies (3 , 23) . Staphylococcus bacteria was most commonly detected in skin (35.5%,36.8%), livers (25.8%,25%), intestines (21%,17.60%), muscles (17.7%,20.6%) from *Cyprinus carpio* and *Silurus glanis*, the occurrence of bacteria in these samples were similar to results of (3 , 23). During storage in relatively hot weather the different predominate organisms of fish will penetrate from the slime through skin in to fish flesh and also from the gills via the blood in to the blood channels and spread to different organs and viscera. Generally the fish intestine were found to be heavy loaded with large numbers of Staphylococcus species (11). The higher numbers of bacterial isolates which indicated in our study in *Cyprinus carpio* (62 isolates) and (68 isolates) in *Silurus glanis* this results were similar to (3 , 24). The high prevalence of Staphylococcus in fish samples indicate the unhygienic handling of fish and degradation of fish is accelerated by Staphylococcus associated with aquatic environments as well as contamination during post - harvest handling (25 , 26). These opportunistic and pathogenic Staphylococcus were also previously isolated by several other researchers from fish in Iraq (27, 28), *S. aureus* has also been detected during these studies and these bacteria produce enterotoxins which are serious causes of gastroenteritis after consumption of fish (29). Because Staphylococci occur both as commensals on skin and mucous membranes and environmental contaminants, infection can be either endogenous or exogenous origin, many infections are opportunistic while some strains of low virulence (30). Most species of isolated Staphylococci were resistant to Ampicillin and some of them were multidrug resistant for more than 2 - 3 different antibiotics, the wide spread presence of antibiotics resistance of microorganisms highlights the importance of good hygienic practice against antibiotic resistant infectious agents (7), most species were sensitive to Ciprofloxacin and this result agree with the result of (7 , 27) .

In conclusion, fish are susceptible to all contaminant organisms that may be found in water, post harvesting , marketing , dealing , fish handlers , this processing will result in microbiological activities leading to loss of fish meat quality so the basic principles for prevention of food borne disease and sanitation should be followed to protect the consumers against the public health hazard, the widespread presence of antibiotic resistance microorganisms should be a priority to reinforce the importance of basic hygiene for fish.

عزل وتشخيص جراثيم المكورات العنقودية من اسماك المياه العذبة وحساسيتها للمضادات الحياتية في مدينة الموصل

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الخلاصة

تم إجراء الفحص الجرثومي لـ 60 عينة سمك (سليمة) محلية من المياه العذبة ، تضمنت 30 سمكة كارب *Cyprinus carpio* و 30 سمكة جري *Silurus glanis* بأوزان مختلفة من الأسواق الشعبية في مدينة الموصل للفترة من ايلول 2011 لغاية ايلول 2012 . أخذت مسحات من الجلد وأجزاء من العضلات والأكباد والامعاء وحضنت في مرق نقيع المخ والقلب لمدة 24 ساعة بدرجة حرارة 37° م (حضان هوائي) ، ثم أخذت عروة زرع مملوءة من المرق وخططت على وسط اكار الدم و اكار الحليب و اكار سكر المانيتول والملح وحضنت الاطباق بدرجة حرارة 37° م ولمدة 24 ساعة . انتخبت مستعمرات المكورات العنقودية وصبغت بصبغة كرام ودرست صفاتها الشكلية وتفاعلاتها الكيموحيوية . كانت نسبة العزل لجراثيم المكورات العنقودية 100% لجميع عينات الاسماك المفحوصة ، شخّصت 130 عزلة من جراثيم المكورات العنقودية لنوعي الاسماك المفحوصة (62) عزلة من الكارب *Cyprinus carpio* و (68) عزلة من الجري *Silurus glanis* ، حيث شخّصت خمسة انواع من المكورات العنقودية بنسب مختلفة من اسماك الكارب *Cyprinus carpio* و اسماك الجري *Silurus glanis* وهي كالتالي: *S. saprophyticus* (29.4% , 29%) ، *S. epidermidis* (22% , 21%) ، *S. hyicus* (17.75% , 17.7%) ، *S. aureus* (17.75% , 19.1%) ، *S. intermedius* (14.5% , 11.8%) على التوالي ، اما نسب عزلات جراثيم المكورات العنقودية من الجلود (35.5% , 36.8%) ، العضلات (17.7% , 20.6%) ، الاكباد (25.8% , 25%) ، الامعاء (21% , 17.60%) من كلا النوعين الكارب والجري على التوالي . كانت نتائج فحص الحساسية لستة مضادات حياتية (امبسلين ، جنتاميسين ، كلورامفينكول ، بولي مكسين ، كو- ترايمكسازول ، سيبروفلوكساسين) مختلفة . ان اغلب عزلات المكورات العنقودية مقاومة للامبسلين لكنها حساسة لسيبروفلوكساسين .

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Antibiotic sensitivity testing was performed using trimethoprim, streptomycin, Rifampin, erythromycin, cephalothin, ciprofloxacin, carbenicillin, amoxiclav, amoxicillin and ampicillin. Isolates showed 50-100% resistance to antibiotics used where all were resistant to rifampin, amoxiclav and amoxicillin and *P. aeruginosa* was resistant to all. Identification of bacterial isolates. To identify bacteria, Gram stain, motility test, colony morphology and color were studied in addition to. Bacteria were isolated from sewage water of different areas of Mosul city to determine the spread of antibiotic resistance in environment. Membrane filter method was used where isolates were grown in MacKonkey, Salmonella-Shigella, Sodium azide, mannitol salt and Pseudomonas isolation agars. Identification of spoilage bacteria. 25 grams of shrimp per specie were aseptically added to 225 ml of peptone water and homogenized [10]. Seven fold dilutions of each homogenate were prepared and 0.1 ml of 10^{-7} dilution was used for enumeration and isolation on nutrient agar. Replicate plates were incubated in aerobiosis and in anaerobiosis at 37 °C. Thereafter counting, three characteristic isolates per plate were purified on Mueller Hinton. Isolation and identification of Staphylococcus bacteria from fish of fresh water and its antibiotics sensitivity in mosul city. Basrah J Vet Res. 2014;13(1):33-42. Staphylococcus bacteria are Gram-positive characterized by irregular clusters, widely distributed in nature and can be found on the skin and mucous membranes. Classification of Staphylococcus. Domain: Bacteria - Single-celled prokaryotes widely distributed in every environment across the world. Ultimately, this allows each of the newly formed cells to have its own cell wall consisting of peptidoglycan. Nutritional Adaptations. Staphylococci species have been shown to circulate between various nutrient-limiting environments including terrestrial habitats and host species among others. In the event of limited nutrients, they persist on given environs for a long time which allows them to survive until conditions improve.