MOLECULAR CHARACTERIZATION AND PATHOGENICITY OF Streptococcus agalactiae SEROTYPE IA ST7 AND III ST283 ISOLATED FROM CULTURED RED HYBRID TILAPIA IN PENINSULAR MALAYSIA

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By

SYUHADA ROSLAN

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Science

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Abstract of the thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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August 2019

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Faculty : Science

Aquaculture is one of the major contributor of economic resources and food production in Malaysia and tilapia (*Oreochromis* spp.) was one of the highest value in import and export market. But the production was reported to decrease from 51, 554 MT to 35, 996 MT from 2012 to 2016 respectively due to streptococcosis. Streptococcosis was caused by *Streptococcus* sp. which is the etiological agent of diseases in human and animals which could lead to different pathological effects. It was suggested that the different pathological effects post infection was caused by genetically distinction of the bacterial strain. Thus, this study was conducted to determine the molecular serotyping, virulence genes profiling and pathogenicity of *Streptococcus agalactiae* isolated from cultured red hybrid tilapia in Malaysia. A total of 256 isolates of *S. agalactiae,* isolated from fish in Peninsular Malaysia. Genotype of the bacterial collections was determined using PCR based on the capsular polysaccharide gene clusters and multilocus sequence typing. Subsequently, two serotypes were identified, which were 11% and 89% for serotype Ia ST7 and III ST283, respectively. The profiles of virulence genes were constructed using m-PCR with different types of the various published primers involving 14 standard virulence genes (*fbsA, fbsB*, *pavA*, *scpB*, *lmb*, *cylE*, *cfb*, *spb1*, *hylB*, *rib*, *bca*, *bac*, *cspA* and *pbp1A/ponA*). Serotype Ia showed different pattern of virulence genes profile compared to serotype III, where it lacked the *lmb, scpB, pavA, fbsB, cyl, bca, cspA* and *bac* genes, thats differentiated the virulence level of the serotype. Median lethal dose 50 (LD_{50}) of *S. agalactiae* in red hybrid tilapia for serotypes Ia and III were calculated at 8.7 x 10^3 CFU/mL and 6.3 x 10^3 CFU/mL, respectively. There was no significant difference ($p > 0.05$) between the rate of mortality of red hybrid tilapia for both serotypes following intraperitoneal challenge. Histopathological

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lesions included meningitis, necrosis of hepatocytes, coagulative tubular necrosis and hypocellular of spleen. Histopathological scoring and independent t-test showed that there was the significant difference ($p < 0.05$) between all lesions in brain, liver (except hepatonecrotic), and spleen but not in kidney. *Streptococcus agalactiae* serotype Ia ST7 showed severe lesions in all organs compared to *S. agalactiae* serotype III ST283. This study concluded that there were two serotypes (Ia and III) of *S.agalactiae* isolated from cultured red hybrid tilapia in Malaysia so far. Moreover, different serotypes of *S. agalactiae* consisted of different virulent characteristics. Red hybrid tilapia was susceptible to both serotypes following intraperitoneal challenge. Serotype Ia showed higher LD_{50} compared to serotype III, but not significant ($p > 0.05$) in term of cumulative mortality.

Keywords: *Streptococcus agalactiae*, molecular serotyping, virulence gene, pathogenicity.

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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan Ijazah Master Sains

CIRI MOLEKUL DAN ANALISIS KEPATOGENAN OLEH *Streptococcus agalactiae* SEROTIPE IA ST7 DAN III ST283 DIPENCIL DARI IKAN TILAPIA MERAH HIBRID DI SEMENANJUNG MALAYSIA

Oleh

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Pengerusi : Mohammad Noor Amal Azmai, PhD
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Akuakultur ialah salah satu dari sumber ekonomi dan penghasilan makanan di Malaysia dan Tilapia (*Oreochromis* spp.) adalah salah satu penyumbang tertinggi dalam pasaran import dan eksport. Walaubagaimanapun, penghasilan tilapia dilaporkan telah menurun dari 51, 554 MT ke 35, 996 MT pada 2012 ke 2016. disebabkan oleh Streptococcosis. Streptococcosis berpunca dari *Streptococcus* sp. iaitu ejen etiologi bagi penyakit manusia dan haiwan. Hal ini dicadangkan bahawa kesan patologi yang berbeza selepas jangkitan disebabkan oleh perbezaan genetik bagi jenis bakteria. Oleh itu, kajian ini dilakukan untuk menentukan penentuan serotipe molekular, profil gen virulen dan analisis patogenisiti *Streptococcus agalactiae* yang dipencil dari ikan tilapia merah di Malaysia. Sebanyak 256 sampel *S. agalactiae* dari Semenanjung Malaysia telah digunakan dalam kajian ini. Genotip bakteria telah ditentu menggunakan kaedah tindak balas rantai polimer berdasarkan penentuan serotipe molekular dari kumpulan gen polisakarida kapsul dan jenis urutan multilokus di mana dua jenis serotipe telah ditemukan iaitu 11% dan 89% untuk serotipe Ia ST7 dan III ST283. Profil gen virulen telah dibina menggunakan kaedah m-PCR berdasarkan primer yang berbeza melibatkan 14 gen virulen stardard (*fbsA, fbsB*, *pavA*, *scpB*, *lmb*, *cylE*, *cfb*, *spb1*, *hylB*, *rib*, *bca*, *bac*, *cspA* dan *pbp1A/ponA*). Serotip Ia menunjukkan corak yang berbeza berbanding serotipe III dimana ianya tiada gen *lmb, scpB, pavA, fbsB, cyl, bca, cspA* dan *bac* gen*,* yang membezakan tahap kevirulenan kedua-dua serotipe. Dos kematian 50 (LD_{50}) bagi serotipe Ia ialah 8.7 x 10^3 CFU/ml manakala 6.3 x 10^3 CFU/ml bagi serotipe III. Tiada perbezaan bermakna antara kematian disebabkan oleh serotipe Ia dan III ($p > 0.05$). Perubahan histopatologi termasuk meningitis, nekrosis sel hepatosit, nekrosis koagulan dengan sitoplasma merah dan hiposel dalam limpa. Penilaian keterukan lesi histopatologi dan analisis t-

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test telah merekod perbezaan yang bermakna ($p < 0.05$) pada lesi otak, hati (kecuali hepatonekrotik), dan limpa tetapi tidak bermakna pada ginjal. Kajian ini merumuskan yang terdapat dua serotipe (Ia dan III) *S. agalactiae* yang dipencil dari ikan tilapia hibrid merah di Malaysia setakat ini. Tambahan pula, serotipe yang berbeza mempunyai karakter virulensi yang berbeza. Ikan tilapia hibrid merah juga mudah dijangkiti oleh kedua-dua serotipe menggunakan kaedah intraperitoneal. Serotipe Ia menunjukkan kadar LD_{50} yang tinggi berbanding serotipe III, tetapi tidak bermakna dari segi kumulatif kadar kematian.

Kata kunci: *Streptococcus agalactiae*, penentuan serotipe molekular, gen virulen, kepatogenan.

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I certify that a Thesis Examination Committee has met on 8 August 2019 to conduct the final examination of Syuhada binti Roslan on her thesis entitled "Molecular Characterization and Pathogenicity of *Streptococcus agalactiae* Serotype Ia ST7 and Serotype III ST283 Isolated from Cultured Red Hybrid Tilapia in Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS AND SYMBOLS

MT	Metric ton
PCR	Polymerase chain reaction
cps	Capsular polysaccharide
ST	Sequence type
MLST	Multilocus sequence typing
BA	Blood agar
H_2O_2	Hydrogen peroxide
O_2	Oxygen
DNA	Deoxyribonucleic acid
rRNA	Ribosomal ribonucleic acid
bp	Base pair
LPS	Lipopolysaccharide
GBS	Group B *Streptococcus*
MLEE	Multilevel event extraction
CG	Clonal group
CC	Clonal complex
TSA	Trypticase soy agar
TSB	Trypticase soy broth
ng	Nanogram
mM	Micromolar
µl	Microliter
$MgCl_2$	Magnesium chloride
dnTPs	Deoxyribonucleotide triphosphate
min	Minutes
secs	Seconds
℃	Degree celcius
h	Hour
rpm	Revolutions per minute
CFU	Colony forming unit
mL	Milliliter
L	Liter
IP	Intraperitoneal injection
hpi	Hour post-infection
LD_{50}	Lethal dose 50
API	Analytical profile index
β	Beta
γ	Gamma
α	Alpha
%	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Aquaculture is one of the biggest industry in Malaysia and ranked top 15 world producer in 2014 with estimated production is 521, 000 tones (FAO, 2016). It was reported that aquaculture 8.9% of total national agriculture domestic product (GDP) which created an estimated 1, 753, 900 million jobs for Malaysian (DOMS, 2016).

However, the progressive effort in development of this sector lead to several effects such as fish stock depletion, national environmental issues, media influences on spreading false information, non-compliance towards halal aquaculture, and diseases that affected cultured fish (Fathi et al., 2018). One of the highest contributor in local import and export market is tilapia (*Oreochromis* spp.). Tilapia (*Oreochromis* spp.) was the second highest harvested freshwater fish in Malaysia with a total production of 35,996 metric tons (MT) in 2016 (DOF, 2018). The first and third highest were recorded for catfish (*Clarias* spp.) and striped catfish (*Pangasianodon* spp.) with a total production of 50,534 MT and 13,914 MT, respectively.

The wholesale value of tilapia was highest at RM 259 million, followed by catfish and striped catfish, at RM 208 million and RM 138 million, respectively. These values significantly indicated that tilapia farming is among the important aquaculture industry in this country (DOF, 2018). It was reported by DOF (2018) that the total production of tilapia was decreased due to several factors especially streptococcosis. In Malaysia, streptococcosis was the caused by *Streptococcus agalactiae* and *S. iniae* (Zamri-Saad et al., 2014).

Streptococcus agalactiae is a Gram-positive bacterium that associated with severe invasive diseases to human and animals. It is a type of bacteria that can cause diseases in aquatic animals, especially fish as well as mammals and reptiles (Amal and Zamri-Saad, 2011). It was reported that *S. agalactiae* strains can be haemolytic or non-haemolytic (Wang et al., 2013).

The common clinical signs and gross lesions showed by fish when infected by this pathogen including exophthalmia, lethargy, intraocular hemorrhage, opaqueness in cornea and classic manifestation of brain infection (Wang et al., 2013). The clinical signs showed by tilapia infected by *S. agalactiae* can cause

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lesion which could be characterized by curvature of the spinal cord, stiffness and bleeding at the base of the fins (Asencios et al., 2016). *Streptococcus agalactiae* infection can cause mortality due to chronic infection and some cases can be categorized as severe acute infection (Pretto-Giordano et al., 2010; Kayansamruaj et al., 2014).

Several potential control and prevention measures of streptococcosis has been suggested by Zamri-Saad et al. (2014) such as selection of hatcheries, treatment of water supply and fish fry, antibiotics and vaccinations. Apart from all suggested prevention which is focusing on the fish management only, the study of the pathogen itself are also important to determine the reason of infection. Yet, there were lack of genetic information of *Streptococcus* sp. reported in Malaysia.

The genomic study is a must for the development of effective vaccine for streptococcosis prevention. In studying epidemiology of *S. agalactiae,* knowing the genetic variation is important to verify the distinct strain, serotype and sequence type of the species. Molecular serotyping through polymerase chain reaction (PCR) and sequencing of serotype-specific gene fragment within capsular polysaccharide (*cps*) genes are among the excellent methods in studying epidemiology of this bacteria (Manning et al., 2005). Generally, serotypes are groups within a single species of microorganisms, such as bacteria or virus, which share distinctive surface structures while *cps* gene is the gene that is related to virulence characteristics of the bacteria (Berridge et al., 2001).

Molecular serotyping of *S. agalactiae* can be discriminated based on two methods, which are based on *cps* serotyping and multilocus sequence typing (MLST) methods (Versalovic et al., 2002; Imperi et al., 2010; Yildirim et al., 2011; Kannika et al.,2017). The molecular typing for *S. agalactiae* is determined by the detection of antigenic structure, *cps* gene clusters using 19 specific primers (Imperi et al., 2010). *Streptococcus agalactiae* has 10 different capsular serotypes which are Ia, Ib, and II-IX (Yao et al., 2013; Li et al., 2014; Kayansamruaj et al., 2014; Kannika et al., 2017). Group B *Streptococcus* (GBS) generally produced one of the 10 antigenically distinct *cps,* which are thought to play a key role in its virulence. In addition, the *cps* gene is a major virulence factor in invasive disease caused by *S. agalactiae* (Chideroli et al.,2017).

Besides, MLST is another reliable approach in studying the epidemiology and molecular typing of bacterial species. MLST produced accurate and portable data in the study of evolutionary and population biology (Urwin and Maiden, 2003). Apart of studying the extracellular antigen, MLST is the study of unambiguous sequence-based typing method which aiming at the variation targeted housekeeping genes that usually of about 500 bp in size (Tien et al., 2011). *Streptococcus agalactiae* consists of 1349 sequence type (ST) around the world (PubMLST, 2019) while the common ST found in Asia such as China and Singapore were the ST7, ST10, ST19, ST283 and ST485 (Kalimuddin et al., 2017; Wang et al., 2018).

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The genotypic characterization for constructing virulence genes profile of *S. agalactiae* is based on 14 virulence genes found in human isolates, which includes *fbsA* (the fibrinogen-binding protein FbsA), *fbsB* (the fibrinogen-binding protein FbsB), *pavA* (a fibrinogen-binding protein), *scpB* (C5a peptidase), *lmb* (laminin-binding protein), *cylE* (β-haemolysin/cytolysin), *cfb* (CAMP factor), *spb1* (haemolysin III), *hylB* (hyaluronate lyase), *rib* (the surface protein rib), *bca* (C-α protein), bac (C-β protein), *cspA* (the serine protease cspA), and *pbp1A/ponA* (penicilin-binding protein A) (Lin et al., 2011). By conducting multiplex PCR analysis, the virulence genes profile can be categorized under three virulence gene categories, which are adhesins, invasins and immune evasins (Willis and Whitfield, 2013; Laczeski et al., 2014).

Each of the virulence gene carries different roles in pathogenicity in the host after infection of the organism. GBS infection can be determined by the virulence factor encoded by these virulence genes including *cps* gene clusters (Hannoun et al., 2009). For example, the *bca* gene coding for Alpha-C protein, a surface protein that helps the bacteria to enter the host cells (Sadowi et al., 2010). Each group of virulence gene plays different role where (1) adhesin promotes the adhesion of bacteria to the host cell surface, (2) invasins allow the adhesive bacteria to cross the mucosal and the blood-brain barrier and (3) immune evasins facilitate escape from host immunity. Usually, pathogenicity analysis was conducted to prove the correlation between serotype and virulence of bacteria by conducting the challenge test of *S. agalactiae* infection on the susceptible fish (Kannika et al., 2017).

1.2 Problem statements

Freshwater aquaculture, especially tilapia farming is one of the major contributor of food source in Malaysia. However, streptococcosis has been emerged as an important disease in this industry and resulted to enormous economic losses (Yanong et al., 2010; Amal and Zamri-Saad, 2011). Nevertheless, streptococcosis also escalate the food insecurity and might possibly harm the health of the consumers. As the genetic information of *S. agalactiae* isolates is still lacking in Malaysia, the molecular research can give a beneficial information to solve this problem. Moreover, it was realized that *S. agalactiae* have several strains with different ST and antigenic properties. Besides, by studying the genetic materials of different strain of the isolates, the comparison of virulence level and infection causes by particular strain can be compare by conducting the bacterial challenge to the host animal. Hence, the molecular epidemiology studies of *S. agalactiae* is needed to provide bacterial information towards the future vaccine strategy for tilapia farming.

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1.3 Objectives

The objectives of this present study are;

1. To identify the molecular serotyping of *Streptococcus agalactiae* isolates from cultured hybrid tilapia in Malaysia based on capsular polysaccharide (*cps*) genes and multilocus sequence typing (MLST).

2. To determine the virulence genes profiling of *Streptococcus agalactiae* based on the standard virulence genes found in human.

3. To investigate the pathogenicity of *Streptococcus agalactiae* serotype Ia ST7 and III ST283 in red hybrid tilapia (*Oreochromis niloticus* x *O. mossambicus*).

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REFERENCES

Aamri F., Padilla D., Acosta F., Caballero M. J., Roo J., Bravo J., Vivas J., & Real F. (2010). Final report of *Streptococcus iniae* in porgy (*Pagrus pagrus*, L.). Journal of Fish Diseases, 33(11): 901-905.

Abbott S. L., Cheung W. K., & Janda J. M. (2003). The genus *Aeromonas*: Biochemical characteristics, atypical reactions and phenotypic identification schemes. Journal of Clinical Microbiology, 41(6): 2348-2357.

Abdullah S., Omar N., Yusoff S. M., Obukwho E. B., Nwunuji T. P., Hanan L., & Samad J. (2013). Clinicopathological features and immunohistochemical detection of antigens in acute experimental *Streptococcus agalactiae* infection in red tilapia (*Oreochromis* spp.). Springerplus, 2: 86.

Abuseliana, A., Daud, H., Abdul Aziz, B., Alsaid, M. (2011). Pathogenicity of *Streptococcus agalactiae* isolated from a fish farm in Selangor to juvenile red tilapia (*Oreochromis* sp.). Journal of Animal and Veterinary Advances, 10 (7): 914-919.

Adigun I. A., Nasir A. A., & Aderibigbe A. B. (2019). Fulminant necrotizing fasciitis following the use of herbal concoction: a case report. Journal of Medical Case Reports, 4: 326.

Aisyhah M. A. S., Amal M. N. A, Zamri-Saad M., Siti-Zahrah A., & Shaqinah N. N. (2015). *Streptococcus agalactiae* isolates from cultured fishes in Malaysia manifesting low resistance pattern towards selected antibiotics. Journal of Fish Diseases, 38: 1093-1098.

Al-Akhrass F., Abdallah L., Berger S., Hanna R., Reynolds N., Thompson S., Hellit R., & Schlievert P. M. (2013). *Streptococcus agalactiae* toxic shock-like syndrome. Medicine, 92(1): 10-14.

Alexander S. K., & Strete D. (2001). Microbiology: A photographic Atlas for the laboratory. The University of Michigan.

Al-Harbi A. (2016). Phenotypic and genotypic characterization of *Streptococcus agalactiae* isolated from hybrid tilapia (*Oreochromis niloticus* x *O. aureus*). Aquaculture, 464: 515-520.

Amal, M.N.A., Zamri-Saad, M., Siti-Zahrah, A., Zulkafli, R., Misri, S., Nur-Nazifah, M. & Shahidan, H. (2010a). Prevalence of *Streptococcus agalactiae* in tilapia kept indifferent water bodies. Journal of Veterinary Resources, 14: 153–162.

Amal, M.N.A., Zamri-Saad, M., Zulkafli, A.R., Siti-Zahrah, A., Misri, S., Ramley, B., Shahidan, H., & Sabri, M.Y.(2010b) Water thermocline

© COPYRIGHT UPM

confirms susceptibility of tilapia cultured in lakes to *Streptococcus agalactiae*. Journal of Animal and Veterinary Advances, 9: 2811–2817.

Amal M. N. A. & Zamri-Saad M. Streptococcosis in Tilapia (*Oreochromis niloticus*): A review. (2011). Pertanika Journal of Tropical Agricultural Science. 34(2): 195-206.

Amal, M.N.A., Zamri-Saad, M., Iftikhar, A.R., Siti-Zahrah, A., Aziel, S., & Fahmi, S. (2012) An outbreak of *Streptococcus agalactiae* infection in cage-cultured golden pompano, *Trachinotus blochii* (Lacepede), in Malaysia. Journal of Fish Diseases, 35: 849–852.

Amal M. N. A., Zamri-Saad M., Siti-Zahrah A., & Zulkafli A. R. (2013). Transmission of *Streptococcus agalactiae* from a hatchery into a newly established red hybrid tilapia, *Oreochromis niloticus* (L.) x *Oreochromis mossambicus* (Peters), farm. Journal of Fish Diseases, 36: 735-739.

Arabestani M. R., Mousavi S. M., & Nasaj M. (2017). Genotyping of clinical *Streotococcus agalactiae* strains based on molecular serotype of capsular (*cps*) gene cluster sequences using polymerase chain reaction. Archives of Clinical Infectious Diseases, 12(1): e36787.

Asencios Y. O., Sánchez F.B., Mendizábal H. B., Pusari K. H., Alfonso H. O., Sayána A. M., Figueiredoc M. A. P., Manrique W. G., de Andrade Belo M. A., Chaupe N. S. (2016). First report of *Streptococcus agalactiae* isolated from *Oreochromis niloticus* in Piura, Peru: Molecular identification and histopathological lesions. Aquaculture Reports, 4: 74–79.

Ansyary H., Kurniawan R. A., Sriwulan S., Ramli R., & Baxa D. V. (2014). Isolation and molecular identification of the etiological agents of streptococcosis in Nile Tilapia (*Oreochromis niloticus*) cultured in net cages in Lake Sentani, Papua, Indonesia. SpringerPlus, 3: 627.

Archenaz R. C. (2017). Molecular Methods for Healthcare-Acquired Infections. Diagnostic Molecular Pathology, 163-177.

Aryal S. (2018). Oxidase Test- Principle, Uses, Procedure, Types, Result Interpretation, Examples and Limitations. Microbiological info.com.

Austin B. & Austin D. A. (2007). Bacterial fish pathogens. Diseases of farmed and wild fish. (4th edition). Chichester. Springer/Prazis Publishing.

Azad I. S., Al-Marzouk A., James C. M., Almatar S., A-Gharabally H., & Qasem J. A. (2012). Outbreak of natural Streptococcosis in hatchery produced silver pomfret (*Pampus argenteus* Euphrasen) larvae in Kuwait. Aquaculture, 330-333: 15-20.

© COPYRIGHT UPM

Baeck, G.W., Kim, J.H., Gomez, D.K., & Park, S.C. (2006). Isolation and characterization of *Streptococcus* sp. from diseased flounder (*Paralichthys olivaceus*) in Jeju Island. Journal of Veterinary Science, 7: 53-58.

Barato P., Martins E. R., Vasquez G. M., Ramirez M., Melo-Christino J., Martinez N., & Iregui C. (2016). Capsule impairs efficient adherence of *Streptococcus agalactiae* to intestinal epithelium in tilapias *Oreochromis* sp. Microbial Pathogenesis, 100: 30-36.

Baron S. In Patterson M. J. (1996). *Streptococcus*. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston, Chapter 13.

Belard S., Toepfner N., Capan-Melser M., Mombo-Ngoma G., Zoleko-Manego R., Groger M., Matsiegui P., Agnandji S. T., Adegnika A. A., Gonzalez R., Kremsner P. G., Menendez C., Ramharter M., & Berner R. (2015). *Streptococcus agalactiae* serotype distribution and antimicrobial susceptibility in pregnant women in Gabon, Central Africa. Scientific Reports, 5: 17281.

Barnett T. C., Cole J. N., Rivera-Hernandez T., Henningham A., Paton J. C., Nizet V., & Walker M. J. (2015). Streptococcal toxins: role in pathogenesis and diseases. Cellular Microbiology, 17(12): 1721-1741.

Berridge B. R., Bercovier H., & Frelier P. F. (2001). *Streptococcus agalactiae* and *Streptococcus difficile* 16S-23S intergenic rDNA: Genetic homogeneity and species-specific PCR. Veterinary Microbiology, 78: 165-173.

Breeding K. M., Ragipani B., David-Lee K., Malik M., Randis T. M., & Ratner A. J. (2016). Real-time PCR-based serotyping of *Streptococcus agalactiae.* Nature, 6: 38523.

Bromage E., & Owens L. (2009). Environmental factors affecting the susceptibility of barramudi to *Streptococcus iniae*. Aquaculture, 290(3-4): 224-228.

Buxton R. (2005). Blood agar plates and hemolysis protocols. American Society for Microbiology, 2016.

Case R. J., Boucher Y., Dahllof I., Holmstrom C., Doolittle W. F., & Kjelleberg S. (2007). Use of 16S rRNA and rpoB genes as molecular markers for microbial ecology studies. Journal of Applied Environmental Microbiology, 73(1): 278-288.

© COPYRIGHT UPM

Carlos A. G. L., Queiroz G. A., Pereira F. L., Tavares G. C., & Figueiredo H. C. P. (2019). *Streptococcus agalactiae* sequence type 283 in farmed fish, Brazil. Emerging Infectious Diseases Journal, 25(4): 776-779.

Carvalho-Castro G. A., Silva J. R., Paiva L. V., Custodio D. A. C., Moreira R. O., Mian G. F., Prado I. A., Chalfun-Junior A., & Costa G. M. (2017). Molecular epidemiology of *Streptococcus agalactiae* isolated from mastitis in Brazilian dairy herds. Brazilian Journal of Microbiology, 43(3): 551-559.

Chakravorty S., Helb D., Burday M., Connell N., & Alland D. (2007). A detailed analysis of 16S ribosomal RNA gene segments for the diagnosis of pathogenic bacteria. Journal of Microbiological Methods, 69(2): 330-339.

Chau M. L., Chen S. L., Yap M., Hartantyo S. H. P., Chiew P. K. T., Fernandez C. J., Wong W. K, Fong R. K., Tan W. L., Tan B. Y. Z., Ng Y., Aung K. T., Mehershashi K. S., Goh C., Kang J. S. L., Barkham T., Leong A. O. K., Gutierrez R. A., & Ng L. C. (2017). Group B *Streptococcus* infections caused by improper sourcing and handling of fish for raw consumption, Singapore, 2015-2016. Emerging Infectious Diseases, 23(12).

Chen M., Wang R., Luo F., Huang Y., Liang W., Huang T., Lei A., Gan X., & Li L. (2015). *Streptococcus agalactiae* isolates of serotypes Ia, III, and V from human and cow are able to infect tilapia. Veterinary Microbiology, 180: 129-135.

Chideroli R. T., Amoroso N., Mainardi R. M., Suphoronski S. A., Padua S. B., Afieri A. F., Alfieri A. A., Mosela M., Moralez A. T. P., Oliveira A. G., Zanolo R., Santis G. W., & Pereira U. P. (2017). Emergence of a new multidrug-resistant and highly virulent serotype of *Streptococcus agalactiae* in fish farms from Brazil. Aquaculture, 479: 45-51.

Dellanoy C. M. J., Crumlish M., Fontaine M. C., Pollock J., Foster G., Dagleish M. P., Turnbull J. F., & Zadoks R. N. (2013). Human *Streptococcus agalactiae* strains in aquatic mammals and fish. Biomedic Central (BMC) Genomic, 13: 41.

Deng Z. B., Zhang Y. W., Geng Y., Wang K. Y., Chen D. F., Ouyang P., Huang X. L., He C. L., Zhong Z. J., Yang Z. X., & Tang L. (2017). A new sequence (ZST-1) of infectious *Streptococcus agalactiae* from Chinese cryprinid fish, *Schizopygopsis pylzovi*. Aquaculture, 468: 496-500.

Dingle K. E., Colles F. M., Wareing D. R. A., Ure R., Fox A. J., Bolton F. E., Bootsma H. J., Willems R. J. L., Urwin R., & Maiden M. C. J. (2001). Multilocus sequence typing system for *Campylobacter jejuni*. Journal of Clinical Microbiology, 39(1): 14-23.

© COPYRIGHT UPM

DOF (Department of Fisheries of Malaysia). (2018). Fisheries statistic. DOF. Malaysia.

DOSM, Selected Agricultural Indicators, Malaysia, 2016.Retrieved 4 September 2019, from Department of Statistics Malaysia.

Donelli G., Vuotto C., & Mastromarino P. (2013). Phenotyping and genotyping are both essential to identify and classify a probiotic microorganism. Journal of Microbiol, Ecology in Health and Disease, 24.

Dramsi S., Caliot E., Bonne I., Guadagnini S., Prevost M. C., Kojadinovic M., Lalioui L., Poyart C., & Trieu-Cort P. (2006). Assembly and role of pili in group B streptococci. Molecular Microbiology, 60(6):1401-1413.

Duarte R. S., Miranda O. P., Bellei B. C., Aparecida M., Brito M. A. V. P., & Teixeira L. M. (2004). Phenotypic and molecular characteristics of *Streptococcus agalactiae* isolates recovered from milk pf dairy cows in Brazil. Journal of Clinical Microbilogy, 42(9): p 4214-4222.

Eldar A., & Ghittino C. (1999). *Lactococcus garvieae* and *Streptococcu iniae* infections in rainbow trout *Onchorhynchus mykiss*: Similar, but different diseases. Diseases of Aquatic Organisms, 36(3): 227-231.

Eskandarian N., Ismail Z., Neela V., Belkum A. V., Desa M. N. M., & Nordin S. A. (2015). Antimicrobial susceptibility profiles, serotype distribution and virulence determinants among invasive, non-invasive and colonizing *Streptococcus agalactiae group* B *streptococcus*) from Malaysian patients. European Journal of Clinical Microbiology & Infectious Diseases, 34(3): 579-584.

Evans J. J., Klesius P. H., Gilbert P. M., Shoemaker C. A., Sarawi M. A., Landsberg J., Duremdez R., Marzouk A., & Zenki S. (2002). Characterization of β-hemolytic Group B *Streptococcus agalactiae* in cultured seabream, *Sparus auratus* L., and mullet, *Liza klunzingeri* (Day), in Kuwait. Journal of Fish Diseases, 25: 505-513.

Evans J. J., Bohnsack J. F., Klesius P. H., Whiting A. A., Garcia J. C., Shoemaker C. A., & Tahakashi S. (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. Journal of Medical Microbiology, 57(Pt 11): 1369–1376.

Evans J. J., Klesius P. H., Pasnik D. J., & Bohnsack J. F. (2009). Human *Streptococcus agalactiae* isolate in Nile Tilapia (*Oreochromis niloticus*). Emerging Infectious Diseases, 15(5): 774-776.

FAO, The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Retrieved on 5 September 2019.

© COPYRIGHT UPM

Fathi S., Harun A.N., Rambat S. & Tukiran N. A. (2018). Current issues in aquaculture: Lesson from Malaysia. Advances Science Letter, 24(1): 503-505.

Foster T. J., Geoghegan J. A., Ganesh V. K., & Hook M. (2014). Adhesion, invasion and evasion: the many functions of the surface proteins of *Staphylococcus aureus*. Nature Reviews Microbiology, 12(1): 49-62.

Fratamico P. M., Debroy C., Liu Y., Needleman D. S., Baranzoni G. M. & Feng P. (2016). Advances in molecular serotyping and subtyping of *Escherichia coli*. Frontier Microbiology.

Fu G. Y., Wan Z. Yi., Xia J. H., Fen L., Liu X. J., & Yue G. H. (2014). The MCP-8 gene and its possible association with resistance to *Streptococcus agalactiae* in tilapia. Fish & Shellfish Immunology, 40: 331-336.

Gera, K., Le, T., Jamin, R., Eichenbaum, Z., & McIver, K. S. (2014). Thephosphoenolpyruvate phosphotransferase system in group A *streptococcus* actsto reduce streptolysin S activity and lesion severity during soft tissue infection. Infection and Immunity, 82: 1192–1204.

Godoy D. T., Carvalho-Castro G. A., Leal C. A., Pereira U. P., Leite R. C., & Figueiredo H. C. (2013). Genetic diversity and new genotyping scheme forfish pathogenic *Streptococcus agalactiae*. Letters in Applied Microbiology, 57(6): 476-483.

Gullet J. C. & Molte F. S. (2015). Quantitative nucleic acid amplification methods for viral infection. Clinical Chemistry, 61(1):72-78.

Gupta E., Bhalla P., Khurana N., & Singh T. (2009). Histopathology for the diagnosis of infectious diseases. Indian Journal of Medical Microbiology, 27(2): 100-106.

Hannoun A., Shehab M., Marie-Therese K., Sabra A., Abi-Rached R., Bazi T., Yunis K. A., Araj G. F. & Matar G. M. (2009). Correlation between Group B Streptococcal genotypes, their antimicrobial reistancce profiles and virulence gene among pregnant woman in Lebanon. International of Microbiology, 2009.

Henriksen S. D. (1978). Chapter I Serotyping of bacteria. Methods in Microbiology, 12: 1-13.

Hooven T. A., Catomeris A. J., Akabas L. H., Randis T. M., Maskell D. J., Peters S. E., Ott S., Santana-Cruz I., Tallon L. J., Tettelin H., & Ratner A. J. (2016) The essential genome of *Streptococcus agalactiae*. BMC Genomics, 17: 4006.

Hu W., Guo W., Meng A., Sun Y., Wang S., Xie Z., Zhou Y., & He C. (2017). A metabolomic investigation into the effects of temperature on

© COPYRIGHT UPM

Streptococcus agalactiae from Nile Tilapia (*Oreochromis niloticus*) based on UPLC-MS/MS. Veterinary Microbiology, 210: 174-182.

Imberty A. & Varrot A. (2009). Microbial recognition of human cell surface glyconjugates. Current Opinion in Structural Biology, 18(5): 567-576.

Imperi M., Pataracchia M., Alfarone G., Baldassarri L., Orefici G., & Creti R. (2010). A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of *Streptococcus agalactiae*. Journal of Microbiological Methods, 80: 212-214.

Iwase T., Tajima A., Sugimoto S., Okuda K., Hironaka I., Kamata Y., Takada K., & Mizunoe Y. (2013). A simple assay for measuring catalase activity: A visual approach. Scientific Reports, 3: 3081.

Janda H. M., & Abbott S. L. (2007). 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, Perils, and Pitfalls. Journal of Clinical Microbiology, 45(9): p 2761-2764.

Jarvinen A., Laakso S., Piiparinen P., Aittakorpi A., Lindfors M., Huopaniemi L., Piiparinen H., Maki M. (2009). Rapid identification of bacterial pathogens using a PCR- and microarray-based assay. BMC Microbiology, 9:161.

Jenkins C., Ling C. L., Ciesielczuk H. L., Lockwood J., Hopskin S., McHugh T. D., Gillespie S. H., & Kibbler C. C. (2012). Detection and identification of bacteria in clinical samples by 16S rRNA gene sequencing: comparison of two different approaches in clinical practice. Journal of Medical Microbiology, 61: 483-488.

Jimenez A., Tibata V., Junca H., Ariza F., Verjan N., & Iregui C. (2011). Evaluating a nested-PCR assays for detecting *Streptococcus agalactiae* in red tilapia (*Oreochromis* sp.) tissue. Aquaculture, 321: 203-206.

Jones N. J. F., Bohnsack S., Takahashi K. A., Oliver M. S., Chan F., Kunst F., Glaser P., Rusniok C., Crook D. W., Harding R. M., Bisharat N., & Spratt B. G. (2003) Multilocus sequence typing system for Group B *Streptococcus*. Journal of Clinical Microbiology, 41: 2530-2536.

Kalimuddin S., Chen S. L., Lim C. T. K., Koh T. H., Tan T. Y., Kam M., et al. (2017). 2015 epidemic of severe *Streptococcus agalactiae* sequence type 283 infections in Singapore associated with the consumption of raw freshwater fish: a detailed analysis of clinical, epidemiological, and bacterial sequencing data. Clinical Infectious Diseases, 64: S145–S152.

Kannika K., Pisuttharachai D., Srisapoome P., Wongtavatchai J., Kondo H., Hirono I., Unajak S., & Areechon N. (2017). Molecular serotyping, virulence gene profiling and pathogenicity of *Streptococcus*

© COPYRIGHT UPM

agalactiae isolated from tilapia farms in Thailand by multiplex PCR. Journal of Applied Microbiology, 122: 1497-1507.

Kayansamruaj P., Pirarat N., Hirono I., & Rodkhum C. (2014). Increasing of temperature induces pathogenicity of *Streptococcus agalactiae* and the up-regulation of inflammatory related genes in infected Nile Tilapia (*Oreochromis niloticus*). Veterinary Microbiology, 172: 265-271.

Kayansamruaj P., Pirarat N., Kondo H., Hirono I., & Rodkhum C. (2015). Genomic comparison between pathogenic *Streptococcus agalactiae* isolated from Nile Tilapia in Thailand and fish-derived ST7 strains. Infection, Genetics and Evolution, 36: 307-314.

Kayansamruaj P., Soontara C., Unajak S., Dong H. T., Rodkhum C., Kondo H., Hirono I., & Areenchon N. (2018). Comparative genomics inferred two distinct populations of piscine pathogenic *Streptococcus agalactiae*, serotype Ia ST7 and serotype III ST283, in Thailand and Vietnam. Genomics, S0888-7543(18)30485-3 *In Press.*

Kerdsin A., Hatrongjit R., Hamada S., & Akeda Y. (2017). Development of a multiplex PCR for identification of β-hemolytic streptococci relevant ti human infections and serotype distribution of invasive *Streptococcus agalactiae* in Thailand. Molecular and Cellular Probes, 36: 10-14.

Kim Y., Farrah S. & Baney R. H. (2007). Membrane damage of bacteria by silanols treatment. Electronic Journal of Biotechnology, 10(2).

Kong F., Ma L., & Gilbert L. (2005). Simultaneous detection and serotype identification of *Streptococcus agalactiae* using multiplex PCR and reverse line blot hybridization. Journal of Medical Microbiology, 54(Pt 12): 1133-1138.

Kong F., Giddig H. F., Berner R., & Gilbert J. L. (2006). *Streptococcus agalactiae* Cβ protein gen (bac) sequence types, based on the repeated region of the cell wall spanning domain: relationship to virulence and a proposed standardized nomenclature. Journal of Medical Microbiology, 55: 829-837.

Kovanen S. M., Kivisto R. I., Rossi M., Schott T., Karkkainen U. M., Tuuminen T., Uksila J., Rautelin L., & Hanninen M. L. (2014). Multilocus sequence typing (MLST) and whole-genome MLST of *Campylobacter jejuni* isolates from human infections in three districts during a seasonal peak in Finland. Journal of Clinical Microbiology, 52(12): 4147-4154.

Kusuda R., & Salati F. (1999). *Enterococcus seriolicida* and *Streptococcus iniae*. In Woo P. T. K. & Bruno D. W. (Eds.). Fish Diseases and Disorders: Viral, bacterial and Fungal Infections (pp. 303-317). London, United Kingdom: CABI Publishing.

© COPYRIGHT UPM

Laczeski M., Pegels E., Oviedo P., Quiroga M., & Vergara M. (2014). Molecular profiles and antimicrobial susceptibility of first isolates of *Streptococcus agalactiae* serotype IX in Argentina. Advances in Microbiology, 4: 474-483.

Lambertsen L., Ekelund K., Skovsted I. C., Liboriussen A., Slotved H. C. (2010) Characterization of invasive group B streptococci from adults in Denmark 1999 to 2004. European Journal of Clinical Microbiology and Infectious Diseases, 29:1071–1077.

Le C., Palanisamy N. K., Yusof M. Y. M., & Sekaran S. D. (2011). Capsular serotype and antibiotic resistance of *Streptococcus pneumonia* isolates in Malaysia. Plos One, 6(5): e19547.

Lehmann & Neumann. (1896). *Streptococcus agalactiae*. International Journal of Systematic and Evolutionary Microbiology, 30:367.

Li Y. W., Liu L., Huang P. R., Fang W., Luo Z. P., Peng H. L., Wang Y. X., & Li A. X. (2014). Chronic streptococcosis in Nile Tilapia, *Oreochromis niloticus* (L.), caused by *Streptococcus agalactiae.* Journal of Fish Diseases, 37: 757-763.

Li W., Wang H., He R., Li Y., Su Y., & Li A. (2016). Major surfome and secretome profile of *Streptococcus agalactiae* from Nile Tilapia (*Oreochromis niloticus*): Insight into vaccine development. Fish & Shellfish Immunology, 55: 737-746.

Lin F. P., Lan R., Sintchenko V., Gillbert G. L., Kong F., & Enrico C. (2011). Computational bacterial genome-wide analysis of phylogenetic profiles reveals potential virulence genes of *Streptococcus agalactiae*. Ploes One, 6(4): e17964.

Lindahl G., Stalhammar-Carlemaln M., & Areschoug T. (2005). Surdace protein of *Streptococcus aglactiae* and related proteins in other bacterial pathogens. Clinical Microbiology Review, 18(1): 102-127.

Liu G., Zhang W., & Lu C. (2013). Comparative genomics analusis of *Streptococcus agalactiae* reveals that isolates from cultured tilapia in China are closely related to human strain A909. Biomedic Central (BMC) Genomic, 14: 775.

Liu Y. M., Zhao J. Z., Li B. B., Yang J. Y., Dong X. G., & Zhang J. J. (2014). A report on the first outbreak of a single clone group A *Streptococcus* (emm-type 89) tonsillopharyngitis in China. Journal of Microbiology, Immunology and Infection, 47: 542–545.

Liu G., Zhu J., Chen K., Gao T., Yao H., Liu Y., Zhang W., & Lu C. (2016). Development of *Streptococcus agalactiae* vaccine for tilapia. Disease of Aquatic Organisms, 122: 163-170.

© COPYRIGHT UPM

Lowry K., Woodman A., Cook J., & Evans D. J. (2014). Recombination in enteroviruses is a biphasic replicative process involving the generation of greater-than genome length 'imprecise' intermediates. Plos One, 19(6): e1004191.

Lusiastuti A. M., Seeger H., Indrawati A., & Zschock M. (2013). The comparison of *Streptococcus agalactiae* isolated from fish and bovine using multilocus sequence typing. Journal of Biosciences, 20(4): 157-162.

Maeland J. A., Brakstad O. G., Bevanger L., & Krokstad S. (2000). Distribution and expression of bca, the gene encoding the C alpha protein, by *Streptococcus agalactiae*. Journal of Medical Microbiology, 49: 193-198.

Maiden M. C., Rensburg M. J., Bray J. E., Earle S. G., Ford S. A., Jolley K. A., & McCarthy N. D. (2013). MLST revisited: the gene-by-gene approach to bacterial genomics. Nature Reviews Microbiology, 11(10): 728-736.

Manning S. D., Lecher D. W., Davies H. D., Foxman B. & Whittam T. S. (2005). DNA polymorphism & molecular serotyping of capsular gene cluster of Group B *Streptococcus*. Journal of Clinical Microbiology, 43(12): 6113-6116.

Marin M., Garca-Lechuz J. M., Alonso P., Villanueva M., Alcala L., Gimeno M., Cercenado E., Sanchez-Somolinos M., Radice C., & Bouza E. (2011). Role of universal 16s rRNA gene PCR and sequencing in diagnosis of prosthetic joint infection. Journal of Clinical Microbiology, p 583-589.

Martins E. R., Melo-Cristino J., & Ramirez M. (2012). Dominance of serotype Ia among group B Streptococci causing invasive infections in nonpregnant adults in Portugal. Journal of Clinical Microbiology, 50: 1219–1227.

Melin M., Trzcinski K., Meri S., Kayhty H., & Vakevainen M. (2010). The capsular serotype of *Streptococcus pneumonia* is more important than the genetic background for resistance to complement. Infection and Immunity, 78(12): p 5262-5270.

Mereghetti L., Sitkiewicz I., Green N. M., & Musser J. M. (2008). Remodeling of the *Streptococcus agalactiae* transcriptome in response to growth temperature. PlosOne, 3(7): e2785.

Meron D., Efrony R., Johnson W. R., Schaefer A. L., Morris P. J., Rosenberg E., Greenberg E. P., & Banin E. (2009). Role of flagella in virulence of the coral pathogen *Vibrio coralliilyticus*. Journal of Applied Environmental Microbiology, 75(17): 5704-6707.

© COPYRIGHT UPM

Mian G. F., Godoy D. T., Leal C. A., Yuhara T. Y., Costa G. M., & Fiqueiredo H. C. (2009). Aspects of the natural history and virulence of *Streptococcus agalactiae* infection in Nile Tilapia. Journal of Veterinary Microbiology, 136(1-2): 180-183.

Milligan-Mhyre K., Charette J. R., Phennicie R. T.,Stephens W. Z., Rawls J. S., Guillemin K. & Kim C. H. (2001). Study of host-microbe interaction in Zebrafish. Methods of Cell Biology, 105: 87-116.

Miragaia M., Carrico J. A., Thomas J. C., Couto I., Enright M. C., Lencastre H. (2008). Comparison of molecular typing methods for characterization of S*taphylococcus epidermidis*: Proposal for clone definition. Journal of Clinical Microbiology,

Morozumi M., Chiba N., Igarashi Y., Mitsuhashi N., Wajima T., Iwata S., & Ubukata K. (2015). Direct identification of *Streptococcus agalactiae* and capsular type by rea-time PCR in vaginal swabs from pregnant women. Journal of Infection and Chemotherapy, 21: 34-38.

Musa N., Wei L. S., Hamdan R. H., Leong L. K., Wee W., Amal M. N., Kutty B. M., Abdullah S. Z. (2009). Streptococcosis in red hybrid tilapia (*Oreochromis niloticus*) commercial farms in Malaysia. Aquaculture Research, 40: 630–32.

Mustafa H. S. I. (2014). In Reiner K. (2012). Catalase test Protocol. *Staphylococcus aureus* can produce catalase enzyme when adding to human WBCs as a Source of H_2O_2 productions in human plasma or serum in the laboratory. Journal of Medical Microbiology, 4: 249-251.

Nagaoka K., Konno S., Murase K., Kikuchi T., & Nakagawa I. (2018). Complete genome sequence of *Streptococcus agalactiae* serotype III, multilocus sequence type 335 strain HU-GS5832, isolated from a human patient in Japan with severe invasive infection. Microbiology Resource Announcement, 7(20): e01303-18.

Ngunyen T. T. T., Ngunyen H. T., Tsai M., Byadgi O., Wang P., Yoshida T., & Chen S. (2017). Genetic diversity, virulence genes, and antimicrobial resistance of *Streptococcus dysgalactiae* isolates from different aquatic animal source. Aquaculture, 479: 256-264.

Nur-Nazifah M., Sabri M. Y., & Siti-Zahrah A. (2014). Development and efficacy of feed-based recombinant vaccine encoding the cell wall surface anchor family protein of *Streptococcus agalactiae* against streptococcosis in *Oreochromis* sp. Fish & Shellfish Immunology, 37: 193-200.

Oviedo P., Pegels E., Laczeski M., Quirogam & Vergara M. (2013). Phenotypic and genotypic characterization of *Streptococcus agalactiae* in pregnant women: first study in a province of Argentina. Brazillian Journal of Microbiology, 44(1).

© COPYRIGHT UPM

Patterson M. J. (1996). *Streptococcus*. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston, Chapter 13.

Pereira U. P., Mian G. F., Oliveira I. C., Benchetrit L. C., Costa G. M., & Figueiredo H. C. (2010). Genotyping of *Streptococcus agalactiae* strains isolated from fish, human and cattle and their virulence potential in Nile tilapia. Veterinary Microbiology, 140(1-2): 186–192.

Perez-Losada M., Arenas M., & Castro-Lanar E. (2018). Microbial sequence typing in genomic era. Infection, Genetics and Evolution, 63: 346-359.

Pett C. A., Polage C. R., & Schreckenberger P. (2005). The role of 16S rRNA gene sequencing in identification of microorganisms misidentified by conventional methods. Journal of Clinical Microbiology, 43(12): 6123-6125.

Pradeep P. J., Suebsing R., Sirthammajak S., Kampeera J., Jitrakorn S., Saksmerprome V., Turner W., Palang I., Vanichviriyakit R., Senapin S., Jeffes A., Kiatpathomchai W., & Withyachumanarnkul B. (2016). Evidence of vertical transmission and tissue tropism of streptococcosis from naturally infected red tilapia (*Oreochromis* spp.). Aquaculture reports, 3: 58-66.

Pretto-Giordano L. G., Muller E. E., de Freitas J. C., & da Silva V. G. (2010). Evaluation on the pathogenesis of *Streptococcus agalatiae* in Nile Tilapia (*Oreochromis niloticus*). Brazilian Archives of Biology and Technology, 53(1): 87-92.

PubMLST Database: *Streptococcus agalactiae* (Group B Streptococcus). (2019, May 1). Retrieved May 15, 2019, from https://pubmlst.org/sagalactiae/.

Ramakrishnan M. A. (2016). Determination of 50% endpoint titer using a simple formula. World Journal of Virology, 5(2): 85-86.

Reiner K. (2010). Catalase test protocol. American Society for Microbiology, 2016.

Robinson J. A., & Ashburner M. (1966). Taking stock of our models: The function and future of stock centres. National Review Genetics, 3: 711-717.

Rodkhum C., Kayansamruaj P., & Pirarat N. (2011). Effect of water temperature on susceptibility to *Streptococcus agalactiae* serotype Ia infection in Nile Tilapia (*Oreochromis niloticus*). Journal of Veterinary Medicine, 41(3): 309-314.

© COPYRIGHT UPM

Rosa-Fraile M., Dramsi S., & Spellerberg B. (2014). Group B streptococcal haemolysin and pigment, a tale of twins. FEMS Microbiology Reviews, 38(5): 932-946.

Rosenfold Y., & Shai Y. (2006). Lipopolysaccharide (Endotoxin)-host defense antibacterial peptides interactions: role in bacterial resistance and prevention of sepsis. Biochimica et Biophysica Acta, 1758(9): 1513-1522.

Sadowy E., Matynia B., Hryniewicz W. (2010). Population structure, virulence factors and resistance determinants if invasive, non-invasive and coloning *Streptococcus agalactiae* in Poland. Journal of Antimicrobial Chemotherapy, 65: 1907-1914.

Salloum M., Mee-Marquet N., Bomelier A. S., Arnault L., & Quentin R. (2010). Molecular characterization and prophage DNA contents of *Streptococcus agalactiae* strains isolated from adult skin and osteoarticular infections. Journal of Clinical Microbiology, 48(4): 1261-1269.

Schubert A., Zakikhany K., Pietrocola G., Meinke A., Speziale P., Eikmanns B. J., & Reinscheid D. J. (2004). The fibrinogen receptor FbsA promotes adherence of *Streptococcus agalactiae* to human epithelial cells. Infection and Immunity, 72(11): 6197–6205.

Sebastiao F. A., Lemos E. G. M., & Pilarski F. (2015). Validation of absolute quantitative real-time PCR for the diagnosis of *Streptococcus agalactiae* in fish. Journal of Microbiological Methods, 119: 168-175.

Shabayek S. & Spellerberg B. (2017). Acid stress response mechanism of Group B Streptococci. Frontiers in Cellular and Infection Microbiology, 7: 359.

Shield P., & Cathcart L. (2010). Oxidase test protocol. American Society for Microbiology.

Shoemaker C. A., Klesius P. H., & Evans J. J. (2001). Prevalence of *Streptococcus iniae* in tilapia, hybrid striped bass, and channel catfish on commercial fish farms in the United States. American Journal of Veterinary Research, 62(2): 174-177.

Soto E., Zayas M., Tobar J., Illanes O., Yount S., Francis S., & Dennis M. M. (2016). Laboratory-controlled challenges of nile tilapia (*Oreochromis niloticus*) with *Streptococcus agalactiae*: Comparison between immersion, oral, intracoelomic and intramuscular routes of infection. Journal of Comparative Pathology, 155: 339-345.

Steimle A., Autenrieth I. B., & Frick J. S. (2006). Structure and function: Lipid A modifications in commensals and pathogens. Journal of International Medical Microbiology, 306(5): 290-301.

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Stenutz R., Weintraub A., & Widmalm G. (2006). The structures of Escherichia coli O-polysaccharide antigens. FEMS Microbiology Reviews, 30(3): 382-403.

Suanyuk N., Kangheae H., Khongpradit R., & Supamattaya K. (2008). *Streptococcus agalactiae* infection in tilapia (*Oreochromis niloticus*) Songklanakarin. Journal of Science and Technology, 27:307–319.

Suhaimi M. E. S., Desa M. N. M., Eskandarian N., Pillay S. G., Ismail Z., Neela V. K., Masri S. N., & Nordin S. A. (2017). Characterization of Group B *Streptococcus* infection based on the demographic, serotype, antimicrobial susceptibility and genotypes of selected isolates from sterile and non-sterile isolation sites in three major hospitals in Malaysia. Journal of Infection and Public Health, 10: 14-21.

Sun Y., Kong F., Zhao Z., & Gilbert G. L. (2005). Comparison of a 3-set genotyping system with multilocus sequence typing for *Streptococcus agalactiae* (Group B *Streptococcus*). Journal of Clinical Microbiology, 43(9): 4704-4707.Tien N., Ho C., Lin H., Shih M., Ho M., Lin H. C., Lin H. S., Chang C., & Lu J. (2011). Multilocus sequence typing of invasive Group B *Streptococcus* in central area of Taiwan. Journal of Microbiology, Immunology and Infection, 44: 430-434.

Suwannasang A., Dangwetngam M., Issaro A., Phromkunthong W., & Suanyuk N. (2014). Pathological manifestations and immune responses of serotypes Ia and III *Streptococcus agalactiae* infections in Nile tilapia (*Oreochromis niloticus*). Songklanakarin Journal of Sciens and Thechnology, 36(5): 499-506.

Thairu Y., Nasir I. A., & Usman Y. (2014). Laboratory perspective of gram staining and its significance in investigations of infectious diseases. Sub-Saharan African Journal of Medicine, 1(4): 168-174.

Thong K., Ling G. Y., Kong L. W., Theam L. C., & Ngeow Y. F. (2004). Macrorestriction analysis of *Streptococcus agalactiae* (group B *Streptococcus*) isolates from Malaysia. Journal of Madical Microbiology, 53: 991-997.

Tien N., Ho C. M., Lin H. J., Shih M. C., Ho M. W., Lin H. C., Chang C. C. & Lu J. J. (2011). Multilocus sequence typing of invasive group B *Streptococcus* in central area of Taiwan. Journal of Microbiol, Immunology and Infection, 44(6): 430-434.

Toranzo A. E., Magarinos B., & Romalde J. L. (2005). A review of the main bacterial fish diseases in mariculture systems. Aquaculture, 246: 37-61.

© COPYRIGHT UPM

Tsai M. A., Wang P. C., Yoshida T., & Chen S. C. (2015). Genetic characteristics of *Streptococcus dysgalactiae* isolated from cage cultured cobia, *Rachycentron canadum* (L.). Journal of Fish Disease, 38: 1037-1046.

Tsai Y. H., Chen P. H., Yu P. A., Chen C. L., Kuo L. T., & Huang K. C. (2019). A multiplex PCR assay from detection of *Vibrio vulnificus, Aeromonas hydrophila,* methicillin resistant *Staphylococcus aureus, Streptococcus pyogenes,* and *Streptococcus agalactiae* from the isolates of patients with necrotizing fasciitis. International Journal of Infectious Diseases, 81:73-80.

Urwin R., & Maiden M. C. J. (2003). Multi-locus sequence typing: A tool for global epidemiology. TRENDS in Microbiology, 11(10): 479.

Van-Sorge N. M., Quach D., Gurney M. A., Sullam P. M., Nizet V., & Doran K. S. (2009). The group B streptococcal serine-rich repeat 1 glycoprotein mediates penetration of blood-brain barrier. Journal of Infectious Diseases, 199(10): 1479-1487.

Verner-Jeffreys D. W., Baker-Austin C., Pond M. J., Rimmer G. S. E., Kerr R., Stone D., Griffin R., White P., STinton N., Denham K., Leigh J., Jones N., Longshaw M., & Feist S. W. (2012). Zoonotic disease pathogens in fish used for pedicure. Emergence of Infectious Diseases, 18(6): 1006-1008.

Versalovic J. & Lupski J. R. (2002). Molecular detection and genotyping of pathogens: more accurate and rapid answers (review). Trends in Microbiology, 10(10).

Wang K., Chen D., Huang L., Lian H., Wang J., Xiao D., Geng Y., Yang Z. & Lai W. (2013). Isolation and characterization of *Streptococcus agalactiae* from Nile Tilapia *Oreochromis Niloticus* in China. African Journal of Microbiology Research, 7(4): 317-323.

Wang T., Birsoy K., Hughes N. W., Krupczak K. M., Post Y., Wei J. J., Lander E. S., & Sabatini D. M. (2015). Identification and characterization of essential genes in the human genome. Science, 350(6264): 1096-10101.

Wang, Y. H., Lu, C. C., Chiu, C. H., Wang, M. H., Yang, T. H., & Chu, C. (2016). Genetically diverse serotypes III and VI substitute major clonal disseminated serotypes Ib and V as prevalent serotypes of *Streptococcus agalactiae* from 2007 to 2012. Journal of Microbiology, Immunology and Infection, 49: 672–678.

Wessels M. R. (1997). Biology of streptococcal capsular polysaccharides. Journal of Applied Microbiology Symposium Supplement, 83: 20S-31S.

© COPYRIGHT UPM

Willis L. M. & Whitfield C. (2013). Structure, biosynthesis, and function of bacterial capsular polysaccharides synthesized by ABC transporter-dependant pathways. Carbohydrate Research, 378: 35-44.

Yang Y., Liu Y., Ding Y., Yi L., Ma Z., Fan H., & Lu C. (2013). Molecular characterization of *Streptococcus agalactiae* isolated from bovine mastitis in Eastern China. PlosOne, 8(7): e67755.

Yanong R. P. E., Francis-Floyd R. (2010). Streptococcal infections of fish, University of Florida, Florida Cooperative Extension Service, 57: 1–6. Available at: http://edis.ifas.ufl.edu/fa057.

Yao K., Poulsen K., Maione D., Rinaudo D., Baldassarri L., Telford J. L., Skov-Sorensen U. B., Members of DEVANI study group, & Kilian M. (2013). Capsular gene typing of *Streptococcus agalactiae* compared to serotyping by latex agglutination. Journal of Clinical Microbiology, p 503-507.

Ye R., Selby C. P., Ozturk N., Annayev Y., & Sancar A. (2011). Biochemical analysis of the canonical model for the mammalian circadian clock. The Journal of Biological Chemistry, 286(29): 25891-25902.

Yildirim I. H., Yildirim S. C., & Kocal N. (2011). Molecular methods for bacterial genotyping and analyzed gene regions (review). Journal of Microbiology and Infectious Diseases, 1(1): 42-46.

Zamri-Saad M., Amal M. N. A., & Siti-Zahrah. (2010). Pathological changes in red hybrid tilapias (*Oreochromis* spp.) Naturally infected by *Streptococcus agalactiae*. Journal of Comparative Pathology, 143: 227-229.

Zamri-Saad M., Amal M. N. A., Siti-Zahrah A., & Zulkafli A. R. (2014). Control and prevention of Streptococcosis in cultured tilapia in Malaysia: A review. Pertanika Journal of Tropical Agricultural Science, 37(4): 389-410.

Zhang D., Li A., Guo Y., Zhang Q., Chen X., & Gong X. (2013). Molecular characterization of *Streptococcus agalactiae* in diseased farmed tilapia in China. Aquaculture, 412-13: 64-69.

Zhang Z., Yu A., Lan J., Zhang H., Hu M., Cheng J., Zhao L., Lin L., & Wei S. (2017). GapA, a potential vaccine candidate antigen against *Streptococcus agalactiae* in Nile Tilapia (*Oreochromis niloticus*). Fish & Shellfish Immunology, 63: 255-260.

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