

Prevalence and characteristics of *Streptococcus agalactiae* (Group B Streptococcus) isolated from freshwater fish and porcine in Hong Kong wet markets

Dulmini Nanayakkara Sapugahawatte, Carmen Li, Priyanga Dharmaratne, Chendi Zhu, Yun Kit Yeoh, Jun Yang, Norman Wai-Sing Lo, Kam-Tak Wong, Margaret Ip*

Department of Microbiology, Faculty of Medicine, Prince of Wales Hospital, The Chinese University of Hong Kong, Sha Tin, Hong Kong (SAR)



Introduction

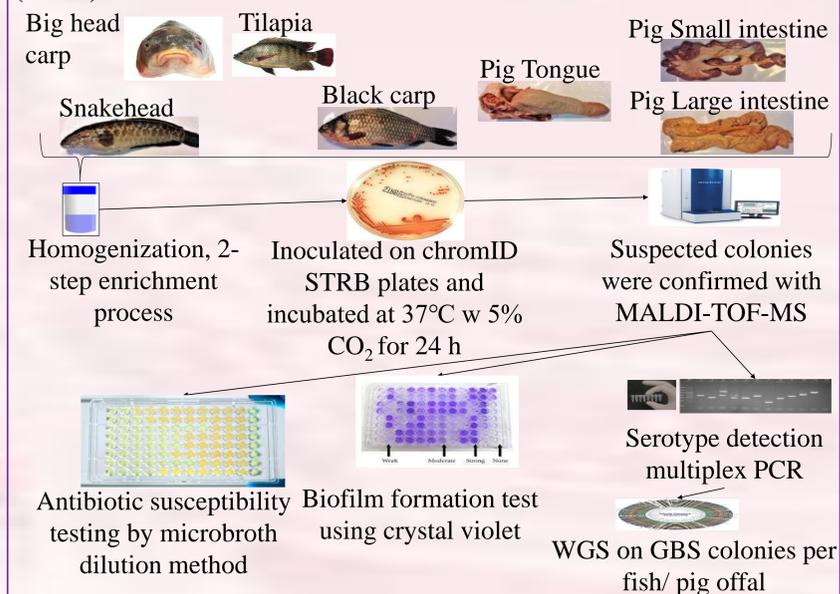
Group B Streptococcus (*Streptococcus agalactiae*, GBS) is a pathogen with diverse host range from domestic and wild animals to humans. In light of the reported foodborne outbreak in Singapore in 2015, which indicated a zoonotic potential of GBS, we aimed to identify and characterize GBS from farmed freshwater fish and pig offal procured from Hong Kong wet markets.

Objectives

Our study sought to isolate and characterize GBS carriage from freshwater fish, and pig organs procured from wet markets across Hong Kong during the period of mid-2016 to 2019 by phenotypic and molecular methods.

Methods

Period: 2016-2019
 Freshwater Fish: 992 (namely 182 tilapia, 314 snakehead fish, 309 black carp and 187 big head carp heads)
 Pig offals: 361 (namely the small and large intestines and tongues)
 GBS strains isolated: 252
 Fish tissue (namely the skin, flesh, gills, heart, spleen, liver and gut) were dissected and homogenized, followed by a 2-step enrichment process before isolating GBS using selective media. Isolates were tested for antimicrobial susceptibilities, biofilm formation, and underwent capsular serotyping and whole-genome sequencing (WGS).



Results

The isolation rates of fish GBS and pig GBS were 19.3% (191 strains) and 16.8% (61 strains), respectively (Table 1). Isolation of GBS from pig was mostly from the tongues (24.8%), followed by the large intestine (13%) and small intestine (3.2%). Isolation rate in fish were most frequent in tilapia (34.1%), followed by black carp (22.3%), snakehead fish (13.6%) and big head carp heads (10.1%). Moderate biofilm formation ability was seen in over 71% of all fish and pig GBS isolates (Table 2). Multidrug resistance was observed in 2 fish GBS strains and 91.8% (56/61) of pig GBS. Molecular characterization revealed that fish GBS was predominantly serotype Ia, ST7 (92.7%, 177/191 of overall fish) while pig GBS was serotype III ST651 (74.8%) (Table 3). Antibiotic susceptibility test revealed most of the fish GBS were sensitive to antibiotics. Seven of the strains showed resistance to at least three classes of antibiotics (Table 3). In contrast, 55 of pig GBS strains were multi-drug resistant, showing resistance to tetracyclines, erythromycin and clindamycin.

Table 1. Prevalence of GBS in freshwater fish and pig offals from Hong Kong wet markets.

Food source (n)	Number	GBS prevalence %, (n)
Freshwater fish	992	19.3% (191)
Tilapia	182	34.1% (62)
Big Head carp heads	187	10.1% (19)
Snakehead fish	314	13.6% (43)
Black carp	309	22.3% (67)
Pig offal	361	16.9% (61)
Tongue	192	24.8% (48)
Small Intestine	92	3.2% (3)
Large Intestine	76	13% (10)

Table 2. Biofilm production ability of fish and pig GBS.

Capacity of biofilm production	Mean of OD _{630nm} value	Fish GBS (n=191) % (n)	Pig GBS (n=61) % (n)	Total (n=252) % (n)
High	> 0.240	0	1.6% (1)	0.4% (1)
Moderate	0.120 – 0.240	71.7% (137)	98.4% (60)	78.2% (197)
Weak/ none	< 0.120	28.3% (54)	0	21.4% (54)

Table 3. Minimum inhibition concentrations (MICs) of GBS against 11 antibiotics.

Food type	No.	Serotypes % (n)				
		Ia	III-2	III-NT	V	NT
Freshwater fish	191	96.3% (184)	-	0.5% (1)	1.04% (2)	2.09% (4)
Pig offals	61	-	3.27% (2)	91.8% (56)	-	4.91% (3)
Total GBS strains collected	252	73% (184)	0.8% (2)	22.6% (57)	0.8% (2)	2.8% (7)

Table 3. Minimum inhibition concentrations (MICs) of GBS against 11 antibiotics.

Class of antibiotic	Antibiotic	Freshwater fish GBS		Pig GBS	
		MIC ₉₀ (mg/L) ^a	Resistance (%,(n))	MIC ₉₀ (mg/L) ^a	Resistance (%,(n))
Penicillins	Penicillin	0.06	0.5 (1)	0.03	0 (0)
Glycopeptides	Vancomycin	0.5	0 (0)	0.25	0 (0)
Aminoglycosides	Gentamicin	>32	98.9 (189)	16	95.1 (58)
	Doxycycline	2	6.2 (12)	16	90.1 (55)
Tetracyclines	Minocycline	1	5.7 (11)	16	90.1 (55)
	Tetracycline	1	6.2 (12)	16	90.1 (55)
Oxazolidonones	Linezolid	2	0 (0)	2	0 (0)
Macrolides	Erythromycin	≤0.12	3.1 (6)	>16	88.5 (54)
Lincosamides	Clindamycin	≤0.12	1.5 (3)	>16	98.3 (60)
Fluoroquinolones	Ciprofloxacin [^]	1	0.5 (1)	0.5	0 (0)
	Levofloxacin	0.5	0 (0)	0.5	0 (0)

MIC breakpoints for GBS were referenced to CLSI [Wayne P.A. 2017]. Breakpoints for defining sensitive strains (in mg/L) of the following antibiotics are in parentheses: CIP ciprofloxacin (≤ 1 mg/L); LEV, levofloxacin (≤ 2 mg/L); GEN, gentamicin (≤ 1 mg/L); TET, tetracycline (≤ 2 mg/L); MIN, minocycline (≤ 2 mg/L); DOX, doxycycline (≤ 2 mg/L); PEN, penicillin (≤ 0.12 mg/L); CLI, clindamycin (≤ 0.25 mg/L); LNZ, Linezolid (≤ 2 mg/L); VAN, vancomycin (≤ 1 mg/L); ERY, erythromycin (≤ 0.25 mg/L). [^]CLSI breakpoint for *Enterococcus* spp was used.

Whole genome sequencing revealed ST7 was dominant in serotype Ia of fish GBS (n=177) while the remaining strains were ST103 (CC103), ST314 and ST931. The serotype V strains were also ST931. Antimicrobial genes were scarce in fish GBS and only the minor ST groups carried genes conferring to tetracyclines and aminoglycoside resistance.

Serotype III pig GBS strains were mainly ST651 (n=45) (CC103) while the remaining strains were ST862 (CC485). Pig GBS genomes contained an average of 7 antibiotic resistance genes, conferring resistance to aminoglycoside, macrolide, phenicol and tetracyclines.

Discussion

- First comprehensive study on GBS carriage and characterization in food animals in locality
- Predominance of serotype Ia was found in freshwater fish
- Serotype Ia was reported more pathogenic in fish than serotype III where meningitis and septicaemia were often observed.
- ST7 strains were absence of antibiotic resistance genes from our WGS study.
- GBS in pigs has been seldom reported
- Serotype III, ST651 and ST862 was found in our pig GBS strains.
- ST862 was the next prevalent ST in GBS positive pregnant women in southern China.
- Multidrug resistance was observed in pig GBS
- WGS also showed multiple antibiotic resistance genes in their genomes
- This raises alarm in the potential of antibiotic resistance in human in One Health perspective and the source of antimicrobial resistance acquisition.

Conclusion

This is the first comprehensive study of food surveillance on *Streptococcus agalactiae* in Hong Kong. Serotype Ia, ST7 and serotype III ST651 were the prevalent strains in fish and pig offals respectively. Antimicrobial resistance was observed more in pigs than fish. The prevalence rate of GBS in food animals and the multi-drug resistance phenotype presented in the strains, especially pig GBS, raise concerns on the zoonotic potential to humans.

Acknowledgement

This project is supported by the Food and Health Bureau, Government of Hong Kong Special Administration Region and Health and Medical Research Fund (HMRF) grant number 17160212 (PI:MI). We thank the summer students Miss Victoria Chu, Miss Nicole Lau, Miss Ingrid Chan, Miss Rachel Lau and Miss Noel Leung for their assistance and participation in this project.

Reference

1. Isolating Group B Streptococcus from a food (fish) sample [Internet]. [cited Dec 25, 2020]. Available from: <https://10minus6cosm.tumblr.com/post/135763399281/isolating-group-b-streptococcus-from-a-food-sample>.
2. Imperi M, Pataracchia M, Alfarone G, Baldassarri L, Orefici G, Creti R. A multiplex PCR assay for the direct identification of the capsular type (Ia to IX) of *Streptococcus agalactiae*. J Microbiol Methods. 2010 Feb;80(2):212-4.
3. Li C, Sapugahawatte DN, Yang Y, Wong KT, Lo NWS, Ip M. Multidrug-Resistant *Streptococcus agalactiae* Strains Found in Human and Fish with High Penicillin and Cefotaxime Non-Susceptibilities. Microorganisms. 2020 July 16;8(7):10.3390/microorganisms8071055.
4. Wayne PA. Performance Standards for Antimicrobial Susceptibility Testing 27th ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2017.