Streptococcosis in Some Fishes: Characterization of whole cell protein of the prevalent Streptococcus spp.

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Abstract

Streptococcus spp. were isolated from the apparently healthy and diseased *Oreochromis niloticus* and *Clarias gariepinus*. The prevalence of infection was 17.5 and 19 % respectively and the disease mainly recorded during summer. Darkness of the skin , exophthalmia and hemorrhaging in the skin and fins were the prominent signs. Kidneys yielded the highest recovery rate of bacterium. The isolated streptococci were identified biochemically as *S. faecalis* , *S. faecium*, *S. pyogene* and *S. pneumoniae*. Lancefield serogrouping of the randomly selected thirty isolates with Streptex kits revealed 23 strains belonging to group D, 5 related to group A and 2 untypeable strains. The most prevalent strains were *S. faecalis and S. faecium*. The SDS PAGE analysis of Whole-cell protein of group D, *S. faecium* had identical protein pattern profile, While, *S. faecalis* showed four distinct groups with a similarity in protein pattern profile. The untypeable strains showed high similarity and one of them had identical protein pattern with one strain of S. *faecalis*, these suggesting that all strains of the same origin.

Introduction

Streptooccosis have been recorded worldwide causing severe economic losses in fish production (**15**). Streptococcus septicemia is considered the largest problem in intensive tilapia rearing systems throughout the world (**39**). Furthermore, the bacterium has been reported to cause mortalities among numerous wild and cultured fish species including, gilthead seabream (**20**), *O. mosambicus* (**9**), *Seriola dumerili* (**32**), African catfish (**19**), Olive flounder (**3**), Silver carp (**34**) and Grey mullet (**10**). The potential transmission of S. *iniae* from fish to human had been reported (**27; 36**). Moreover, fish pathogenic, Lactococcus *garvieae* and *S. agalactiae* casuses neonatal meningitis in human (**5; 28**). The present work was planned to describe the clinical signs and prevalence of streptococcosis among the examined fishes; to identify the recorded isolates and to find the relationship between the whole-cell protein fingerprints and the serotypes of the isolated strains.

Materials and Methods

1- Examined fishes:

Clinically diseased and apparently healthy fishes were collected from El-Riah El-Tawfiki and their tributaries, and private fish farms at Kafer El-Sheikh and Sharkia Governorates during the period from February 2005 to January 2006. They were 320 *Oreochromis niloticus* weighted 60 ±5 g and 100 *Clarias gariepinus* weighted 200 ±10 g and transported to the fish diseases laboratory at Fac. Vet. Med. (Moshtoher) under accurate conditions (**37**) and examined immediately.

2- Clinical examination:

The fishes were submitted to external examination and necropsy using the methods described (**31**). Signs and lesions observed were recorded.

3- Bacterial isolation:

Tissue specimens were taken for microbiologic examination from skin and underlying musculature, liver, spleen, kidneys, heart, and brain and inoculated into Brain heart infusion broth then incubated at 30 ± 1°C for 24 h. They were streaked on Trypticase soya agar, 5% heep blood agar, MacConkey's agar and Streptococcus selective agar. Pure colonies were picked up and submitted to phenotypic characterization.

4- Characterization of bacterial isolates:

Colonies character on different media was recorded. Moreover, hemolytic activity on 5% sheep blood agar was detected (**38**). Cell morphology was determined by gram-stained smears and only gram-positive catalase-negative isolates were subjected to biochemical characterization using sets of biochemical reactions (**6; 18; 22**). Optachin sensitivity test was also done (**4**).

5- Lancefield grouping:

Thirty isolates were randomly selected and subjected to Lancefield streptococcal grouping (**7**), the isolates were serogrouped with Streptex kits for grouping of streptococcus groups A, B, C, D, E, F and N (Welcome Diagnostic A Division, Dartford, England).

6- Characterization of whole-cell proteins:

6.1- Preparation of whole-cell proteins

Nine strains were selected; 7 of lancefield group *D (S. faecalis* and *S. faecium)* and 2 untypeable with lancefield antisera. The bacteria were grown in 250 ml of Todd-Hewitt broth for 24 h at 35°C. Pellets obtained by centrifugation at 16.000 xg for 20 min and frozen for 24 hr. The bacteria thawed at room temperature and treated with a Tris-EDTA-lysozyme solution for 2h at 37°C and sonicated at 80 w in 15-s bursts for 2 min with cooling. Then centrifugated at low-speed to remove the bacterial debris. The supernatant fluid was pelleted at 100.000 xg for 1hr. The pellets was then suspended in distilled water and the protein concentration was determined **(29)**.

6.2- Electrophoresis (SDS-PAGE) of the whole cell proteins:

The whole cell protein of each isolate was separated by using Sodium dodecyl sulphate polyacrylamid gel electrophoresis (SDS-PAGE) of the whole cell protein were performed **(26)** using prestained high molecular weight standard marker (bio-Rad). The gel was stained with Coomassie brilliant blue and destained according to Elliott *et al.* (1990) .Dice index of similarity was determined with each isolates **(21)** and Dendrogram was constructed (Advanced American Biotechnology UPGMA , USA.)

Results and Discussion

The clinical signs varied with species affected, darkness of the skin, uni- or bilateral exophthalmia corneal turbidity, redness of the skin and hemorrhaging at the base of the fins (Fig 1A,B) were the prominent signs observed in naturally infected *O. niloticus*. Skeletal deformity (Fig 1C) and abdominal distension were also recorded. Internally the lesions recorded were paleness of liver with gray white lesions; distended gall bladder; and congestion and enlargement of Kidneys and spleen. In some cases, ascetic fluid in abdominal cavity was found. These findings came nearly similar to those recorded before (**8; 34; 35**). While, the infected *C. gariepinus* showed hemorrhaging in the skin, fins, mouth barbells and belly region (Fig 1D). Internally, there were congestion and enlargement of the liver, spleen and kidneys. The intestine was congested filled with yellow viscous material tinged with blood. These results came in agreement with some previous reports (**13; 19**).

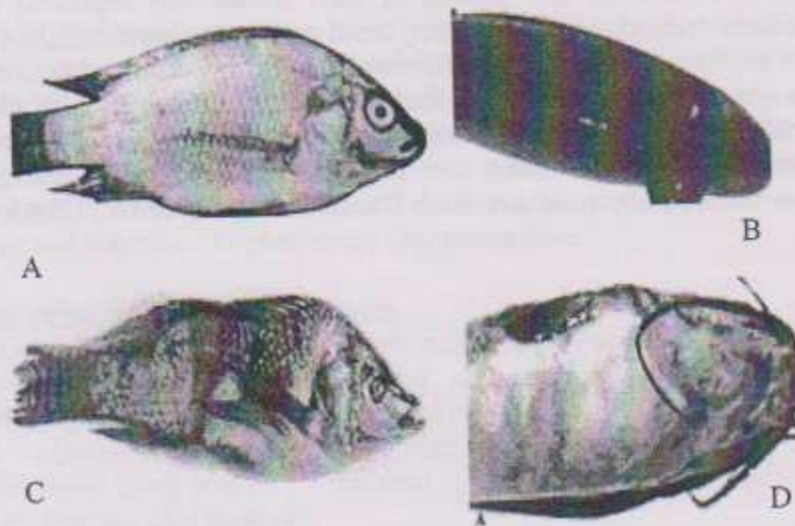


Figure (1): Showing hemorrhaging in the skin and fins (A), darkness of the skin with exophthalmia (B) and skeletal deformity (C) in naturally infected *O. niloticus* ; and hemorrhaging allover the body and fins (D) of infected *C. gariepinus*.

As shown in Fig. 2, the prevalence of streptococcosis was 17.5 and 19% in *O. niloticus* and *C. gariepinus* respectively. These findings came in a partial agreement with those recorded before (**13; 19**), but either lower than the results obtained in some investigations (**16**) or higher than others (**11; 12**). The higher rate of infection, recorded in wild (26.25%) than in cultured (8.75%) *O. niloticus*, disagreed with those obtained previously (**8; 30**). These findings may be due to water pollution. Moreover, the results revealed that the disease mainly recorded during summer (32.38%). These observations came in agreement with that reported before (**8; 19**) and in disagreement with others (**23**) attributed the chronic mortalities to *S. dysgalactiae* during winter. These variations may be due to bacterium species, fish species and size as well as environmental factors.

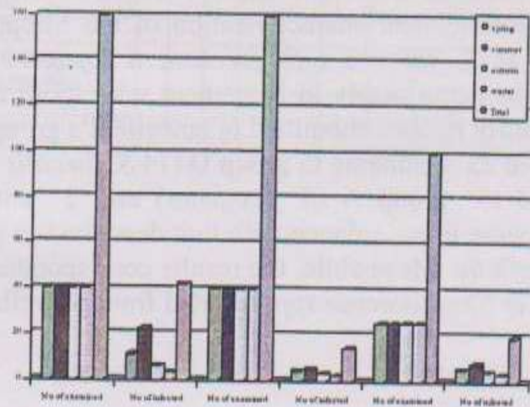


Figure (2): Prevalence and Seasonal prevalence of streptococcosis among naturally infected *O. niloticus* and *C. gariepinus*.

The bacterium could be isolated at varying rate from tissue specimens (table 1) and kidneys from both *O. niloticus and C. garipeinus* showing the highest recovery rate (33.33 and 31.57%) respectively. These observations supported the results of Dena (2004) and El-Refaee (2005). In the same respect, It has been indicated that the bacterium can be detected in higher rate from liver, gills and brain respectively(**1; 16; 35**). The difference in tissue recovery of streptococcus species may be attributed to fish species, and the mode of infection and the course of disease.

Bacterial colonies developed on Trypticase soya agar, 5% Sheep blood agar, and Streptococcus selective agar and unable to grew on MacConkey's agar. The colonies appeared gray white or dewdrop-like and showed hemolytic characters on blood agar. These observations came nearly similar to that observed before (**8; 11; 19**). The results revealed that all isolates were gram positive, catalase and oxidase negative and showed typical phenotypic and biochemical characterization of the Streptococcus (table 2) and typeable as *S. faecalis* , *S. faecium, S. pyogene* and *S. pneumoniae*. These results came nearly in agreement with those recorded before (**2; 13; 22; 34**). Thirty isolates submitted to lancefield's grouping and Streptex kits test revealed 23 belonging to group D (14 *S. faecalis* and 9 *S. faecium*) and 5 related to group A (*S. pyogenes*) and 2 untypeable isolates. These findings were in accordance with that described in previous reports (**14; 40**) for *S. difficile.* Meanwhile, the results corresponded to that recorded by others (**35**) for *Streptococcus spp*. isolated from Nile tilapia.

Table (1): Recovery percentage of Streptococcus species from the examined fish.

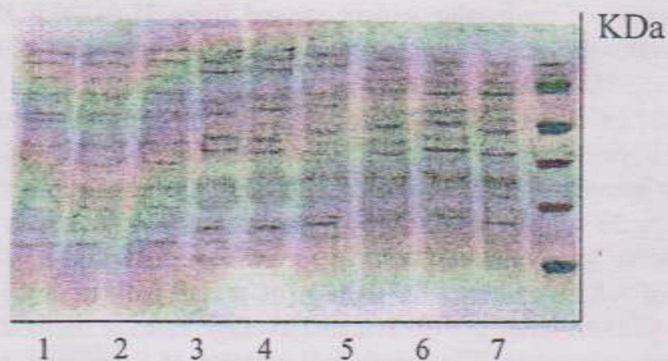
Type of recovered bacteria		Tissue samples						No. of isolate	%
		Skin	Liver	Spleen	Kidneys	Heart	Brain		
O. niloticus (wild)	*S. faecalis*	2	7	7	11	2	0	29	69.04
	S. faecium	0	2	4	3	2	0	11	26.19
	S. pyogene	0	0	1	1	0	0	2	4.76
	S. pneumoniae	0	0	0	0	0	0	0	0.0
Total		2	9	12	15	4	0	42	-
O. niloticus (cultured)	*S. faecalis*	0	2	0	2	1	0	5	35.71
	S. faecium	0	2	1	2	1	0	6	42.85
	S. pyogene	0	1	0	0	0	0	1	7.14
	S. pneumoniae	1	0	0	0	1	0	2	14.28
Total		1	5	1	4	3	0	14	-
C. gariepinus	*S. faecalis*	1	2	1	3	0	2	9	47.36
	S. faecium	0	2	1	3	1	0	7	36.84
	S. pyogene	1	1	0	0	0	0	2	10.52
	S. pneumoniae	1	0	0	0	0	0	1	5.26
Total		3	5	2	6	1	2	19	-

Table (2): Biochemical characterization of the isolated *Streptococcus species*.

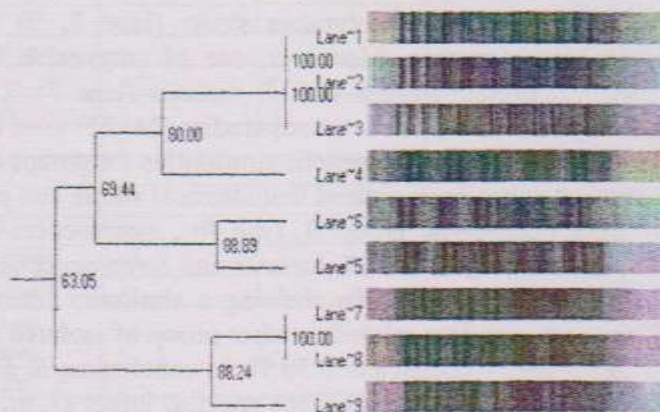
Test	*S. faecalis*	*S. faecium*	*S. pyogenes*	*S. pneumoniae*
No. of isolates	43	24	5	3
Shape of cells	Cocci arranged in chains	Cocci arranged in short chains	Cocci arranged in pairs & chains	Cocci arranged in pairs
Motility	-	-	-	-
Haemolysis on BA	α / γ	α / γ	β	A
Growth on ordinary media TSA	+	+	+	+
MacConkey agar	-	-	-	-
Catalase	-	-	-	-
Oxidase	-	-	-	-
Gelatin liquefaction	-	-	-	-
Starch hydrolysis	±*	-	-	-
esculin hydrolysis	+	+	-	-
Indole test	-	-	-	-
Citrate utilization	±*	-	-	-
Methyl-red	±	-	-	-
Voges proskauer	±	+	-	-
H_2S production (TSI)	±*	-	-	-
Growth at 6.5% NaCl	+	+	-	-
Table (2): Continue				
Optachin sensitivity	-	-	-	+
Sugar fermentation				
Arabinose	-	±	-	±
Mannitol	-	±	+	-
Glucose	±	±	+	+
Sucrose	±	+	-	-
Lactose	±	-	-	-
Maltose	±	-	-	+
Fructose	-	-	-	-

* only two strains were positive for these biochemical test.

The SDS polyacrylamide gel electrophoresis of whole cell protein of the tested strains (Fig 3), revealed that *Streptococcus spp.* possessed 11-18 protein bands of molecular weight ranged from 21.0-229.9 KDa and the major bands were 35.0 (7 strains) and 32.0 KDa (6 strains) With respect to the species, SDS-PAGE analysis of whole cell protein of *S. faecium* had three major bands of molecular weight 37.0, 31.0 and 28.0 KDa. While, the whole cell protein profiles of *S. faecalis* showed three major bands of molecular weight 44.0, 35.0, and 26.3 KDa. In the same respect, It has been found (**19**; **33**) that the most common characteristic bands of isolated *Streptococcus spp* and *Enterococcus faecalis* were 18 and 35 KDa. Cluster analysis of whole-cell protein pattern of the tested streptococcus strains based on the similarity coefficient and average linkage method. They were grouped into six types. The whole-cell proteins of S *faecium.* had identical protein pattern (Fig 4 lanes1, 2, 3) while, The whole-cell proteins of *S. faecalis* grouped into four distinct types. *S. faecalis* strain (lane 4) showed high similarity with *S. faecium* and *S.faecalis* (lane 5,6) revealed high similarity in between. The untypable strain (lane 8, 9) showed high similarity in protein pattern. Moreover, one of untypeable stain (lane 8) showed identical protein pattern with *S. faecalis* (lane 7). These findings came in consistence with some previous studies (**24**; **25**) used the whole cell protein pattern to determine the genetic similarities for groups of bacteria. In the same respect, It has been noticed that identical whole cell protein pattern is present in nonhemolytic group B, type Ib , streptococci isolates from human, fish and mice (**17**), and *S. faecalis* and *Streptococcus sp*[3] was very closed to the standard *S. faecalis* showing a similarity level 0.83% (**19**). While, *S. faecium* was less related to other group of isolated streptococcus species with similarity coefficient 0.50 %. In conclusion, *S. faecium* and *S. faecalis* are the prevalent streptococcus species, infect *O. niloticus* and *C. gariepinus* and the kidneys are the best organ for bacterium isolation. The *S. faecium* strains had identical whole cell protein pattern while, *S. faecalis* showed four distinct group with a similarity in protein patterns. The untypeable strains showed high similarity and one of them had identical protein pattern with one strain of *S. Faecalis*



Fiure (3): SDS-PAGE profiles of whole cell proteins lanes 1-9 represent the tested streptococcus strains and lane 10 is the protein marker.



Figure(4): Similarity dendrogram and migration pattern of streptococcus strains isolated from fish lane 1-9.

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مرض الميكروب السبحى المكور في بعض الأسماك: خصائص البروتين الخلوي لأنواع الميكروب السبحى المكور السائدة

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تم عزل الميكروب السبحى المكور من أسماك البلطي النيلي والقرموط الأفريقي السليمة ظاهريا والمريضة وكان معدل نسب الإصابة ١٧,٥ و ١٩% علي التوالي ووجدت بأعلى نسبة في فصل الصيف، وكانت العلامات المرضية الأكثر وضوحا جحوظ بالعين وانزفة علي الجلد والزعانف. وقد بينت الكلي أعلي معدل تواجد للبكتريا. وقد صنفت المعزولات بيوكيمائيا إلي الميكروب السبحى المكور فيكاليز و الميكروب السبحى المكور فيشيم و الميكروب السبحى المكور بيوجين و الميكروب السبحى المكور نيموني. واظهر التصنيف باستخدام Lancefield serogroups لعدد ثلاثون عترة منتقاة عشوائيا عن ٢٣ عترة تنتمي إلي group D و٥ عترات تنتمي إلي group A وعترتان لا تنتمي لأي من Lancefield serogroups . وكانت العترات الأكثر انتشارا هي الميكروب السبحى المكور فيكاليز و الميكروب السبحى المكور فيشيم. وأظهر التفريد الكهربي لعترات الميكروب السبحى المكور فيشيم المختبرة أن منظومة البروتين الخلوي متماثلة تماما بينما الميكروب السبحى المكور فيكاليز أظهر وجود أربع مجموعات منفصلة تبدو متشابهة في منظومة البروتين الخلوى وكذلك العترات الغير محددة النوع أظهرت وجود تشابة كبير في المنظومة البروتينية لهما في حين أظهرت أحدى هذه العترات تماثلا تاما في المنظومة البروتينية مع إحدى عترات الميكروب السبحى المكور فيكاليز مما يفترض أن كل هذه العترات من أصل واحد.