Journal of Animal and Veterinary Advances 9 (20): 2640-2646, 2010
ISSN: 1680-5593

Streptococcus agalactiae the Etiological Agent of Mass Mortality in Farmed Red Tilapia (*Oreochromis* sp.)

[1]Ali Abuseliana, [1]Hassan Daud, [2]Saleha Abdul Aziz, [2]Siti Khairani Bejo and [1]Milud Alsaid
[1]Aquatic Animal Health Unit, Department of Veterinary Clinical Studies,
[2]Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine,
University Putra Malaysia, Serdang 43400, Selangor, Malaysia

Abstract: Streptococcal infection was reported to cause significant mortality and high economical loss in freshwater and saltwater fish species including tilapia species, worldwide. Recently, few disease outbreaks affecting cultured red tilapia in Selangor state, Malaysia was investigated. Affected fish showed loss of appetite, serpentine swimming and exophthalmia. Sick and healthy appeared fish were clinically examined and samples from brain, liver, spleen and kidney were collected for agent isolation. All isolates were gram-positive, oxidase-negative, catalase-negative, β-haemolytic cocci and were characterized as a Group B *Streptococcus agalactiae* (GBS) using commercial identification kits (Streptococcal grouping Kit, RapID™ STR System and BBL Crystal GP ID Kit). The isolates were sensitive to amoxicillin, ampicillin, erythromycin, chloramphenicol, linomycin, rifampicin, vancomycin, gentamicin, sulfamethoxazole + trimethoprime and tetracycline. In contrast, they were resistant to neomycin, amikacin, kanamycin and streptomycin. Specific Polymerase Chain Reaction (PCR) and 16S rRNA sequencing technique results confirmed the isolates as a GBS. Results of this current study indicated that the *Streptococcus agalactiae* infection started to spread and warrants focusing on ways to prevent and control the disease before it become endemic.

Key words: Bacteriology, identification, *Oreochromis* sp., *Streptococcus agalactiae*, warrants, kanamycin

INTRODUCTION

According to Department of Fisheries Malaysia 2007 statistics, the aquaculture industry in Malaysia contributed about 16% to national seafood supply and tilapia (*Oreochromis* sp.) considered one of the three major freshwater species farmed in Malaysia. The rapid increases in global aquaculture industry have exposed many diseases that were not known in aquaculture fields. Like livestock's, fish farms exposed to different kinds of diseases which can cause huge economical damages to farming activity. However, bacteria were often associated with multifactorial nature fish diseases which were responsible for most of the diseases that cause mortality and losses in fish farms (Inglis *et al*., 1993; Plumb, 1999; Yin, 2004). Fish streptococcosis is one of bacterial diseases affecting both captive and wild fish in freshwater, estuarine and marine environments. Moreover, it is one of the fish diseases that is reported in intensive aquaculture systems causing high economic loss to fish farmers and can cause mortality >50% over 1 week (Inglis *et al*., 1993; Lio-Po and Lim, 2002; Yanong and Francis-Floyd, 2006). Disease caused by Streptococci has become a major problem in cultured freshwater and saltwater fish species worldwide. *Streptococcus* and *Enterococcus* sp. are commonly associated with streptococcal infection in farmed fish (Inglis *et al*., 1993).

Streptococcus iniae, *streptococcus parauberis* and *Streptococcus agalactiae* reported to be responsible for warm water streptococcosis (Toranzo *et al*., 2005). Amongst the *streptococci* sp., *Streptococcus agalactiae* reported to have a broad host range, infecting both terrestrial and aquatic animals including tilapia (Evans *et al*., 2002). *Streptococcus agalactiae* is a Group B Streptococcus (GBS); it is Gram-positive coccus in pairs or short chains, catalase negative, oxidase negative and CAMP (Christie, Atkins and Munch-Petersen) positive. *Streptococcus agalactiae* grow on nutrient media such as Trypticase Soy Agar (TSA), Blood Agar (BA) and Brain Heart Infusion Agar (BHIA). Its colonies are grayish white, mucoid, pin head size, round, convex and β-hemolytic on blood agar (Murray *et al*., 2005; Evans *et al*., 2002). *Streptococcus agalactiae* has been isolated from numerous fish species in natural outbreaks of disease and has been shown to be pathogenic in experimental trials using different routes of infections (Evans *et al*., 2002; Toranzo *et al*., 2005; Musa *et al*., 2009). The clinical signs of *S. agalactiae* infected fish include loss of appetite, swimming abnormalities, unilateral or bilateral exophthalmia, corneal opacity and

Corresponding Author: Hassan Daud, Aquatic Animal Health Unit, Department of Veterinary Clinical Studies, Faculty of Veterinary Medicine, University Putra Malaysia, Serdang 43400, Selangor, Malaysia

external hemorrhages; the internal organs showing many changes such as spleen enlargement, ascites as well as pale liver discoloration (Suanyuk *et al*., 2005). Lately, a commercial tilapia farm in Selangor suffered from high morbidity and mortality and the infected fish showed abnormal swimming, eyes opacity and exophthalmia. Samples were collected and microbiological and molecular techniques were used for isolation and identification of the ethological agent.

MATERIALS AND METHODS

Bacteriology: A total of 120 fish samples were collected from commercial tilapia farm in Selangor State, Malaysia. The samples were consists of 40 diseased fish, 40 freshly dead fish (<6 h) and 40 healthy appearance fish. The diseased fish samples were shown clinical signs such as lethargy, abnormal swimming, eye opacity and exophthalmia. Samples from liver, spleen, kidney and brain of sick and healthy fish were collected aseptically and cultured on Blood Agar (BA) and Brain Heart Infusion Agar (BHIA) (Becton, Dickinson and Company, USA). Kidney and brain of freshly died fish were aseptically collected and streaked on BA and BHIA. The plates were then incubated at 37°C for 24 h. Pure colonies were then sampled and subjected to morphological and biochemical and molecular tests for characterization.

Isolate characterization: Colony morphology, hemolysis and Gram-staining smears were observed. Identification was carried out using conventional biochemical tests. The isolates were further characterized using the RapID™ STR System (remel, USA) and BD BBL crystal™ Identification systems (Becton, Dickinson and company, USA). The systems were used according to manufacturer instructions and the results were compared with the analytical database of the manufacturer. The isolates were serogrouped according to Lancefield (1993) using a commercial Streptococcal grouping Kit (Oxoid, UK) for grouping of streptococci groups A-G.

Antimicrobial susceptibility test (Disk diffusion method): A sterile cotton swab was dipped onto the adjusted suspension then streaked over the entire surface of Mueller-Hinton agar plates (Becton, Dickinson and Company, USA) and left for 5 min. Amoxicillin (25 μg), ampicillin (10 μg), gentamycin (10 μg), erythromycic (15 μg), kanamycin (30 μg), streptomycin (10 μg), chloramphenicol (30 μg), tetracycline (30 μg), sulfamethoxazole + trimethoprime (23.75 + 1.25 μg), vancomycin (30 μg), amikacin (30 μg), linomycin (15 μg), rifampicin (5 μg) and neomycin (30 μg) (Biomerieux, France) disks were applied on the agar surface using disk dispenser. The plates were inverted and incubated at 35°C for 24 h. The resistant and sensitive patterns of the isolates were determined by measuring the zone of inhibition diameter for each antibiotic disc. The inhibition zone diameter below 13 mm was considered resistant between 13 and 17 mm moderately susceptible and above 17 mm considered as susceptible.

Extraction of bacterial DNA: The isolates were grown over night on brain-heart infusion agar at 37°C. The colonies were picked and re-suspended in 500 μL of sterilized Double Distilled Water (DDW) and washed two times; bacterial pellet was suspended in 500 μL DDW and boiled for 10 min and then centrifuged at 6,000× for 5 min. The supernatant containing bacteria DNA was stored at -20°C until used.

Polymerase Chain Reaction (PCR): Three representative isolates Sd1, Sd20 and Sh1 were chosen to run PCR assay and the positive control was *Streptococcus agalactiae* ATCC 13813 reference strain. Two sets of oligonucleotide primers capable of detecting specific sequence of the 16 S rRNA gene of *Streptococcus agalactiae* designed by Martinez *et al*. (2001) were purchased from commercial company (First BASE Laboratories, Malaysia). The primers with the following sequence: F1 (Forward), 5'-GAGTTTGATCATGG CTCAG-3' and IMOD (Reverse), 5'-ACCAACATGT GTTAATTACTC-3' which were expected to give an amplicon of 220 bp. The PCR amplification mixture consists of reaction buffer, 50 μL of master mix (mix of Taq DNA polymerase, dNTPs and Magnesium chloride) (Promega Corporation, USA), 5 μL primer mix (10 μL reverse primer plus 10 μL forward primer plus 80 μL Tris-EDTA), 10 μL DNA template and 35 μL deionized distilled water in a total volume of 100 μL. The amplification was carried out in a PCR thermal cycler (DNA Engine®, BIO-RAD, Mexico). The PCR cycles were conducting as described by Duremdez *et al*. (2004) with an initial denaturation at 94°C for 4 min followed by 35 cycles, each consisted of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min and final extension at 72°C for 1 min. The PCR products were determined by agarose gel electrophoresis and photographed under UV light.

Sequencing of 16S rRNA gene: One of the PCR confirmed *S. agalactiae* (Sd1) was used to run 16S rRNA sequencing. The PCR amplification of 16S rRNA gene was undergone with universal primers (5'-GAT TAG ATA CCC TGG TAG TCC AC-3' as a forward primer and 5'-CCC GGG AAC GTA TTC ACC G-3'as a reverse primer) (Sun *et al*., 2008). The PCR was performed in 50 μL reaction mixture contained 1×PCR buffer with 1.5 mmol L^{-1} MgCl2, 200 μmol L^{-1} each dNTPs, 0.2 μmol L^{-1} each primer, 10 μL template and 2.5 units Taq polymerase. Reaction were carried out by using thermal cycler (DNA Engine PTC-200; Bio-Rad, USA) started with initial denaturation

for 2 min at 98°C followed by 35 cycles consisting of 96°C for 1 min, 60°C for 1 min and 72°C for 1 min and a final extension period 72°C for 10 min. After amplification, 5 μL of reaction product was electrophoresed by 1.5% agarose gel, then stained with EB and viewed under UV light.

The amplified gen was purified by using PCR purification protocol as recommended by manufacturer's instruction (QIAquick PCR purification kit, QIAGE Hilden, Germany).

The purified product was then sequenced commercially (First BASE Laboratories Sdn. Bhd) and the sequences were compared with the 16S rRNA gene sequences in the Gene Bank database using the National Center for Biotechnology Information Blast search tool.

RESULTS AND DISCUSSION

A streptococcus isolates were obtained from 80 (100%) dead and sick fish (mostly from spleen, kidney and brain) and named from Sd1-Sd80, whereas only 2 (5%) streptococcus isolates were obtained from healthy fish spleen and named Sh1 and Sh2.

The colonies were β-hemolytic on blood agar, grayish white, pin head size approximately 1 mm in diameter, circular and slightly convex. The isolates were Gram positive cocci, catalase negative, oxidase negative, esculin negative, lactose negative, sorbitol negative, trehalose positive and growth in media containing 6.5% NaCl negative (Table 1).

The isolates reacted similarly in Rap ID™ STR System (Table 2). The results probabilities were 99.81% and the identification for all isolates was group B (*S. agalactiae*). The positive and negative reactions for BD BBL Crystal™ Identification System were shown in (Table 3) and all isolates results were similar. The results obtained from BBL Crystal mind software confirmed that the isolates were *S. agalactiae*. Using the streptococcal grouping kit,all isolates reacted serologically with the group B antiserum and classified into Lancefield's serologic group B. The sensitivity of 82 *S. agalactiae* isolates to 14 commercial antibiotics was tested. After 24 h incubation, all isolates were found to be sensitive to amoxicillin,ampicillin, erythromycin, chloramphenicol, linomycin, rifampicin, vancomycin, gentamicin, sulfamethoxazole+trimethoprime and tetracycline but were resistant to neomycin, amikacin,

Table 1: Conventional biochemical characteristics of *S. agalactiae* isolates used in this study compared to fish *S. agalactiae* isolated from other countries

	Streptococcus agalactiae			
Tests	Current isolates, Malaysia	Duremdez, *et al.* (2004) Kuwait	Salvador *et al.* (2005) Brazil	Yuasa *et al.* (2008) Thailand
Gram stain	+, cocci	+, cocci	+, cocci	+, cocci
Hemolysis	β type	β type	no	β type
Catalase	-	-	-	-
Oxidase	-	-	-	-
Esculin	-	-	-	-
Sorbitol	-	-	-	-
Lactose	-	-	-	-
Trehalose	+	+	+	+
Growth in media containing 6.5% NaCl	-	-	-	*NA

*NA: Not Available

Table 2: Biochemical characteristics of *S. agalactiae* using RapID™ STR system

Tests	Results
ARG L-arginine	+
ESC Esculin	-
MNL Mannitol	-
SBL Sorbitol	-
RAF Raffinose	-
INU Inulin	-
GLA p-Nitrophenyl-α, D-galactoside	-
GLU p-Nitrophenyl-α, D-glucoside	+
NAG p-Nitrophenyl-n-acetyl-β, D-glucosaminide	-
PO_4 p-Nitrophenylphosphate	+
TYR Tyrosine β-naphthylamide	-
HPR Hydroxyproline β-naphthylamide	-
LYS Lysine β-naphthylamide	+
PYR Pyrrolidine β-naphthylamide	-
HEM Hemolysis on BA	+

Table 3: Biochemical characteristics of *S. agalactiae* using BD BBL Crystal™ identification system (Gram-positive ID Kit)

Tests	Results	Tests	Results
FGC 4MU-β-D-glucoside	-	SUC Sucrose	+
FVA L-valine-AMC	-	MNT Mannitol	-
FPH L-plenylalanine-AMC	+	MTT Maltotriose	+
FGS 4MU-α-D-glucoside	+	ARA Arabinose	-
FPY L-pyroglutamic acid-AMC	-	GLR Glycerol	-
FTR L-tryptophan-AMC	+	FRU Fructose	+
FAR L-arginine-AMC	+	BGL p-nitrophenyl-β-D-glucoside	-
FGA 4MU-N-acetyle-β-D-glucosaminide	-	PCE p-nitrophenyl-β-D-cellobioside	-
FHO 4MU-phosphate	+	PLN Proline and Leucine-p-nitroanilide	+
FGN 4MU-β-D-glucuronide	-	PHO p-nitrophenyl-phosphate	+
FIS L-isoleucine-AMC	-	PAM p-nitrophenyl-α-D-maltoside	-
TRE Trehalose	+	PGO o-nitrophenyl-β-D-glucoside and p-nitrophenyl-α-D-galactoside	-
LAC Lactose	-	URE Urea	-
MAB Methyle-α and β-glucoside	+	ESC Esculin	-
ARG Arginine	+		

Table 4: Antimicrobial sensitivity test of *S. agalactiae* isolated from diseased tilapia

Antimicrobial disc (μg)	*S. agalactiae
Amoxicillin (25)	S++
Ampicillin (10)	S++
Erythromycin (15)	S++
Chloramphenicol (30)	S+
Linomycin (15)	S+
Rifampicin (5)	S+
Sulfamethoxazole+Trimethoprime (23.75+1.25)	S+
Vancomycin (30)	S
Tetracycline (30)	S
Gentamicin (10)	S
Streptomycin (10)	R
Kanamycin (30)	R
Neomycin (30)	R
Amikacin (30)	R

*(R) Resistant, (S) Sensitive, (S+) More sensitive, (S++) Most sensitive

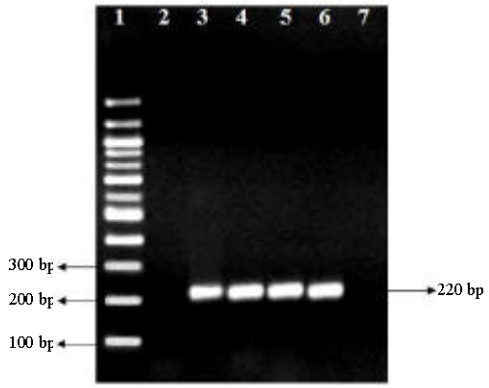

Fig. 1: Agarose gel showing PCR amplification generated by *S. agalactiae* species specific primer. Lane 1, 100 bp ladder; Lane 2, negative control (no DNA); Lane 3, positive control; Lane 4, Sd1 isolate; Lane 5, Sd20 isolate; Lane 6, Sh1 isolate

kanamycin and streptomycin (Table 4). All three isolates and reference strain used in PCR gave the expected size fragment of approximately 220 bp and no bands were formed for negative controls (Fig. 1). BLAST analysis of the 16S rRNA product sequence revealed that the isolate exhibited similarity of 99% to *S. agalactiae* strain HNwc isolated from pond-cultured tilapia in China and *S. agalactiae* strain SO304015 isolated from cultured Japanese horse mackerel in Japan. The distance tree clarified the close relation between the isolate and other *S. agalactiae* recorded in gene bank database (Fig. 2).

Red tilapia (*Oreochromis* sp.) was first introduced to Malaysia in the middle of 1980s. From that date on, tilapia production was increased rapidly. However, the first identification of *S. agalactiae* as an infectious disease to tilapia was in 1997 and now has been reported to cause high mortality among tilapia populations in many tilapia rearing cages and farms around Malaysia (Musa *et al*., 2009; Siti-Zahrah *et al*., 2008). The same pathogen has been reported to cause streptococcosis in tilapia and other fish species around the world as described by Hernandez *et al*., 2009; Evans *et al*., 2008; Suanyuk *et al*., 2008; Duremdez *et al*., 2004. All sick fish sampled for the present study showed either abnormal swimming, eye opacity or exophthalmia. Internally, a congested brain and enlarged liver and spleen were observed. Evans *et al*. (2002) reported clinical signs such as erratic swimming, C-shaped body curvature, ocular opacity, periorbital and intraocular haemorrhage, purulence and exophthalmia in cultured seabream *Sparus auratus* (L.) and wild mullet *Liza klunzingeri* due to streptococcosis. In the present study, *S. agalactiae* isolates were found only in brain, kidney and spleen. The involvement of the brain contributed to the abnormal behavior (Evans *et al*., 2002).

The colonies phenotypic characteristics, Gram's stain and the conventional biochemical tests are generally tests performed to identify *S. agalactiae* isolates in most laboratory practices. This study isolates phenotypic characteristics and biochemical tests results were similar to *S. agalactiae* isolated from another tilapia farms in Malaysia (Musa *et al*., 2009; Siti-Zahrah *et al*., 2008). Furthermore, it was similar to those isolated from Thailand (Yuasa *et al*., 2008; Suanyuk *et al*., 2005) and Kuwait (Duremdez *et al*., 2004). The phenotypical and biochemical profile of *S. agalactiae* isolated from Brazil was similar to this study isolates except it was non-hemolytic on blood agar (Salvador *et al*., 2005).

Commercial biochemical kits have been used many years ago for identification and characterization of bacteria species (Buller, 2004). Nearly all β-hemolytic Streptococci have specific cell wall carbohydrate antigen which allow classification of streptococci to groups (Lancefield, 1993). The Streptococcal Grouping Kit considered a rabid test for identification of group B Streptococcus.

The kit used latex mixed with group specific antibodies for each antigen group A-G each latex group agglutinate with its specific antigen. The Lancefield group B corresponds to a single streptococcal species which is *S. agalactiae* (Evans *et al*., 2002). The RapID™ STR System is a qualitative rapid system for the identification of medically important streptococci; it's just need 4 h to obtain the result.

The biochemical tests in this system based on microbial degradation of specific substrates detected by various indicator systems. All this study isolates gave same result as a *S. agalactiae* and the identification report gave 99.81% probability for all isolates. Many of tests used in BBL Crystal ID System were modified from classical methods such as fermentation, oxidation, degradation and hydrolysis of various substances. All isolates gave same results which were identical to *S. agalactiae* this study results were slightly similar to *S. agalactiae* isolated from Malaysian tilapia farms by Musa *et al*. (2009) but they were different with negative

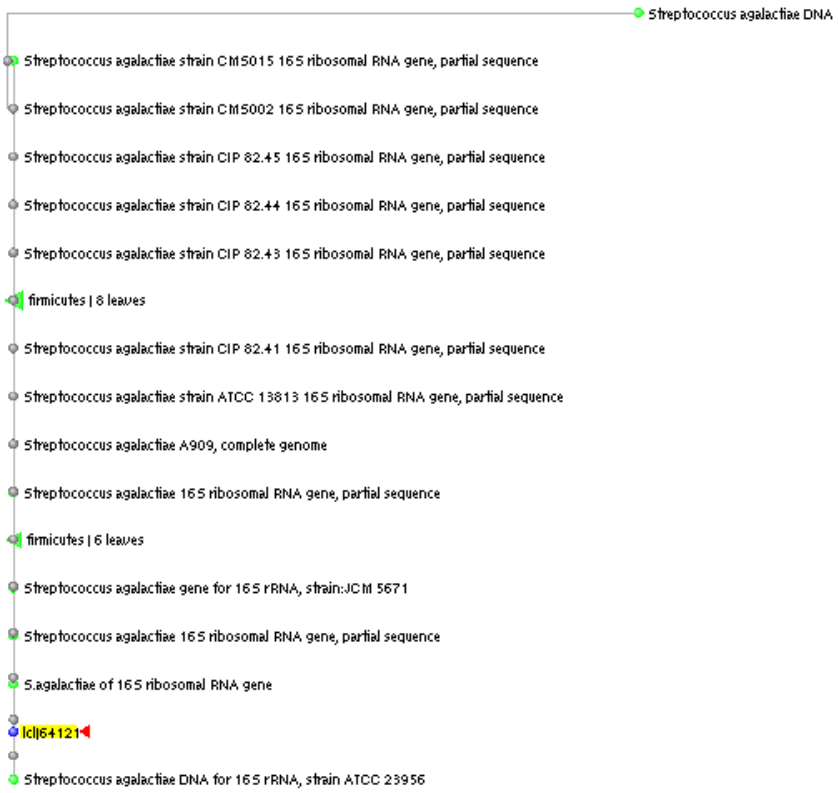

Fig. 2: Gene Bank distance tree showing the relation of our isolate to other *S. agalactiae* found in Gene Bank database

results in L-valine-AMC, L-isoleucine-AMC, p-nitrophenyl-β-D-glucoside and o-nitrophenyl-β-D-glucoside and p-nitrophenyl-β-D-galactoside. These differences may due to the variations between species strains.

In the current study, the susceptibility rate of all *S. agalactiae* isolates to amoxicillin, ampicillin and erythromycin was high, while it was moderate or less sensitive to chloramphenicol, linomycin, rifampicin, vancomycin, gentamicin, sulfamethoxazole + trimethoprime and tetracycline. The sensitivity of this study isolates to amoxicillin, ampicillin, erythromycin, chloramphenicol, rifampicin (rifampin) and sulfamethoxazole + trimethoprime was corresponding to *S. agalactiae* isolated from Kuwait (Duremdez *et al*., 2004; Evans *et al*., 2002) but it was different in gentamicin resistant. The *S. agalactiae* isolated from Thailand (Suanyuk *et al*., 2005) and Kuwait (Duremdez *et al*., 2004) were resistant to sulfamethoxazole + trimethoprime in contrast to current isolates. This study isolates were resistant to neomycin, amikacin, kanamycin and streptomycin. These results matched previous publications in streptomycin (Evans *et al*., 2002), kanamycin (Musa *et al*., 2009; Duremdez *et al*., 2004) and neomycin (Duremdez *et al*., 2004) resistant. To date, there have been no documented cases of any level of resistance among *S. agalactiae* isolated from fish to amoxicillin, ampicillin and erythromycin used in this study. Streptococcosis caused by *S. agalactiae* continues to be an important cause of high morbidity in tilapia farm around Asia. Oxytetracycline and erythromycin still the drug of choice for treatment and prophylaxis in case of streptococcosis in Malaysia (Musa *et al*., 2009). Differences in resistance and sensitivity to antibiotics among the same species could be due to environmental variability, serotype variety and frequent and non-guide use of chemotherapy in the aquaculture field.

The *S. agalactiae* isolates tested gave 220 bp clear bands. The gene was amplified with 16S rRNA species-specific oligonucleotide primers; Forward, 5'-GAGTTTGATCATGG CTCAG-3' and Reverse, 5'-ACCAACATGT GTTAATTACTC-3'. These primers were designed and tested by Martinez *et al*. (2001). The PCR technique used in this study for *S. agalactiae* confirmation was certainly more relaiable than the conventional methods because the species-specific primers were capable to confirm the organism to the species level without doubt. Other publications also affirmed the technique to be reliable, sensitive, specific and time-effective (Duremdez *et al*., 2004). The Blast result of 16S rRNA sequences was confirmed that the isolates of this study were a strain of *S. agalactiae*. The data obtained from Gene Bank showed that the *S. agalactiae* isolates were very close to the Hnwc strain isolated

from tilapia farms in China and the SO304015 strain isolated from cultured Japanese horse mackerel in Japan. *S. agalactiae* has been isolated from various fish species and reported to be a serious pathogen in fresh and marine water fish worldwide (Musa *et al*., 2009; Johri *et al*., 2006; Suanyuk *et al*., 2005; Duremdez *et al*., 2004; Evans *et al*., 2002). More studies need to be carried out in Malaysia on the distribution of *S. agalactiae* in tilapia to allow selection of therapeutics that can be more effective for streptococcosis treatment. The finding of the current study would enable us to formulate a suitable measure to prevent and control future disease outbreaks.

CONCLUSION

The methods employed in this study were able to identify all *S. agalactiae* isolates from fish samples. The conventional biochemical tests were accurate and helpful for primary identification. The other commercial kits were reliable, rapid and convenient for identification of *S. agalactiae*. The PCR assay and 16S r RNA sequencing were more accurate to confirm *S. agalactiae* isolates identification.

ACKNOWLEDGEMENT

Researchers extend the sincere thanks to all who contributed to the success of this research whether with advice or with technical assistance in faculty of veterinary medicine, Universiti Putra Malaysia.

REFERENCES

Buller, N.B., 2004. Bacteria from Fish and Other Aquatic Animals: A Practical Identification Manual. CABI Publishing, Cambridge, MA, USA.

Duremdez, R., A. Al-Marzouk, J.A. Qasem, A. Al-Harbi and H. Gharabally, 2004. Isolation of *Streptococcus agalactiae* from cultured silver pomfret, *Pampus argenteus* (Euphrasen) in Kuwait. J. Fish Dis., 27: 307-310.

Evans, J., P. Klesius, P. Gilbert, C. Shoemaker and M. Al-Sarawi *et al*., 2002. Characterization of B-haemolytic group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L. and wild mullet, Liza klunzingeri (Day) in Kuwait. J. Fish Dis., 25: 505-513.

Evans, J.J., J.F. Bohnsack, P.H. Klesius, A.A. Whiting, J.C. Garcia, C.A. Shoemaker and S. Takahashi, 2008. Phylogenetic relationships among *Streptococcus agalactiae* isolated from piscine, dolphin, bovine and human sources: A dolphin and piscine lineage associated with a fish epidemic in Kuwait is also associated with human neonatal infections in Japan. J. Med. Microbiol., 57: 1369-1376.

Hernandez, E., J. Figueroa and C. Iregui, 2009. *Streptococcosis* on a red tilapia, *Oreochromis* sp. farm: A case study. J. Fish Dis., 32: 247-252.

Inglis, V., R.J. Roberts and N.R. Bromage, 1993. Vibriosis in Bacterial Diseases of Fish. Chapter 6, Blackwell Scientific Publications, London, pp: 109-121.

Johri, A.K., L.C. Paoletti, P. Glaser, M. Dua and P.K. Sharma *et al*., 2006. Group B Streptococcus: Global incidence and vaccine development. Nature Rev. Microbiol., 4: 932-942.

Lancefield, R.C., 1993. A serological differentiation of human and other groups of haemolytic streptococci. J. Exp. Med., 59: 571-591.

Lio-Po, G.D. and L.H. Lim, 2002. Infectious Diseases of Warmwater Fish in Fresh Water. In: Diseases and Disorders of Finfish in Cage Culture, Woo, P.T., D.W. Bruno and L.H. Lim (Eds.). CABI Publishing, Wallingford, pp: 231-281.

Martinez, G., J. Harel and M. Gottschalk, 2001. Specific detection by PCR of *Streptococcus agalactiae* in milk. Can. J. Vet. Res., 65: 68-72.

Murray, P.R., K.S. Rosenthal, G.S. Kobayashi and M.A. Pfaller, 2005. Medical Microbiology. 5th Edn., Mosby Inc., St. Louis MO. USA., pp: 291-296.

Musa, N., L.S. Wei, N. Musa, R.H. Hamdan and L.K. Leong *et al*., 2009. Short communication: Streptococcosis in red tilapia (*Oreochromis niloticus*) commercial farm in Malaysia. Aquac. Res., 40: 630-632.

Plumb, J.A., 1999. Health Maintenance and Principal Microbial Diseases of Cultured Fishes. Iowa State University Press, Ames.

Salvador, R., E.E. Muller, J.C. Freitas, J.H. Leonhadt, L.G. Pretto-Giordano and J.A. Dias, 2005. Isolation and characterization of *Streptococcus* spp. group B in Nile tilapias (*Oreochromis niloticus*) reared in hapas nets and earth nurseries in the northern region of Parana State, Brazil. Ciencia Rural, 35: 1374-1378.

Siti-Zahrah, A., B. Padilah, A. Azila, R. Rimatulhana and R. Shahidan, 2008. Multiple Streptococcal Species Infection in Cage-Cultured Red Tilapia but Showing Similar Clinical Signs. In: Diseases in Asian Aquaculture VI, Bondad-Reantaso, M., C. Mohan, M. Crumlish and R. Subasinghe (Eds). Asian Fisheries Society, Manila, pp: 313-320.

Suanyuk, N., F. Kong, D. Ko, G.L. Gilbert and K. Supamattaya, 2008. Occurrence of rare genotypes of *Streptococcus agalactiae* in cultured red tilapia *Oreochromis* sp. and Nile tilapia *O. niloticus* in Thailand-relationship to human isolates. Aquaculture, 284: 35-40.

Suanyuk, N., H. Kanghear, R. Khongpradit and K. Supamattaya, 2005. Streptococcus agalactiae infection in tilapia (*Oreochromis niloticus*). Songklanakarin J. Sci. Technol., 27: 307-319.

Sun, W., L. Dong, K. Kaneyama, T. Takegami and N. Segami, 2008. Bacterial diversity in synovial fluids of patients with TMD determined by cloning and sequencing analysis of the 16S ribosomal RNA gene. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endodontol., 105: 566-571.

Toranzo, A.E., B. Magarinos and J.L. Romalde, 2005. A review of the main bacterial fish disease in mariculture system. Aquaculture, 246: 37-61.

Yanong, R.P. and R. Francis-Floyd, 2006. Streptococcal Infection of Fish. Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, Florida.

Yin, L.K., 2004. Current Trendsin the Study of Bacterial and Viral Fish and Shrimp Diseases. World Scientific Publishing Co., Singapore.

Yuasa, K., T. Kamaishi, K. Hatai, M. Bahnnan and P. Borisutpeth, 2008. Tow Cases of Streptococcal Infections of Cultured Tilapia in Asia. In: Diseases in Asian Aquaculture VI, Bondad-Reantaso, M., C. Mohan, M. Crumlish and R. Subasinghe (Eds.). Fish Health Section, Asian Fisheries Society, Manila, pp: 259-268.