Bull. Eur. Ass. Fish Pathol., 24(3) 2004, 137

First isolation of *Staphylococcus epidermidis* from cultured gilthead sea bream (*Sparus aurata*) in Turkey

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Abstract

This study describes the first isolation of *Staphylococcus epidermidis* from gilthead sea bream (*Sparus aurata*), reared in the southwest of Turkey. During the spring of 2003 (April-May), the infection appeared in juvenile gilthead sea bream (3-5 g) in a net cage. The outbreak occurred after a sudden increase of water temperature in the middle of April. The fish losses were up to 12 % in one day. Affected fish showed haemorrhages on the fins and gills, the liver was anaemic and the abdomen was slightly distended as a result of ascitic fluid in the abdominal cavity. The gram-positive *S. epidermidis* was isolated from the anterior kidney, spleen and liver of sick fish. *S. epidermidis* was identified by morphological, physiological and biochemical features, using conventional methods and the ID 32 Staph system.

Introduction

Staphylococcus epidermidis has been reported previously as a fish pathogen in some marine and freshwater fish in Japan, Taiwan and Greece (Kusuda and Sugiyama 1981; Wang et al., 1996; Varvarigos, 2001). Several severe epizootics have been described in farmed fish i. e. in red sea bream (*Chrysophrys major)* and yellowtail (*Seriola quinqueradiata*) in Japan (Kusuda and Sugiyama, 1981); grass carp (*Ctenopharyngedon idella*) (Wang et al., 1996) and tilapia (*Oreochromis* spp.) in Taiwan (Huang et al., 1999) and sea bream (*Sparus aurata*) and sea bass (*Dicenthrarchus labrax*) at different fish farm locations in Greece (Varvarigos, 2001). Sugiyama and Kusuda (1981) thought that the bacteria originated from water or fish rather than from human beings, because of the pronounced antigenic differences when compared to human strains of *S. epidermidis*. This suggestion is supported by ecological studies, which have clearly demonstrated the presence of *S. epidermidis* in the aquatic environment (Gunn et al., 1982, Austin and Austin 1999). Staphylococci may be present in the fish throughout the year, but the disease is induced by a sudden rise in water temperatures or other stress factors in the aquatic environment. It usually appears in the spring and causes problems throughout the summer (Varvarigos, 2001). Staphylococcal infections in fish were also observed by Fryer and John, (1993) during specific and severe stress imposed on the fish by the envi-

ronment. In fish, typical signs of staphylococcal infections are exophthalmia, congestion and ulceration on the tail (Kusuda and Sugiyama, 1981). Staphylococcal infections involve systemic disease characterized by septicaemia (Varvarigos, 2001).

This study describes isolation of *S. epidermidis* in juvenile gilthead sea bream (*Sparus aurata*) in a net cage farm, located in the coastal region of the Aegean Sea in Turkey.

Materials and Methods

Sampling

The outbreak was restricted to a single net cage farm with a total of twenty-eight cages in the Aegean Sea. The disease was observed in six net cages of juvenile gilthead sea bream (*S. aurata*) during the spring, from April to May 2003. Moribund, diseased fish ranging in size from 3-5g were collected from the farm and transported to the Fish Disease Laboratory in Egirdir Fisheries Faculty at Süleyman Demirel University.

Isolation and Identification of Bacteria

For bacterial isolation, samples were taken from the kidney, spleen and liver of each fish and streaked on trypticase soy agar (TSA, Merck). The plates were incubated at 30°C for 48-72 hours. Based on the morphology, only one type of colony growth could be determined after 72 hours in all samples. Pure cultures of the isolates were obtained on TSA. Routine tests for determination of biochemical characteristics of the bacteria were carried out as described in Baird-Parker (1963,1965a, b), Cowan and Steel (1970), Collins and Lyne (1976), Kinkelin *et al*. (1985) and NCCLS (2001). A presumptive identification of the strain was performed by Gram staining, catalase, oxidase activity, motility, resistance to O/129 vibriostat and the glucose oxidation-fermentation test. The strain was identified by testing several biochemical reactions as described in Bergery's Manual (Holt *et al.* 1994). The haemolytic ability of the bacteria was determined by growth on 5% sheep blood agar. For detection of the coagulase, rabbit plasma with lyophilized-EDTA (Merck) was used. Samples were also plated on Brain heart infusion agar (Difco), Baird Parker's agar (Merck) and Mannitol salt phenol red agar (Merck) and incubated at 30°C. To determine the physiological characteristics of the bacteria, samples were inoculated into trypticase soy broth (TSB, Merck) and growth was determined at 4°C and at 45°C. The salt requirements/tolerance of the strain was determined in TSB containing 0 % and 15% NaCl. The ID 32 Staph system test strips (Biomerieux SA/69280 Marcy-l' Etiole, France) were also used for bacteriological diagnosis.

Antimicrobial Sensitivity

Antibiogram tests for the isolate were performed using the disc diffusion techniques on Mueller Hinton agar (Oxoid). NCCLS standards were used for evaluation of the results. Also, ATB_VET (14 289 Biomerieux) strip system was used to obtain the antibiotic sensitivity of the isolated strain.

Results and Discussion

The external symptoms of the infected fish were congestion and haemorrhages on the pectoral and caudal fins, dark coloration of the skin, lethargy, and excessive secretion of mucus on the skin and gills. The mouth and lower jaw were haemorrhagic and the gills were anaemic. Internal symptoms included ascitic fluid in the abdominal cavity, with an

Characteristics	Response	Characteristics	Response
Colony pigment	White	**Acid production from:**	
Colony diameter on TSA (mm)	1	Glucose	+
Gram Stain	+	Mannitol	-
Morphology	Spherical Cocci	Inositol	-
Motility	-	Sorbitol	-
Oxidation-Fermentation(O-F)	F	Rhamnose	-
Sensitivity to O/129	-	Sucrose	+
Production of:		Melibiose	-
Catalase	+	Amygdalin	-
Oxidase	-	Arabinose	-
Coagulase (rabbit plasma)	-	Saccharose	+
ß galactosidase	-	Lactose	+
H_2S	-		
Indol	-	**Growth on:**	
Lysine decarboxylase	-	Baird Parker's agar	+
Ornitine decarboxylase	-	Mannitol salt agar	+
Arginine dehydrolase	-	Trypticase soy agar	+
Phosphatase	+	Brain Heart infusion agar	+
Urease	+	0% NaCl	+
Voges Proskauer (VP) reaction	+	15%NaCl	+
Methyl red test	+	Growth at:	
Nitrate reduction	+	4 °C	-
Degradation of:		45 °C	+
Blood (Haemolysis)	ß		
Gelatin	-	**Utilisation of:**	
Starch	-	Citrate	-

(+): positive reaction; (-): negative reaction.

Table 1: Phenotypic characteristics of isolated *Staphylococcus epidermidis*.

enlarged, pale liver showing congested blood vessels with inflamed dark areas. The gall bladder was filled with dark greenish or light yellowish bile. The spleen was also enlarged. Congestion and haemorrhages were seen in some regions of the brain. The stomach was empty in some fish. Although all these symptoms are similar to those observed by Varvarigos (2001) in sea bass and sea bream, infections with *S. epidermidis* have been reported to induce different symptoms in tilapia (Huang *et al.*, 1999) such as white and yellowish nodules in the anterior kidney and spleen. On TSA agar samples from the internal organs at 30°C in 18-24 hours resulted in growth of usually small, convex, regular, white colonies. The isolated bacteria were Gram-positive cocci, which were seen as single cells or as irregular grape-like clusters under a light microscope. All the isolates were identified as *S. epidermidis*.

The biochemical and physiological characteristics of the bacteria are given in Table 1 and the results of the ID 32 Staph system tests in Table 2. The isolated strain was also growing on Mannitol salt agar and Baird Parker's agar, and on brain heart infusion agar. On blood agar, â haemolysis was obtained. The strain was coagulase negative. Catalase and phosphatase were produced but not oxidase, lysine decarboxylase, ornitine decarboxylase, H_2S and indol. Nitrates were reduced. Methyl red test and Voges proskauer reaction were positive. Urea was degraded by the strain but starch was not. Growth occurred even at 45°C and with 0-15 % NaCl. Citrate was not utilized.

The antimicrobial sensitivities of the isolate were determined by the disc method and the results are listed in Table 3. The isolate was resistant to penicillin (10Units), ampicillin (10mcg), ampicillin-sulbactam (20mcg), methicillin/oxacillin (1mcg), erythromycin (30mg), tercoplanin (30mcg), gentamycine (10mcg), oxytetracycline (30ìg), streptomycine (10ìg), tobramycine (10mcg) and oxacillin (1ìg). It was sensitive to vancomycin and amikacin (30mcg), cipofloxacin (5mcg), norfloxacin (10mcg), trimethoprim (1,25ìg), sulphamethoxazole (23,75ìg), furazolidone (100ìg) and chloramphenicol (30 ìg). When tested with the ATB-VET (14 289) strips, the

URE	ADH	ODC	ESC	GLU	FRU	MNE	MAL	LAC	TRE	MAN	RAF	NIT
+	-	-	-	+	+	-	+	+	-	-	-	+
VP	ßGAL	ArgA	PAL	PyrA	NOVO	SAC	NAG	TUR	ARA	ß GUR	RIB	CEL
+	-	-	-	-	-	+	-	-	-	-	-	-

URE:Urease, ADH:Arginine dihydrolase, ODC:ornithine decarboxylase, ESC:Esculin (Hydrolysis), GLU:Glucose, FRU:Fructose, MNE:Mannose, MAL:Maltose, LAC:Lactose TRE:Trehalose, MAN:Mannitol, RAF:Raffinose, NIT:Nitrates,VP: Acetoin production, âGAL: âGalactosidase, ArgA:arginine Arymlamidase, PAL:Alkaline phosphatase, PyrA: Pyrrolidonyl Arymlamidase, NOVO:Novobiocine, SAC:Sucrose, NAG; N-Acetyl-Glucosamine, TUR: Turanose, ARA: Arabinose, âGUR: âGulucuronidase, RIB:Ribose, CEL:Celiobese.

Table 2: ID 32 Staph system test results for *Staphylococcus epidermidis.*

Antibiotics	Zone size (mm)	Sensitivity
Penicillin (10 units)	10	R
Ampicillin (10 mcg)	12	R
Ampicillin-sulbactam (20 mcg)	18	R
Methicillin - Oxacillin (1mcg)	0	R
Erythromycin (30 mcg)	0	R
Vancomycin (30mcg)	15	S
Tercoplanin (30mcg)	13	R
Gentamycin (10mcg)	12	R
Amikacin (30 mcg)	20	S
Cipofloxacin (5 mcg)	31	S
Norfloxacin (10mcg)	30	S
Trimethoprim (1,25μg) - Sulphamethoxazole(23,75 μg)	26	S
Oxytetracycline (30μg)	0	R
Furazolidone (100)	20	S
Streptomycin (10μg)	8	R
Chloramphenicol (30 μg)	30	S
Tabromycin (10mcg)	0	R
Oxalicilin (1μg)	0	R

S: sensitive, R: resistant

Table 3: Sensitivity of isolated *Staphylococcus epidermidis* to antibiotics

strain was sensitive to chloramphenicol, doxycyclin, lincomycin, pristanamycin, tylosin, enrofloxacin, nitrofurantoin, fusidic acid and rifampicin (Table 4).

Two species of staphylococcus have been reported to cause staphylococcosis in fish: *S. aureus* (Shah and Tyagi, 1986) and *S. epidermidis* (Kusuda and Sugiyama, 1981; Huang et al., 1999; Wang et al., 1996). The first reports of fish pathogenic strains of *S. epidermidis* were from severe epizootics in farmed yellowtail and red sea bream in Ja-

Antibiotics	(mg/l)	Sensitivity
Penicillin	0,25	R
Amoxicillin	4	R
Amox-clav.ac	2	R
Oxacillin	2	R
Cephalothin	8	R
Cefoperazon	4	R
Streptomycin	8	R
Spectinomycin	64	R
Kanamycin	8	R
Gentamicin	4	R
Apramycin	16	R
Chloramphenicol	8	S
Tetracyclin	4	R
Doxycyclin	4	S
Erythromycin	1	R
Lincomycin	2	S
Pristinamycin	2	S
Tylosin	2	S
Colistin	4	R
Cotrimoxazol	2/38	R
Sulfamethizol	100	R
Flumequin	4	R
Oxolinic ac.	2	R
Enrofloxacin	0,5	S
Nitrofurantoin	25	S
Fusidic ac.	2	S
Rifampicin	4	S
Metronidazol	4	R

S: sensitive, R: resistant

Table 4: ATB-VET (14 289) Kit results for *Staphylococcus epidermidis*

pan from July 1976 to September 1977. The description of the disease was not very detailed, but typical signs included exophthalmia, congestion and ulceration in the fish (Kusuda and Sugiyama, 1981). The results from the identification tests in the present study are in accordance with those reported from the outbreaks in red sea bream and yellowtail in Japan (Kusuda and Sugiyama, 1981; Austin & Austin, 1999) and in sea bream and sea bass in Greece (Varvarigos, 2001). Characteristic for this disease is that the outbreaks are related to stress and the mortality of fish peak over the course of a few days. However, the condition often persists and the mortality gradually reappears. Available information suggests that in the aquatic environment, *S. epidermidis* should be considered as opportunistic bacteria with potential to become pathogenic for fish under stressful conditions. It is not possible to eliminate the bacteria from the fish or from the environment (Varvarigos, 2001).

In conclusion, this study represents the first reported case of *Staphylococcus epidermidis* isolated from juvenile gilthead sea bream, in the south-western part of Turkey in 2003.

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