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Serological and molecular typing of *Tenacibaculum maritimum* from New Zealand farmed salmon, *Oncorhynchus tshawytscha*

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ABSTRACT

Tenacibaculum maritimum is a cosmopolitan bacterial pathogen with the potential to cause significant losses in a broad range of farmed and wild marine fish species. This study investigated the antigenic diversity of *T. maritimum* isolated in culture from farmed Chinook (king) salmon (*Oncorhynchus tshawytscha*) from the Marlborough Sounds in New Zealand. A total of 36 isolates were examined using antibody serotyping and rapid molecular serotyping via multiplex PCR (mPCR) targeting genes encoding O-antigen biosynthesis enzymes. Serological analysis using three different polyclonal antisera developed against *T. maritimum* isolated from farmed Atlantic salmon (*Salmo salar*) in Tasmania, Australia revealed that there are three putative serotypes of *T. maritimum* that occur in New Zealand. The predominant serotype was defined by a positive reaction to all three Tasmanian antisera (antisera A, B and C), designated as serotype ABC. This serotype was found at all nine farm locations tested and represented 81% of all isolates examined. The same library of isolates was evaluated by mPCR serotyping and found three O-AGC types among tested isolates. O-AGC Type 3 was not only the predominant type (72%) present, but it also had a wide distribution, having been isolated at eight of the nine farms. Two other O-AGC types (O-AGC Type 2–1 and O-AGC Type 3–2) were identified, providing evidence of genetic variation. However, there was only partial concordance between the two serotyping techniques, which is likely linked to differences in the way serotypes are defined in the two approaches that were used. Nevertheless, in broad terms there is good evidence of intraspecific antigenic variation within our library of isolates, and collectively these data will be of crucial importance for assessing the pathogenicity of the isolates and the subsequent development of a vaccine for this emerging disease in New Zealand marine salmon farms.

1. Introduction

Genus *Tenacibaculum* from the family Flavobacteriaceae contains Gram-negative, filamentous-rod bacteria, which are present predominantly in the marine environment (Suzuki et al., 2001) and include species pathogenic to many marine fish species (Avendaño-Herrera et al., 2006; Fernández-Álvarez and Santos, 2018). Of the bacterial pathogens belonging to the *Tenacibaculum* genus, *Tenacibaculum maritimum* (formerly known as *Flexibacter maritimus*) is frequently isolated from diseased fish globally and has been the subject of considerable research since the late 1970s (Bridel et al., 2020; Hikida et al., 1979; Lagadec et al., 2021), with several complete and draft genome sequences now available (Pérez-Pascual et al., 2017; Lopez et al., 2022a). *T.maritimum* is one of the eight pathogenic species in the genus *Tenacibaculum* responsible for causing tenacibaculosis and is of considerable economic significance in marine aquaculture (Avendaño-Herrera et al., 2006; Fernández-Álvarez and Santos, 2018; Nowlan et al., 2020). Tenacibaculosis has been identified in >30 marine fish species, which is indicative of the lack of strict fish-host specificity of *T. maritimum* (Table 1).

In New Zealand, tenacibaculosis is a priority emerging disease in

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Table 1
Susceptible marine fishes to *Tenacibaculum maritimum* and known antigenic groups in each country.

Susceptible marine fish species	Country	Source and key references	Known serotypes	Known O-AGC type
Atlantic salmon *Salmo salar*	Australia	Farmed (Handlinger et al., 1997)	O3 (Lopez et al., 2022a) A, B, C (A. Angelucci, pers. comm.)	2–1, 3–0, 3–1, 3–2 (Lopez et al., 2022a)
	Canada	Farmed (Ostland et al., 1999; Frisch et al., 2018; Bateman et al., 2022)	ND	ND
	Chile	Farmed (Apablaza et al., 2017)	ND	ND
	Spain	Farmed (Pazos et al., 1993; Avendaño-Herrera et al., 2004)	O4 (Lopez et al., 2022a)	4–0 (Lopez et al., 2022a)
	Scotland	Farmed (Ferguson et al., 2009)	ND	ND
	Ireland	Farmed (Downes et al., 2018)	ND	ND
Chinook salmon *Oncorhynchus tshawytscha*	New Zealand	Farmed (Kumanan et al., 2022; Brosnahan et al., 2019; Johnston et al., 2020)	AB, B, ABC (This study)	2–1, 3–0, 3–2 (This study)
	USA	Farmed & wild (Chen et al., 1995)	ND	ND
	Canada	Wild (Bass et al., 2022)	ND	ND
Coho salmon *Oncorhynchus kisutch*	Canada	Wild (Bass et al., 2022)	ND	ND
Rainbow trout *Oncorhynchus mykiss*	Australia	Farmed (Handlinger et al., 1997)	ND	2–1, 3–0, 4–0 (Lopez et al., 2022a)
	Chile	Farmed (Valdes et al., 2021)	ND	ND
Sockeye salmon *Oncorhynchus nerka*	Canada	Wild (Bateman et al., 2022; D.F·O, 2020; Pacific Salmon Foundation, 2022)	ND	ND
Barramundi *Lates calcarifer*	Singapore	Farmed (Rafidah et al., 2015)	ND	ND
	Vietnam	Farmed (Dong et al., 2017)	ND	ND
Black bream *Acanthopagrus butcheri*	Australia	Wild (Handlinger et al., 1997)	ND	ND
Black sea bream *Acanthopagrus schlegeli*	Japan	Farmed (Masumura and Wakabayashi, 1977; Rahman et al., 2015)	ND	1–0 (Lopez et al., 2022a)
Black damselfish *Neoglyphieodon melas*	Egypt	Captive (Haridy et al., 2015)	ND	ND
Dover Sole *Solea solea*	Scotland	Farmed (McVicar and White, 1982)	O2 (Lopez et al., 2022a)	2–1 (Lopez et al., 2022a)
Greenback flounder *Rhombosolea tapirina*	Australia	Wild (Handlinger et al., 1997)	ND	ND
Gurnard *Chelidonichthys lucernus*	Italy	Farmed (Magi et al., 2007)	O3 (Magi et al., 2007)	ND
Gilthead seabream *Sparus aurata*	Greece	Farmed (Kolygas et al., 2012)	ND	ND
	Spain	Farmed (Avendaño-Herrera et al., 2004)	O1 (Avendano-Herrera et al., 2005b)	1–0, 1–1 (Lopez et al., 2022a)
Japanese flounder *Paralichthys olivaceus*	Japan	Farmed (Rahman et al., 2015; Baxa et al., 1986)	ND	3–0 (Lopez et al., 2022a)
	Korea	Farmed (Jang et al., 2009)	ND	2–1 (Lopez et al., 2022a)
Lumpsucker *Cyclopterus lumpus*	Norway	Farmed (Småge et al., 2016)	ND	ND
Northern anchovy *Engraulis mordax*	USA	Wild (Chen et al., 1995)	ND	ND
Orbicular batfish *Platax orbicularis*	French Polynesia	Farmed (Lopez et al., 2022b; Alix et al., 2020)	O1, O3 (Lopez et al., 2022a)	1–0, 3–0, 3–1 (Lopez et al., 2022a)
Pacific sardine *Sardinops sagax*	USA	Wild (Chen et al., 1995)	ND	ND
Picasso triggerfish *Rhinecanthus assasi*	Egypt	Captive (Haridy et al., 2015)	ND	ND
Puffer fish *Takifugu rubripes*	Japan	Not stated (Rahman et al., 2014)	NUF1081 (Rahman et al., 2014)	ND
Red sea bream *Pagrus major*	Japan	Farmed (Masumura and Wakabayashi, 1977; Rahman et al., 2015)	O1, O2, O1/O2 (Lopez et al., 2022a)	1–0 (Lopez et al., 2022a)
Rock bream *Oplegnathus fasciatus*	Japan	Farmed (Wakabayashi et al., 1986)	ND	ND
Sand tiger shark *Carcharias taurus*	Italy	Captive-bred (Florio et al., 2016)	ND	ND
Seabass *Dicentrarchus labrax*	Greece	Farmed (Kolygas et al., 2012)	ND	ND
	France	Farmed (Bernardet et al., 1994)	O1, O3, O2 (Lopez et al., 2022a; Avendano-Herrera et al., 2005a)	1–0, 3–1, 2–1 (Lopez et al., 2022a)
	Malta	Farmed (Yardımcı and Timur, 2015)	O1 (Lopez et al., 2022a)	1–1, 2–1, 3–0 (Lopez et al., 2022a)
	Turkey	Farmed (Yardımcı and Timur, 2015)	O1 (Yardımcı and Timur, 2015)	ND
Sole *Solea senegalensis*	Spain	Farmed (Cepeda and Santos, 2002; Piñeiro-Vidal et al., 2007; Vilar et al., 2012)	O1, O3 (Avendano-Herrera et al., 2005b)	ND
	Portugal	Farmed (Mabrok et al., 2016)	O3 (Mabrok et al., 2016)	ND
Striped trumpeter *Latris lineata*	Australia	Wild (Handlinger et al., 1997)	ND	ND
Turbot *Scophthalmus maximus*	Spain	Farmed (Alsina and Blanch, 1993; Devesa et al., 1989; Pazos et al., 1993; Piñeiro-Vidal et al., 2007)	O2 (Avendano-Herrera et al., 2005b)	2–1, 3–0, 4–0 (Lopez et al., 2022a)
	Italy	Wild (Magi et al., 2007)	O3 (Magi et al., 2007)	ND

(*continued on next page*)

farmed marine Chinook (king) salmon (*Oncorhnychus tshawytscha*) (see Kumanan et al., 2022). Globally, New Zealand is the largest aquaculture producer of Chinook salmon for a niche market, contributing >70% of worldwide Chinook salmon production (New Zealand Salmon Farmers Association, 2022; Araujo et al., 2021). Over 85% of the production of this anadromous species is in sea-pens located in the South Island of New Zealand. Since 2012, salmon mortalities in sea-pens located in the Marlborough Sounds have been associated with gross pathology of tenacibaculosis (Brosnahan et al., 2019; Norman et al., 2013; Ministry for Primary Industry, 2020; Kumanan et al., 2022). The disease is prevalent during the summer months when the seawater temperature exceeds 16 °C. Affected Chinook salmon exhibit varying degrees of epidermal damage, including ulcerative epidermal lesions, skin spots, tail fin erosion and gill erosion (Johnston et al., 2020). This clinical pathology in New Zealand Chinook salmon is consistent with typical tenacibaculosis, which is characterized by a wide range of lesion severity from small areas of scale loss to ulcerative skin conditions exposing skeletal muscles (Frisch et al., 2018). Targeted surveillance using optimized diagnostic techniques has revealed that *T. maritimum* and other *Tenacibaculum* spp. are readily isolated from lesions of moribund fish (Kumanan et al., 2022). Significantly, there is an increasing number of reports of marine fish species susceptible to tenacibaculosis and a paucity of serological information (Table 1). These factors alongside the emergence of tenacibaculosis in New Zeland farmed Chinook salmon (Brosnahan et al., 2019; Kumanan et al., 2022) indicate a growing need to identify *T. maritimum* serotypes to guide appropriate disease prevention in New Zealand.

In many countries, the use of antimicrobials has been the primary treatment option to manage tenacibaculosis (Nowlan et al., 2021; Irgang et al., 2021). However, in line with the New Zealand Veterinary Association's goal for primary production to be antibiotic free by 2030 (New Zealand Veterinary Association, 2019), the New Zealand salmon industry do not use antibiotics (New Zealand King Salmon, 2016, High Country Salmon, 2022, Sanford, 2022). There is concern that extensive use of antimicrobials in aquaculture may increase the prevalence of antimicrobial resistance (AMR) and thus reduce future treatment options and increasing the probability of AMR transfer to human pathogens (Zhao et al., 2021; Barnes et al., 2022; Preena et al., 2020; Reverter et al., 2020; Bondad-Reantaso et al., 2023). Alternatively, vaccination is an efficient disease prevention method that can be used to tackle AMR in aquaculture (Bondad-Reantaso et al., 2023). At present, there are no registered fish vaccines available in New Zealand. Internationally, there is a single commercially available vaccine for prevention of *T. maritimum*: ICTHIOVAC®-TM (serotype O2) vaccine for turbot, *Scophthalmus maximus,* which is reported to provide short-term immunity for six months (ICTHIOVAC® TM-Hipra Laboratories; Miccoli et al., 2019).

Successful vaccination relies on the diligent selection of a strain(s) or its antigen(s) (Barnes et al., 2022). A significant challenge in developing an effective vaccine against *T. maritimum* is the antigenic variability present on the lipopolysaccharide (LPS) constituent of the outer membrane of the pathogen (Van Gelderen et al., 2010; Avendaño-Herrera et al., 2004; Yardimci and Timur, 2016; Fernández-Álvarez and Santos, 2018). LPS is an essential outer membrane constituent of Gram-negative bacteria (Whitfield et al., 2020). The highly variable repeating oligosaccharide units present in the immunogenic O-polysaccharide (O-antigen) region of LPS results in the antigenically diverse O-specific serotypes (Whitfield and Trent, 2014). Antigenic studies have revealed the presence of at least four O-serotypes (O1, O2, O3 and O4) within the species (Lopez et al., 2022a). Hence, a vaccine formulated against a single strain of *T. maritimum* may not generate immunity against strains with different O-antigen composition (Romalde et al., 2005). An effective vaccine should provide protection against a broad range of *T. maritimum* strains that are present within major production regions (Tinsley et al., 2011; Romalde et al., 2005; Barnes et al., 2022). This would require a formulation of a multi-valent vaccine or a vaccine targeting selected serotypes (incorporating the most virulent strains) to efficiently control the disease and prevent outbreaks (Barnes et al., 2022). Multi-valent vaccines have successfully provided broad spectrum protection against other antigenically heterogenous fish pathogens such as *Yersinia ruckeri* (see Tinsley et al., 2011), *Aeromonas hydrophila* (see Shirajum Monir et al., 2020) and *Flavobacterium psychrophilum* (see Hoare et al., 2017). Therefore, investigating the diversity of *T. maritimum* strains in discrete farming regions is needed to ensure that effective autogenous vaccines are developed and deployed.

Using two bacterial typing methods, we aimed to identify the antigenic diversity of New Zealand *T. maritimum* isolates and compare them to strains from Australia and other countries. First, conventional serotyping (antigen-antibody assay) was used to study serological relationships between New Zealand isolates and serotypes of known pathogenicity in the Tasmanian (Australia) Atlantic salmon industry (Van Gelderen et al., 2010; C. Angelucci, NRE Tasmania, pers. commun.). Second, we used the multiplex PCR (mPCR) based serotyping scheme to detect the variation among genes of the O-antigen biosynthesis cluster (O-AGC) of *T. maritimum* associated with salmon disease in New Zealand (Lopez et al., 2022a). The variation present in the O-AGC region is considered responsible for the diversity of the bacterial O-antigen and the resulting multiple subtypes (Liu et al., 2021), and this knowledge has enabled the comparison of local (New Zealand) O-AGC variants with isolates from other countries and hosts for the first time. This type of assessment provides the basis on which emerging serotypes in New Zealand marine farming environments can be characterized in the future.

2. Materials and methods

2.1. Source and identification of bacterial strains

Tenacibaculum maritimum isolates were collected from nine Chinook salmon farms in the Marlborough Sounds located on the north coast of the South Island, New Zealand (Fig. 1). Four of the farms are in the Pelorus Sound (sites A-D, Fig. 1), while five farms are in Queen Charlotte Sound (sites *E*- I, Fig. 1). The 36 isolates used in this study were collected between January 2020 and August 2021 (Table 2). A total of 35 *T. maritimum* isolates were recovered in culture using Marine Shieh's Selective Medium (MSSM; Kumanan et al., 2022; Wilson et al., 2019) from the skin of Chinook salmon; one isolate was recovered from seawater. Isolates were assessed for colony morphology, which was consistently pale white, irregular, flat and strongly adhered to the surface of the medium (Kumanan et al., 2022). To confirm the identity of

Table 1 (*continued*)

Susceptible marine fish species	Country	Source and key references	Known serotypes	Known O-AGC type
White seabass *Atractoscion nobilis*	USA	Farmed and wild (Chen et al., 1995; Drawbridge et al., 2021)	ND	ND
Yellowtail *Seriola quinqueradiata*	Japan	Farmed (Rahman et al., 2015; Baxa et al., 1986)	ND	1–0 (Lopez et al., 2022a)
Yellow eye mullet *Aldrichetta forsteri*	Australia	Wild (Handlinger et al., 1997)	ND	ND

Please consult key references for the various methodologies used to serotype isolates. ND: not determined.

Fig. 1. *Tenacibaculum maritimum* isolates were collected from moribund Chinook salmon (*Oncorhynchus tshawytscha*) in the Marlborough Sounds, New Zealand. Isolates were obtained from nine farms: (A) Waihinau Bay, (B) Waitata, (C) Kopaua, (D) Forsyth, (E) Ruakaka, (F) Te Pangu, (G) Clay Point, (H) Ngāmahau, (I) Ōtānerau.

bacterial isolates exhibiting a characteristic *T. maritimum* morphology (Kumanan et al., 2022), genomic DNA (gDNA) was extracted using AllPrep DNA/RNA Mini Kit (Qiagen, Hilden, Germany). Species-specific primers targeting a 155 base pair (bp) region of the *T. maritimum* 16S rRNA (forward 5′-TGCCTTCTACAGAGGGATAGCC-3′; reverse 5′-CTATCGTTGCCATGGTAAGCCG-3′) were used in combination with the detection probe (5' HEX-CACTTTGG AATGGCATCG–BHQ1 3′; (Fringuelli et al., 2012) for PCR analysis as previously described (Kumanan et al., 2022).

Three serologically distinct strains of *T. maritimum* obtained from the Department of Natural Resources and Environment Tasmania (NRE Tas), Australia and reference strains obtained from the National Collection of Industrial, Food and Marine Bacteria (NCIMB) were included in this study for comparative purposes (Table 2). The groups of *T. maritimum*, isolated from Tasmanian farmed Atlantic salmon, were defined based on a preliminary serotyping process, which recognized two serotypes, designated A (TCFB 4635) and B (TCFB 3289) (L. Schmidtke and J. Carson, unpublished data, 1995) and were subsequently expanded to include a third serotype designated C (TCFB 4574) (see Table 3; C. Angelucci, NRE Tas).

2.2. Serotyping (dot-blot analysis)

2.2.1. Bacterial preparation

Tenacibaculum maritimum broth cultures were grown in 40 mL Marine Shieh's Medium broth (Wilson et al., 2019) on a shaker at 180 rpm for 48 h at 22 °C. In the event of bacterial aggregates (Fig. 2B), a new broth preparation was made from a single colony and re-cultured until a homogenous suspension was obtained. Microscopy of Gram-stained samples from 48 h cultures was performed to check purity. Broth cultures were transferred to sterile tubes and centrifuged for 30 min at 2900 x*g* at room temperature (RT). The supernatant was removed and the bacterial pellet was washed twice using phosphate buffered saline (PBS) (0.01 mol L^{-1}, pH 7.4) by centrifugation at 2900 x*g* at RT. Washed bacterial pellets were resuspended in PBS and formalin inactivated. Cell integrity was assessed on Gram-stained cells under oil immersion at 1000× magnification using an Olympus BX5 microscope.

2.2.2. Antisera and dot-blot technique optimization

Rabbit antisera were developed to three *Tenacibaculum maritimum* isolates from Tasmania, Australia (TCFB 3289, TCFB 4635 and TCFB 4574) using the services of the Walter and Eliza Hall Institute Antibody Facility, Victoria, Australia. These isolates were prepared by culturing in Marine Sheih's Medium broth at 22 °C with shaking at 180 rpm for 24 h, then cells were centrifuged (3000 rpm, RT, 15 min) and washed twice with sterile PBS. Cells were formalin inactivated with 2% v/v formalin and then washed again twice with sterile PBS (Angelucci, NRE Tas). Each of these isolates represent a serotype and antisera were originally assigned by NRE Tasmania as serotype A (anti 4635), serotype B (anti 3289) and serotype C (anti 4574) (see Table 3). A preliminary dot-blot analysis conducted with minor modifications made to the method described by Avendaño-Herrera et al. (2004) showed cross-reaction

Table 2
List of *Tenacibaculum maritimum* isolates used in this study.

O-AGC genetic typing	Conventional serotyping using whole cell (formalin inactivated)				*Tenacibaculum maritimum* strains	Location	Host	Year
	Dot-blot analysis using antisera to:			Serotype				
	TCFB 4635 (A)	TCFB 3289 (B)	TCFB 4574 (C)					
1	++	−	−	A	NCIMB 2153	Japan	*Acanthopagrus schlegeli*	1976
	++	++	++	ABC	NCIMB 2154^{T}	Japan	*Pagrus major*	1977
2–1	−	+	−	B	NCIMB 2158	Scotland	*Solea solea*	1981
	+	++	−	AB	CCCM 125	Waitata, New Zealand *	*Oncorhynchus tshawytscha*	2021
	+	+	−	AB	CCCM 133	Ōtānerau, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	+	++	++	ABC	CCCM 126	Waitata, New Zealand *	*Oncorhynchus tshawytscha*	2021
	+	++	++	ABC	CCCM 129	Clay Point, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	+	++	++	ABC	CCCM 139	Clay Point, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	+	++	++	ABC	CCCM 140	Ngāmahau, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	+	++	++	ABC	CCCM 152	Te Pangu, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	−	++	−	B	TCFB 3289~	Tasmania, Australia	*Salmo salar*	2014
3–0	−	++	−	B	CCCM 013	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	−	++	−	B	CCCM 015	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	−	+	−	B	CCCM 103	Te Pangu, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	−	+	−	B	CCCM 127	Ruakaka, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	+	+	−	AB	CCCM 105	Ruakaka, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	+	++	++	ABC	CCCM 001	Waihinau, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	+	ABC	CCCM 004	Waihinau, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	+	ABC	CCCM 005	Waihinau, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	+	ABC	CCCM 006	Waihinau, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	++	ABC	CCCM 010	Waihinau, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	++	ABC	CCCM 014	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	+	ABC	CCCM 017	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	+	ABC	CCCM 018	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	++	ABC	CCCM 019	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	++	ABC	CCCM 020	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	++	ABC	CCCM 034	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	+	ABC	CCCM 035	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	++	++	++	ABC	CCCM 038	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	++	ABC	CCCM 039	Forsyth, New Zealand *	*Oncorhynchus tshawytscha*	2020
	+	++	++	ABC	CCCM 085	Waihinau, New Zealand *	*Oncorhynchus tshawytscha*	2020
	++	++	++	ABC	CCCM 101	Clay Point, New Zealand [Y]	*Oncorhynchus tshawytscha*	2020
	+	++	++	ABC	CCCM 104	Te Pangu, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	+	+	+	ABC	CCCM 106	Kopaua, New Zealand *	*Oncorhynchus tshawytscha*	2021
	+	++	+	ABC	CCCM 108	Ngāmahau, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	++	++	++	ABC	CCCM 122	Ōtānerau, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021

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Table 2 (*continued*)

O-AGC genetic typing	Conventional serotyping using whole cell (formalin inactivated)				*Tenacibaculum maritimum* strains	Location	Host	Year
	Dot-blot analysis using antisera to:			Serotype				
	TCFB 4635 (A)	TCFB 3289 (B)	TCFB 4574 (C)					
	+	++	+	ABC	CCCM 131	Ōtānerau, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
3–1	++	–	–	A	TCFB 4635~	Tasmania, Australia	*Salmo salar*	2018
3–2	++	++	++	ABC	CCCM 102	Waihinau, New Zealand [*]	*Oncorhynchus tshawytscha*	2020
	++	++	++	ABC	CCCM 123	Ruakaka, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
	+	++	++	ABC	CCCM 128	Ruakaka, New Zealand [Y]	*Oncorhynchus tshawytscha*	2021
4	–	–	++	C	TCFB 4574~	Tasmania, Australia	*Salmo salar*	2017

The notation * and [Y] indicate *O. tshawytscha* farms located at Pelorus Sound and Queen Charlotte Sound, respectively; superscript[T] is the type strain; TCFB = Tasmanian Collection of Fish Bacteria; CCCM = Cawthron Culture Collection of Microorganisms (New Zealand isolates); NCIMB = National Collection of Industrial, Food and Marine Bacteria; (–) faint detection; (+) medium detection; (++) strong detection.

Table 3
Tenacibaculum maritimum serotypes and respective antisera obtained from the Department of Natural Resources and Environment Tasmania (NRE Tas), Australia.

Reference materials	Serotypes*	Dilution and adsorbed / un-adsorbed antisera used for dot-blot analysis
Isolate TCFB 4635	A	NA
Isolate TCFB 3289	B	NA
Isolate TCFB 4574	C	NA
Rabbit anti- *T. maritimum* (TCFB 4635) serum	Antiserum A	1:64,000 (un-adsorbed)
Rabbit anti- *T. maritimum* (TCFB 3289) serum	Antiserum B	1:160,000 (adsorbed)
Rabbit anti- *T. maritimum* (TCFB 4574) serum	Antiserum C	1:20,000 (adsorbed)

*Serotypes 'A', 'B' and 'C' were as arbitrarily notated by the Centre for Aquatic Animal Health & Vaccines, NRE Tas and were assigned independent of serotypes or serotypes published elsewhere (unpublished data).

across the collection of NZ strains with both antisera Anti-3289 and Anti-4574. Subsequently, the antisera were cross-adsorbed and titrated to increase the specificity of the antisera to their corresponding antigens (see Supplementary text 1.1 and Supplementary fig. 1.1). Second, since there is a tendency for *T. maritimum* to auto-agglutinate (Fernández-Álvarez and Santos, 2018), we compared two methods of cell inactivation; formalin inactivation, using 0.5% formalin at RT and heat fixed (100 °C for 60 min, (Avendaño-Herrera et al., 2004) using five representative isolates (see Supplementary text 1.2). The comparison established that the method of inactivation did not influence the dot-blot outcome (see Supplementary text 1.2 and Supplementary Fig. 1.2).

Tenacibaculum maritimum isolates (Table 2) were enumerated using a counting chamber (0.02 mm Helber Bacteria Counting Chamber, Hawksley, UK) under 400× magnification, standardized to a cell count of 1×10^9 cells/mL and held at 4 °C until used for serotyping. The cell aggregates were dispersed using a Dounce homogenizer and cell diluted 1:40 in sterile PBS. The serological assays were carried out using the method described by Avendaño-Herrera with minor modifications (Avendaño-Herrera et al., 2004) (Supplementary text 1.3) using whole cell preparation inactivated with formalin. Dot blot reactions of unknowns were scored once a positive reaction was observed in the positive control (TCFB 4635, TCFB 3289 and/or TCFB 4574) of respective antisera (Table 3). Reactions were scored according to colour intensity: intense staining more intense or equal to the positive control were recorded as "++", medium staining as "+" and absence of reaction as "-". Each isolate was tested four times with each antiserum and corresponding positive controls were included on each membrane (Table 3). PBS and formalin inactivated *Vibrio parahaemolyticus* (NCTC 10884; National Collection of Type Cultures) cells grown in marine broth at 22 °C for 48 h were used as a negative control.

2.3. O-AGC typing

Bacterial gDNA was extracted from 48 h cultures of *T. maritimum* strains in Table 2 (excluding isolates originated from Tasmania), following the method described in Section 2.1. The genetic loci within the O-antigen genomic cluster (O-AGC) were amplified using primers developed by Lopez et al., 2022a. O-AGC types of TCFB isolates were predicted in silico from genome sequences deposited at PubMLST database https://pubmlst.org/bigsdb?db=pubmlst_tenacibaculum_isolates&page=query (id 133 = TCFB 3289; id 135 = TCFB 4574 and id 137 = TCFB 4635). mPCR was performed for the rest of the isolates mentioned in Table 2. Each mPCR reaction contained 10 μL of 1× MyFi mix (BioLine, London, England), 0.5 μL of each primer (10 μM), 1 μL gDNA (90–100 ng) and 5 μL of PCR grade water, and the cycling reaction performed as described by Lopez et al. (2022a). To verify the test method, positive controls NCIMB 2154[T] and NCIMB 2158 (Lopez et al. (2022a) were included. The gDNA of other *Tenacibaculum* species isolated from New Zealand Chinook salmon (i.e., *T. dicentrarchi*, CCCM20/030 and *T. soleae,* CCCM20/023) were included to confirm the specificity of the primers towards *T. maritimum*. Five microliters of each PCR product were electrophoresed (70 V for 45 min) on a 2% agarose gel stained with 5% *v*/v RedSafe™-20,000× (iNtRonbio) and run in 1 X Tris-acetate-EDTA (TAE) buffer along with 5 μL of AccuRuler 100 bp DNA Ladder (MaestroGen). The bands of amplified genes were then visualized using FireReader V10 (UVITEC, Cambridge, UK). To verify the amplicons, mPCR products were purified using DNA Clean & Concentrator-5 (ZymoResearch) and sequenced using Sanger technology by an external contractor (Otago Genomics Facility, University of Otago, Dunedin, New Zealand) using the PCR primers described in Lopez et al. (2022a).

3. Results

3.1. Bacterial strain

All isolates had consistent colony morphology: pale white, flat and strongly adhered to the surface of MSSM. They were confirmed as *T. maritimum* by species-specific PCR. Although most of the isolates produced homogenous *T. maritimum* suspensions when cultured in broth, some isolates formed bacterial aggregates (Fig. 2B), but this was not specific to certain isolates and occurred sporadically. After 48 h of incubation at 180 rpm, cultures yielded *T. maritimum* densities of 10^7–10^9 cells/mL. Spherical cells (Fig. 2D) were seen occasionally in

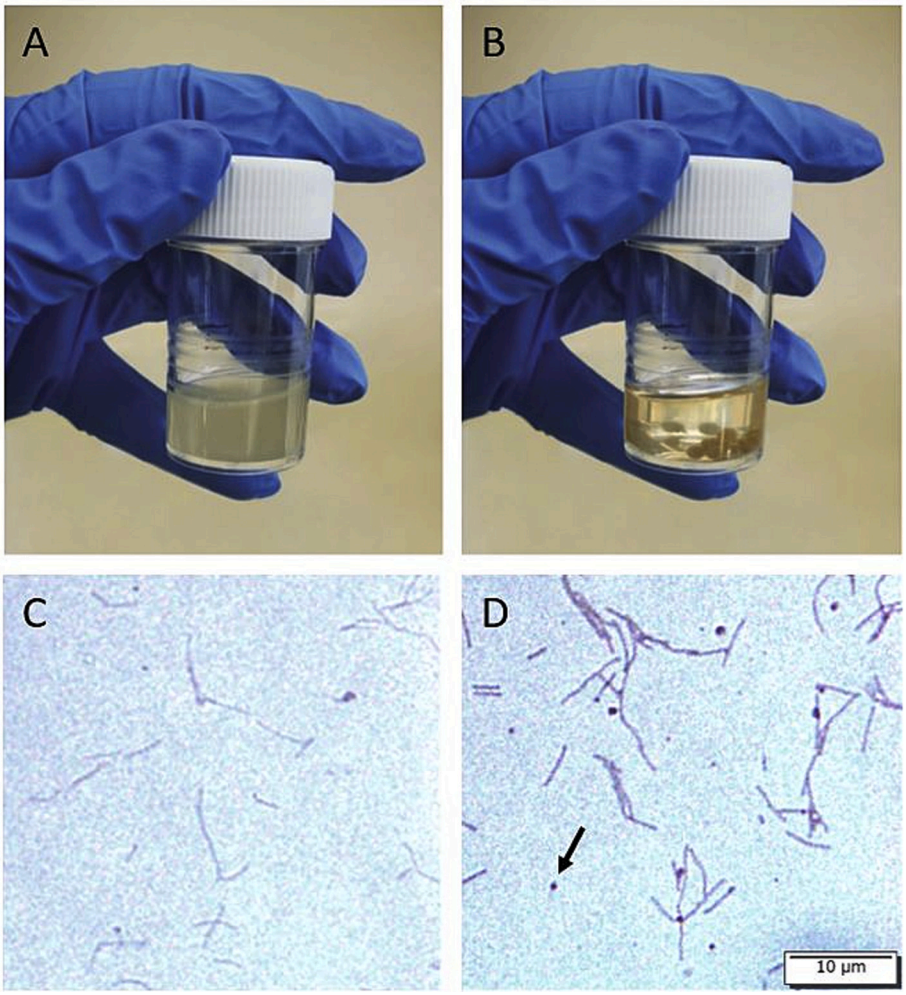

Fig. 2. (A and B) Examples of *Tenacibaculum maritimum* culture grown in broth with the same incubation conditions. (A) Broth exhibiting homogenous suspension of *T. maritimum*. (B) Isolate that formed bacterial aggregates. (C and D) Gram-stained cells of *T. maritimum* under oil immersion at 1000× magnification. (C) Heat inactivated cells showing minimal aggregation. (D) Formalin inactivated cells showing multicellular clumps. The arrow in figure D indicates a spherical cell of *T. maritimum*.

culture and have been observed by Piñeiro-Vidal et al. (2012) in *T. dicentrarchi* and Wang et al. (2008) in *T. aiptasiae*; spherical cells appear to be a feature of filamentous bacteria described as 'flexibacteria' (Lewin and Lounsbery, 1969). Heat and formalin inactivation did not affect the cell integrity of *T. maritimum*; however, fewer cell aggregates were observed in heat inactivated samples compared to formalin inactivated samples (Fig. 2C and D).

3.2. Dot-blot

Antisera developed for *T. maritimum* isolated from Atlantic salmon in Tasmania bound *T. maritimum* isolates from Chinook salmon and the reference cultures (NCIMB 2153, NCIMB 2154^T and NCIMB 2158) (Table 2). The specificity of each antiserum toward its homologous positive control (Tasmanian Atlantic salmon isolates) and the reactions with some representative New Zealand isolates of *T. maritimum* is given is Fig. 3.

Serological analysis using the three antisera revealed some serological diversity among New Zealand *T. maritimum* isolates (Table 2). Three distinct serological groups were present among the isolates tested. Of the 36 New Zealand isolates examined in this study, 29 (81%) reacted with all three antisera (antisera A, B and C: designated as serotype ABC), three isolates (8%) were recognized by antisera A and B (designated as serotype AB) and 4 (11%) were detected by antiserum B only (Fig. 4). All three of these serological groups were detected in both Pelorus Sound and Queen Charlotte Sound in the Marlborough Sounds region (Fig. 4). Serotype ABC was present at all nine farms and was the predominant type of isolate tested in Chinook salmon farms in the Marlborough Sounds. This antigenic combination was also observed for the reference isolate NCIMB 2154^T isolated from Japan (*Pagrus major*), but not the isolates from Scotland or Australia (Table 2). Dot-blot analysis revealed the presence of serotype B in New Zealand, which is also present in Australian Atlantic salmon (TCFB 3289) and Dover sole in Scotland (NCIMB 2158) (Table 2).

3.3. Molecular typing/mPCR

The mPCR assay performed on 36 New Zealand *T. maritimum* isolates identified three O-AGC types: Type 2–1, 3–0 and 3–2 groups in Chinook salmon farms (Table 2 and Fig. 5). Of the 36 isolates tested, 26 isolates (72%) were classified as O-AGC Type 3, which was predominant in the Pelorus Sound and Queen Charlotte Sound sites (Fig. 4) and found in all farms except for one. The second O-AGC group of Type 2–1 corresponded to seven isolates (19%) across five farms in both Pelorus and Queen Charlotte Sounds (Fig. 4). The final group, O-AGC Type 3–2, corresponded to three isolates (8%). All three O-AGC groups were present in Pelorus Sound and Queen Charlotte Sound (Fig. 4). mPCR typing of NCIMB 2154 and NCIMB 2158 is consistent with typing by Lopez et al. (2022a) and provides confidence in the O-AGC typing results of the New Zealand *T. maritimum* isolates (Fig. 5). The *T. maritimum* mPCR assay was specific to *T. maritimum*, as no product from *T. soleae* (CCCM20/023) or *T. dicentrarchi* (CCCM20/030) gDNA was observed in either the singleplex or mPCR for the *T. maritimum* O-AGC construct (data not shown). The amplicons were confirmed to be the correct target gene by

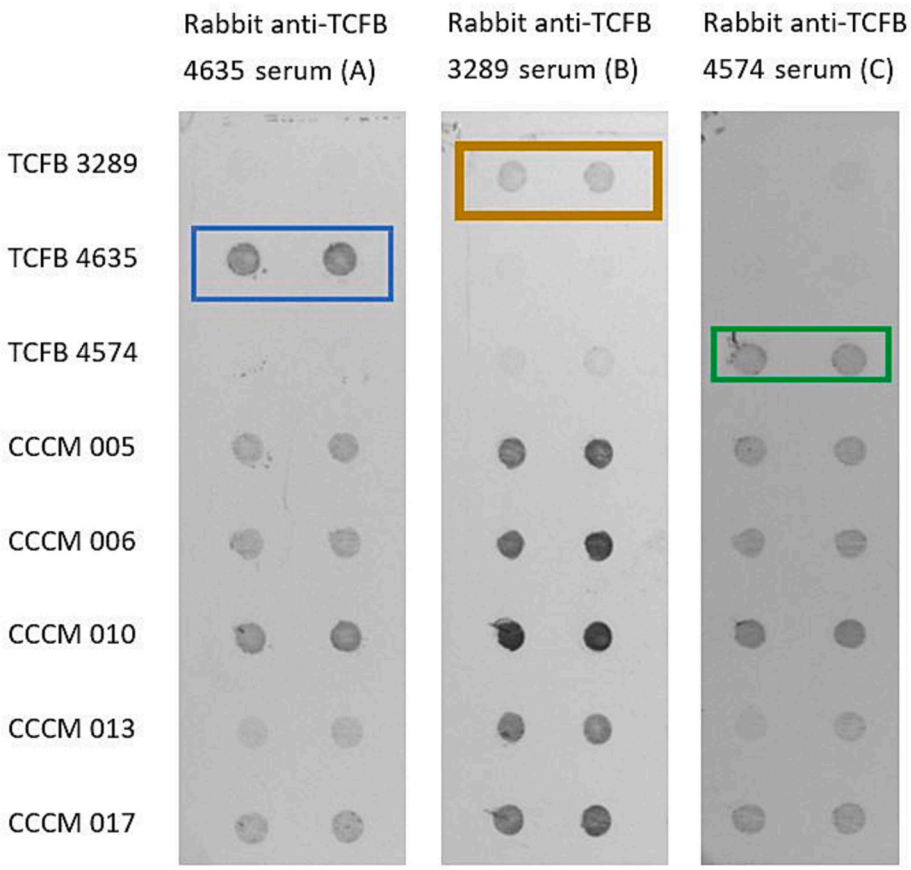

Fig. 3. Dot-blot serotyping showing the specificity of each antiserum toward its positive control (TCFB 3289, 4635 and 4574). Representative New Zealand *Tenacibaculum maritimum* isolates (CCCM 005, 006, 010 and 017) detected by all three antisera (A, B and C); isolate CCCM 013 was only detected by Antiserum B.

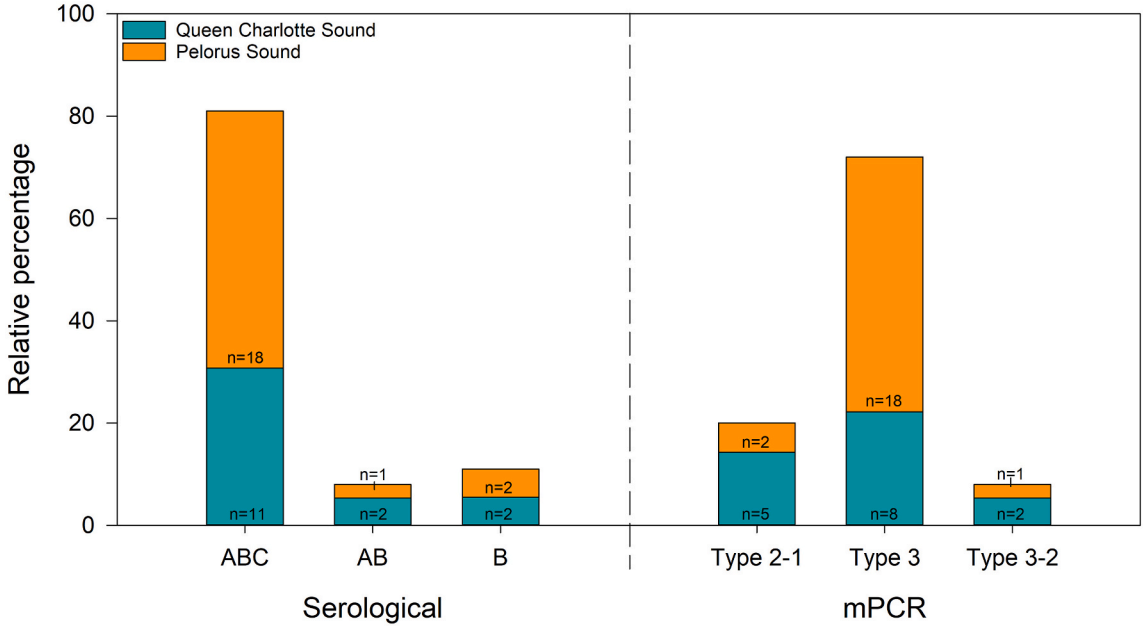

Fig. 4. The relative percentage of *Tenacibaculum maritimum* serological and O-AGC (mPCR) groups present in the library of isolates collected from Chinook salmon (*Oncorhynchus tshawytscha*) farms in the Marlborough Sounds, New Zealand (total 36 isolates).

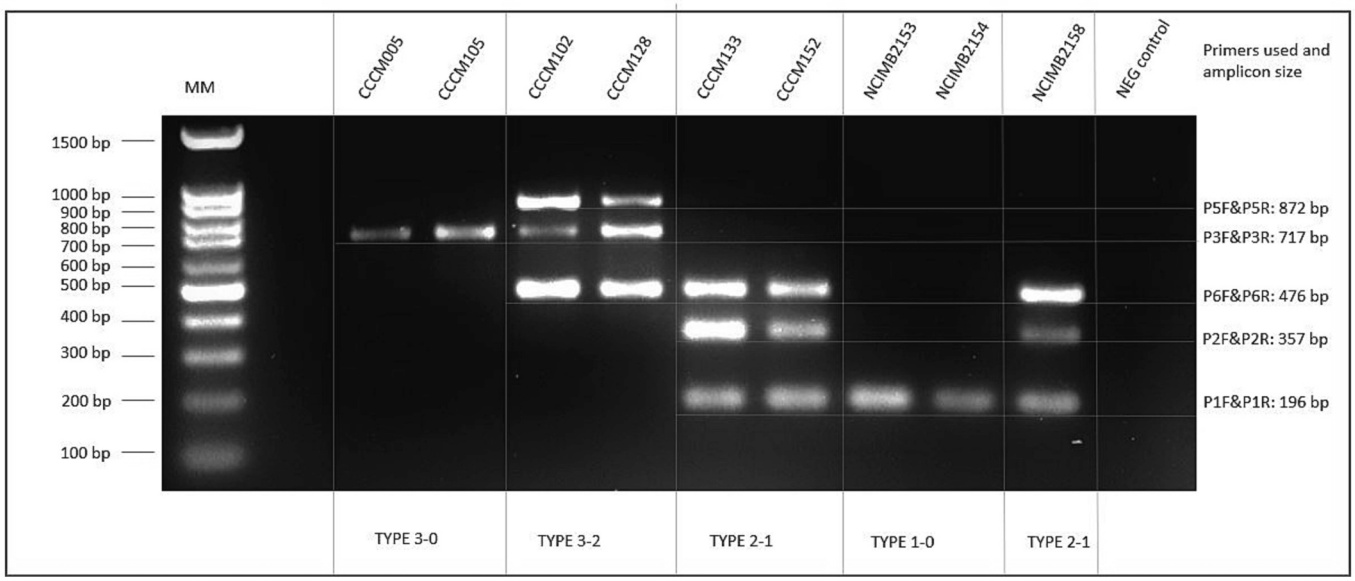

Fig. 5. Representative genetic profile of *Tenacibaculum maritimum* O-AGC groups by mPCR present in New Zealand Chinook salmon (*Oncorhynchus tshawytscha*) farms; data for *Tenacibaculum maritimum* isolates held in Cawthron's Culture Collection of Microorganisms (CCCM). Reference *T. maritimum* cultures were obtained from the National Collection of Industrial, Food and Marine Bacteria (NCIMB) and used as positive controls.

Sanger sequencing technology.

4. Discussion

The increasing incidence of *T. maritimum* associated mortalities in New Zealand (Kumanan et al., 2022; Brosnahan et al., 2019) along with New Zealand's warming seas threaten the Chinook salmon industry (NIWA, 2022; Lane et al., 2022). To mitigate this emerging infectious disease threat (Kumanan et al., 2022; Brosnahan et al., 2019), a timely, reliable and effective prevention strategy is required. As a first step toward disease prevention, we have taken a pragmatic approach to study the antigenic properties of *T. maritimum*. This is the first study reporting the antigenic diversity of *T. maritimum* isolated from Chinook salmon exhibiting signs of tenacibaculosis in New Zealand. Utilizing antisera from Tasmania, we found at least three *T. maritimum* serotypes (ABC, AB and B) among the isolates tested in this study. There are also multiple *T. maritimum* serotypes that have been identified in isolates from Atlantic salmon (C. Angelucci, NRE Tasmania, pers. comm.; Van Gelderen et al., 2010). Originally, *T. maritimum* serotypes were thought to be host specific (Avendaño-Herrera et al., 2004); however, increased investigation of this pathogen among multiple vulnerable host species has identified that serotypes are not strictly host specific (Lopez et al., 2022a; Van Gelderen et al., 2010). Our results align with these previous findings, as our data show antigenic variation in isolates obtained from a single species, *Onchorhynchus tshawytscha*.

A total of 29 *T. maritimum* isolates (81%) from New Zealand Chinook salmon were positive with all three Tasmanian antisera (antisera A, B and C), and were allocated to serotype ABC. This suggests that the majority of New Zealand *T. maritimum* isolates share antigenic components with each of the three Tasmanian *T. maritimum* serotypes. This novel serotype occurred across all Chinook salmon farms located at Pelorus Sound and Queen Charlotte Sound in New Zealand and was not previously reported to occur in Tasmania (C. Angelucci, pers. comm.). We also noted that isolate NCIMB 2154^{T}, which was isolated from diseased red seabream in Japan, was also assigned to serotype ABC. In addition, we found New Zealand isolates that only typed as serotype B, and these were detected in eight of the nine farms; the reference isolate NCIMB 2158 from Dover sole, Scotland, also tested as serotype B. Isolates specific to serotype B have also been reported in Tasmanian Atlantic salmon (C. Angelucci, pers. comm.). Notably, the antigenic variability reported in NCIMB 2154 in this study has previously been observed in an O-antigen serotyping scheme where this isolate was assigned to serotype O1 and O2 (Avendaño-Herrera et al., 2004; Fernández-Álvarez and Santos, 2018). However, NCIMB 2158 was specific to serotype B in this study and was only assigned to group O2 in a previous study by Avendaño-Herrera et al. (2004).

Three isolates from New Zealand Chinook salmon were designated as serotype AB, as were some isolates from Tasmanian Atlantic salmon (C. Angelucci, pers. comm.); none of the NCIMB reference strains were identified as serotype AB. Serotype C was based on an isolate from Tasmanian Atlantic salmon, and none of the New Zealand or NCIMB strains were assigned to this serotype. Based on the samples tested, serotype C appears to be unique to Tasmanian Atlantic salmon.

Serotyping by antigen-antibody reaction of *T. maritimum* isolates provides insight into phenotypic strain variation based on surface antigens of New Zealand isolates from Chinook salmon. This forms the basis for comparing Atlantic salmon isolates in Tasmania with isolates from Chinook salmon in New Zealand. Nevertheless, antibody-based serotyping schemes can encounter drawbacks, including the selection of suitable strains for antiserum production, establishing specificity along with choice of assay method, subjective interpretation, and standardization and harmonization of serotyping schemes (Lopez et al., 2022a; Sloan et al., 2017). For example, in this study, we found it difficult to produce homogenous bacterial preparations for dot-blot assay because of the tendency of *T. maritimum* to sporadically form cell aggregations, a problem that was also observed by Rahman et al. (2014). Bacterial flocculation, cell clumping and strong adherence to culture flasks added further difficulties when estimating cell densities. Serotyping of *T. maritimum* isolates by antigen-antibody reaction in this study describes types with indistinct boundaries. Consequently, allocation of the New Zealand strains of *T. maritimum* to particular serotypes should be considered preliminary; however, our results do provide relevant phenotypic information as to the likely strain variation and distribution across different farms and production areas.

Recent sequencing of the *T. maritimum* whole genome (Bridel et al., 2020; Pérez-Pascual et al., 2017) has confirmed the use of genomic loci encoding antigenic variability as a means of establishing less ambiguous serotyping (Lopez et al., 2022a). The variation in the O-AGC genomic cluster of *T. maritimum* among available whole genome sequences distinguishes eight O-AGC subtypes, which fall into four major O-AGC

types corresponding to four serotypes (Lopez et al., 2022a). We assessed the suitability of this rapid profiling system on our collection of New Zealand *T. maritimum* isolates. Molecular subtyping revealed three O-AGC types (Type 2–1, Type 3–0 and Type 3–2) across the 36 New Zealand *T. maritimum* isolates that were analyzed. Of the three O-AGC groups identified in New Zealand, Type 3–0 was the most abundant (72%) across both Pelorus and Queen Charlotte Sounds. *Tenacibaculum maritimum* of sequence Type 3–0 were also isolated from farmed Tasmanian Atlantic salmon, orbicular batfish (French Polynesia), olive flounder (Japan) and turbot (Malta and Spain) (Lopez et al., 2022a). The globally predominasnt subtype 2–1 was the next most abundant type comprising 20% of New Zealand isolates that were isolated from Pelorus and Queen Charlotte Sounds. This subtype has been reported to occur in a variety of locations and hosts including Dover sole from Scotland (NCIMB 2158), white seabass (USA), turbot (France and Spain), European seabass (Malta, France, Italy and Spain), orbicular batfish (French Polynesia), Atlantic salmon (Australia), rainbow trout (Australia) and olive flounder (South Korea) (Lopez et al., 2022a). O-AGC subtype 3–2 was detected in only 8% of New Zealand isolates from Pelorus Sound and Queen Charlotte Sound. Moreover, this subtype has only been detected in New Zealand Chinook salmon, Atlantic salmon and striped trumpeter from Australia (Lopez et al., 2022a). The wide range of fish species from which these O-AGC types of *T. maritimum* have been isolated indicates there is no host or geographical specificity associated with the O-AGC types.

In this study, serotype ABC was found across all three O-AGC types (Type 2–1, 3–0 and 3–2) identified in the 36 New Zealand isolates of *T. maritimum* that were tested. Contrary to the expectations of the proposed typing scheme by, Lopez et al. (2022a), we did not see a clear correlation between antibody-based serotyping and O-AGC typing in this study. For example, NCIMB 2153 and 2154^{T} were O-AGC Type 1, but different serotypes were assigned by antibody-based assay. This indicates that factors other than LPS O-antigen may play a role in serological classification (Fratamico et al., 2016). It has long been known that multiple antigens such as somatic (O-antigen), flagella (H-antigen) and capsular polysaccharide [K-antigen/capsular polysaccharide (CPS)] comprise the bacterial envelope and serological assignment of bacteria is influenced by factors such as antigen expression, immunodominance and availability (Orskov et al., 1977; Rochat et al., 2017; Stenutz et al., 2006; Whitfield and Roberts, 1999). Bacterial LPS and CPS are both involved in producing an immunological response in the host (Perera et al., 2021; Evrard et al., 2010; Zhang et al., 2019). CPS has been widely studied in some bacterial species, including *Klebsiella* and *Salmonella* for antigenic typing (Choi et al., 2020; Perera et al., 2021; Wyres et al., 2016). Notably, LaFrentz et al. (2007) reported two distinctive carbohydrate-banding patterns correspond to the LPS and CPS in *F. psychrophilum*, a closely related species to *T. maritimum* and member of the family *Flavobacteriaceae.* It was also suggested the CPS is an important virulence factor for the cell adherence of *F. psychrophilum*. To the best of the authors' knowledge, there are no detailed published reports about the CPS region of *Tenacibaculum maritimum* although the presence of a capsular structure was reported by Avendaño-Herrera (2005) using electron microscopy. In this study, antisera developed against whole inactivated *T. maritimum* was used for serotyping, and one would expect the antisera would recognize all antigenic components present on the cell surface of bacteria, including LPS (O) and CPS (K), which contrasts with the O-AGC mPCR typing that is limited to O-antigen (Lopez et al., 2022a). We suspect, the absence of K:O based genetic typing may have contributed to the lack of concordance between serotyping and O-AGC mPCR types observed in this study as well as in *F. psychrophilum* isolates as reported previously (Rochat et al., 2017). Given the nature of *T. maritimum* to produce exopolysaccharides, it is essential to further investigate the potential involvement of CPS in parallel with LPS as described in *Klebsiella* species (Wyres et al., 2016; Kubler-Kielb et al., 2013). The known virulence mechanism of CPS to evade host innate immunity by its antiphagocytic ability (Pettis and Mukerji, 2020) should not be ignored in determining serotype and pathogenicity, and CPS diversity should be accounted for during a vaccine development (Lin et al., 2022; Barai et al., 2022). Nevertheless, this preliminary study has value because the antigenic relationship of *T. maritimum* between Australia and New Zealand has been assessed, and importantly we have identified the predominant serotype in New Zealand Chinook salmon, a finding that will aid the epizootiological assessment of disease outbreaks.

In conclusion, our study reports the first antigenic characterization of *T. maritimum* associated with Chinook salmon exhibiting clinical signs of tenacibaculosis. Antigenic variation identified by both antibody and mPCR based typing will allow us to investigate whether there is an association between serotype assignment and the degree of *T. maritimum* virulence through a pathogen challenge model, which is essential for appropriate strain selection for an effective vaccine (Barnes et al., 2022; Tinsley et al., 2011; Shirajum Monir et al., 2020; Hoare et al., 2017). Results obtained from this work will enable the formulation of an autogenous vaccine comprising local antigens. This approach is expected to be more effective and efficient than using an existing commercial vaccine, which may not be serotype specific and can be subject to extended delays due to regulatory and policy approval. Critically, such imported vaccines may not be effective against local or emerging serotypes (Barnes et al., 2022). It is important to note that antigenic characterization of a fish pathogen is not a one-time analysis; instead, it should be a continuous process as part of disease surveillance in aquaculture to enable the detection of emerging serotypes that may cause disease in immunized fish (Barnes et al., 2022). The mPCR typing in parallel with serological techniques can serve as a time-effective screening tool to monitor emerging *T. maritimum* variants.

Declaration of Competing Interest

The authors have no competing interests to declare.

Data availability

Link will be provided a proof stage

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aquaculture.2023.740055.

References

Alix, M., Gasset, E., Bardon-Albaret, A., Noel, J., Pirot, N., Perez, V., Coves, D., Saulnier, D., Lignot, J.-H., Cucchi, P.N., 2020. Description of the unusual digestive tract of *Platax orbicularis* and the potential impact of *Tenacibaculum maritimum* infection. PeerJ 8, e9966. https://doi.org/10.7717/peerj.9966.

Alsina, M., Blanch, A.R., 1993. First isolation of *Flexibacter maritimus* from cultivated turbot (*Scophthalmus maximus*). Bulletin of European Association of Fish Pathologists 13, 157–160.

Apablaza, P., Frisch, K., Brevik, Ø.J., Småge, S.B., Vallestad, C., Duesund, H., Mendoza, J., Nylund, A., 2017. Primary isolation and characterization of *Tenacibaculum maritimum* from Chilean Atlantic Salmon mortalities associated with a *Pseudochattonella* spp. algal bloom. J. Aquat. Anim. Health 29, 143–149. https://doi.org/10.1080/08997659.2017.1339643.

Araujo, B.C., Symonds, J.E., Glencross, B.D., Carter, C.G., Walker, S.P., Miller, M.R., 2021. A review of the nutritional requirements of Chinook salmon (*Oncorhynchus tshawytscha*). New Zealand journal of marine and freshwater research 1-30. https://doi.org/10.1080/00288330.2021.1985530 ahead-of-print.

Avendaño-Herrera, R., 2005. Avances en el conocimiento del patógeno de peces Tenacibaculum maritimum: implicaciones en el diagnóstico y prevención de la enfermedad.

Avendaño-Herrera, R., Magariños, B., López-Romalde, S., Romalde, J.L., Toranzo, A.E., 2004. Phenotypic characterization and description of two major O-serotypes in *Tenacibaculum maritimum* strains from marine fishes. Dis. Aquat. Organ. 58, 1–8. https://doi.org/10.3354/dao01226.

Avendano-Herrera, R., Magarinos, B., Morinigo, M.A., Romalde, J.L., Toranzo, A.E., 2005a. A novel O-serotype in *Tenacibaculum maritimum* strains isolated from cultured sole (*Solea senegalensis*). Bull. Eur. Assoc. Fish Pathol. 25, 70–74.

Avendano-Herrera, R., Toranzo, A.E., Romalde, J.L., Lemos, M.L., Magarinos, B., 2005b. Iron uptake mechanisms in the fish pathogen *Tenacibaculum maritimum*. Appl. Environ. Microbiol. 71, 6947–6953.

Avendaño-Herrera, R., Toranzo, A.E., Magariños, B., 2006. Tenacibaculosis infection in marine fish caused by *Tenacibaculum maritimum*: a review. Dis. Aquat. Organ. 71, 255–266. https://doi.org/10.3354/dao071255.

Barai, L., Hasan, M.R., Haq, J.A., Ahsan, C.R., 2022. Salmonellacidal antibody response to *Salmonella enterica* serovar Typhi in enteric fever and after vaccination with Vi capsular polysaccharide. Int. J. Infect. Dis. 121, 120–125. https://doi.org/10.1016/j.ijid.2022.05.022.

Barnes, A.C., Silayeva, O., Landos, M., Dong, H.T., Lusiastuti, A., Phuoc, L.H., Delamare-Debouteville, J., 2022. Autogenous vaccination in aquaculture: a locally enabled solution towards reduction of the global antimicrobial resistance problem. Rev. Aquac. 14, 907–918. https://doi.org/10.1111/raq.12633.

Bass, A.L., Bateman, A.W., Connors, B.M., Staton, B.A., Rondeau, E.B., Mordecai, G.J., Teffer, A.K., Kaukinen, K.H., Li, S., Tabata, A.M., Patterson, D.A., Hinch, S.G., Miller, K.M., 2022. Identification of infectious agents in early marine Chinook and Coho salmon associated with cohort survival. Facets 7, 742–773. https://doi.org/10.1139/facets-2021-0102.

Bateman, A.W., Teffer, A.K., Bass, A., Ming, T., Kaukinen, K., Hunt, B.P.V., Krkošek, M., Miller, K.M., 2022. Atlantic salmon farms are a likely source of *Tenacibaculum maritimum* infection in migratory Fraser River Sockeye salmon. Can. J. Fish. Aquat. Sci. 79, 1225–1240. https://doi.org/10.1139/cjfas-2021-0164.

Baxa, D.V., Kawai, K., Kusuda, R., 1986. Characteristics of gliding bacteria isolated from diseased cultured flounder, *Paralichthys olivaceous*. Fish Pathology 21, 251–258. https://doi.org/10.3147/jsfp.21.251.

Bernardet, J.-F., Kerouault, B., Michel, C., 1994. Comparative study on *Flexibacter maritimus* strains isolated from farmed sea bass (*Dicentrarchus labrax*) in France. Fish Pathology 29, 105–111. https://doi.org/10.3147/jsfp.29.105.

Bondad-Reantaso, M., Mackinnon, B., Karunasagar, I., Fridman, S., Alday-Sanz, V., Brun, E., Groumellec, M., Li, A., Surachetpong, W., Bin, Dall'occo, A., Urbani, R., Caputo, A., 2023. Review of alternatives to antibiotic use in aquaculture. Rev. Aquac. 2023, 1–31. https://doi.org/10.1111/raq.12786.

Bridel, S., Bourgeon, F., Marie, A., Saulnier, D., Pasek, S., Nicolas, P., Bernardet, J.-F., Duchaud, E., 2020. Genetic diversity and population structure of *Tenacibaculum maritimum*, a serious bacterial pathogen of marine fish: from genome comparisons to high throughput MALDI-TOF typing. Vet. Res. 51, 60. https://doi.org/10.1186/s13567-020-00782-0.

Brosnahan, C.L., Munday, J.S., Ha, H.J., Preece, M., Jones, J.B., 2019. New Zealand *rickettsia*-like organism (NZ-RLO) and *Tenacibaculum maritimum*: distribution and phylogeny in farmed Chinook salmon (*Oncorhynchus tshawytscha*). J. Fish Dis. 42, 85–95. https://doi.org/10.1111/jfd.12909.

Cepeda, C., Santos, Y., 2002. First isolation of *Flexibacter maritimus* from farmed Senegalese sole (*Solea senegalensis, Kaup*) in Spain. Bulletin-European Association of Fish Pathologists 22, 388–392.

Chen, M.E., Henry-Ford, D., Groff, J.M., 1995. Isolation and characterization of *Flexibacter maritimus* from marine fishes of California. J. Aquat. Anim. Health 7, 318–326. https://doi.org/10.1577/1548-8667(1995)007<0318:IACOMF>2.3.CO;2.

Choi, M., Hegerle, N., Nkeze, J., Sen, S., Jamindar, S., Nasrin, S., Sen, S., Permala-Booth, J., Sinclair, J., Tapia, M.D., Johnson, J.K., Mamadou, S., Thaden, J.T., Fowler, J.V.G., Aguilar, A., Terán, E., Decre, D., Morel, F., Krogfelt, K.A., Brauner, A., Protonotariou, E., Christaki, E., Shindo, Y., Lin, Y.-T., Kwa, A.L., Shakoor, S., Singh-Moodley, A., Perovic, O., Jacobs, J., Lunguya, O., Simon, R., Cross, A.S., Tennant, S. M., 2020. The diversity of lipopolysaccharide (O) and capsular polysaccharide (K) antigens of invasive *Klebsiella pneumoniae* in a multi-country collection. Front. Microbiol. 11, 1249. https://doi.org/10.3389/fmicb.2020.01249.

D.F.O, 2020. Advice from the Assessment of the Risk to Fraser River Sockeye Salmon due to Tenacibaculum maritimum transfer from Atlantic Salmon farms in the Discovery Islands area, British Columbia [Online]. Fisheries and Oceans Canada Science Advisory Secretariat Research Document 2020/044. Available: https://waves-vagues.dfo-mpo.gc.ca/library-bibliotheque/40940809.pdf [Accessed].

Devesa, S., Barja, J.L., Toranzo, A.E., 1989. Ulcerative skin and fin lesions in reared turbot, *Scophthalmus maximus* (L.). J. Fish Dis. 12, 323–333. https://doi.org/10.1111/j.1365-2761.1989.tb00321.x.

Dong, H.T., Taengphu, S., Sangsuriya, P., Charoensapsri, W., Phiwsaiya, K., Sornwatana, T., Khunrae, P., Rattanarojpong, T., Senapin, S., 2017. Recovery of *Vibrio harveyi* from scale drop and muscle necrosis disease in farmed barramundi, *Lates calcarifer* in Vietnam. Aquaculture 473, 89–96. https://doi.org/10.1016/j.aquaculture.2017.02.005.

Downes, J.K., Yatabe, T., Marcos-Lopez, M., Rodger, H.D., Maccarthy, E., O'connor, I., Collins, E., Ruane, N.M., 2018. Investigation of co-infections with pathogens associated with gill disease in Atlantic salmon during an amoebic gill disease outbreak. J. Fish Dis. 41, 1217–1227. https://doi.org/10.1111/jfd.12814.

Drawbridge, M., Shane, M., Silbernagel, C., 2021. The status of white seabass, *Atractoscion nobilis* as a commercially ready species for marine US aquaculture. J. World Aquacult. Soc. 52 https://doi.org/10.1111/jwas.12772.

Evrard, B., Balestrino, D., Dosgilbert, A., Bouya-Gachancard, J.L., Charbonnel, N., Forestier, C., Tridon, A., 2010. Roles of capsule and lipopolysaccharide O antigen in interactions of human monocyte-derived dendritic cells and *Klebsiella pneumoniae*. Infect. Immun. 78, 210–219. https://doi.org/10.1128/iai.00864-09.

Ferguson, H., Delannoy, C., Nicolson, J., Sutherland, D., Crumlish, M., Hay, S., 2009. Jellyfish as vectors of bacterial disease for farmed fish. Nature Precedings. https://doi.org/10.1038/npre.2009.3631.1.

Fernández-Álvarez, C., Santos, Y., 2018. Identification and typing of fish pathogenic species of the genus *Tenacibaculum*. Appl. Microbiol. Biotechnol. 102, 9973–9989. https://doi.org/10.1007/s00253-018-9370-1.

Florio, D., Gridelli, S., Fioravanti, M.L., Zanoni, R.G., 2016. First isolation of *Tenacibaculum maritimum* in a captive sand tiger shark (*Carcharias taurus*). J. Zoo Wildl. Med. 47, 351–353. https://doi.org/10.1638/2015-0064.1.

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*. Front. Microbiol. 7, 644. https://doi.org/10.3389/fmicb.2016.00644.

Fringuelli, E., Savage, P.D., Gordon, A., Baxter, E.J., Rodger, H.D., Graham, D.A., 2012. Development of a quantitative real-time PCR for the detection of *Tenacibaculum maritimum* and its application to field samples. J. Fish Dis. 35, 579–590. https://doi.org/10.1111/j.1365-2761.2012.01377.x.

Frisch, K., Småge, S.B., Johansen, R., Duesund, H., Brevik, Ø.J., Nylund, A., 2018. Pathology of experimentally induced mouthrot caused by *Tenacibaculum maritimum* in Atlantic salmon smolts. PloS One 13, e0206951. https://doi.org/10.1371/journal.pone.0206951.

Handlinger, J., Soltani, M., Percival, S., 1997. The pathology of *Flexibacter maritimus* in aquaculture species in Tasmania, Australia. J. Fish Dis. 20, 159–168. https://doi.org/10.1046/j.1365-2761.1997.00288.x.

Haridy, M., Hasheim, M., Abd El-Galil, M., Sakai, H., Yanai, T., 2015. Pathological findings of *Tenacibaculum maritimus* infection in Black damselfish, *Neoglyphieodon melas* and Picasso triggerfish, *Rhinecanthus assasi* in Red sea, Egypt. Veterinary Science & Technology 6, 1. https://doi.org/10.4172/2157-7579.1000214.

High Country Salmon, 2022. Environment and sustainability [Online]. New Zealand. Available. https://www.highcountrysalmon.co.nz/pages/environment-sustainability [Accessed 10 November 2022].

Hikida, M., Wakabayashi, H., Egusa, S., K., M., 1979. *Flexibacter* sp., a gliding bacterium pathogenic to some marine fishes in Japan. Bulletin of The Japanese Society for the Science of Fish 45, 421–428. https://doi.org/10.2331/suisan.45.421.

Hoare, R., Ngo, T.P.H., Bartie, K.L., Adams, A., 2017. Efficacy of a polyvalent immersion vaccine against *Flavobacterium psychrophilum* and evaluation of immune response to vaccination in rainbow trout fry (*Onchorynchus mykiss L.*). Vet. Res. 48, 43. https://doi.org/10.1186/s13567-017-0448-z.

Icthiovac® Tm-Hipra Laboratories. Available: https://www.hipra.com/portal/en/hipra/animalhealth/products/detail-global/icthiovac-tm [Accessed 12 November 2022].

Irgang, R., Mancilla, M., Avendaño-Herrera, R., 2021. Florfenicol and oxytetracycline susceptibility patterns in Chilean isolates of *Tenacibaculum dicentrarchi*: an emerging pathogen for farmed salmonids. J. Fish Dis. 44, 1043–1046. https://doi.org/10.1111/jfd.13380.

Jang, Y.-H., Jeong, J.-B., Yeo, I.-K., Kim, K.-Y., Harikrishnan, R., Heo, M.-S., 2009. Biological characterization of *Tenacibaculum maritimum* isolated from cultured olive flounder in Korea and sensitivity against native plant extracts. Journal of Fish Pathology 22, 53–65.

Johnston, H., Symonds, J., Walker, S., Preece, M., Lopez, C., Nowak, B., 2020. Case definitions for skin lesion syndromes in Chinook salmon farmed in Marlborough Sounds, New Zealand. J. Fish Dis. 44, 141–147. https://doi.org/10.1111/jfd.13317.

Kolygas, M.N., Gourzioti, E., Vatsos, I.N., Athanassopoulou, F., 2012. Identification of *Tenacibaculum maritimum* strains from marine farmed fish in Greece. Vet. Rec. 170, 623. https://doi.org/10.1136/vr.100778.

Kubler-Kielb, J., Vinogradov, E., Ng, W.I., Maczynska, B., Junka, A., Bartoszewicz, M., Zelazny, A., Bennett, J., Schneerson, R., 2013. The capsular polysaccharide and lipopolysaccharide structures of two carbapenem resistant *Klebsiella pneumoniae* outbreak isolates. Carbohydr. Res. 369, 6–9. https://doi.org/10.1016/j.carres.2012.12.018.

Kumanan, K., Von Ammon, U., Fidler, A., Symonds, J.E., Walker, S.P., Carson, J., Hutson, K.S., 2022. Advantages of selective medium for surveillance of *Tenacibaculum* species in marine fish aquaculture. Aquaculture 558. https://doi.org/10.1016/j.aquaculture.2022.738365.

Lafrentz, B.R., Lindstrom, N.M., Lapatra, S.E., Call, D.R., Cain, K.D., 2007. Electrophoretic and Western blot analyses of the lipopolysaccharide and glycocalyx of *Flavobacterium psychrophilum*. Fish Shellfish Immunol. 23, 770–780. https://doi.org/10.1016/j.fsi.2007.02.005.

Lagadec, E., Småge, S.B., Trösse, C., Nylund, A., 2021. Phylogenetic analyses of Norwegian *Tenacibaculum* strains confirm high bacterial diversity and suggest circulation of ubiquitous virulent strains. PloS One 16, e0259215. https://doi.org/10.1371/journal.pone.0259215.

Lane, H.S., Brosnahan, C.L., Poulin, R., 2022. Aquatic disease in New Zealand: synthesis and future directions. N. Z. J. Mar. Freshw. Res. 56, 1–42. https://doi.org/10.1080/00288330.2020.1848887.

Lewin, R.A., Lounsbery, D.M., 1969. Isolation, cultivation and characterization of flexibacteria. J. Gen. Microbiol. 58, 145–170. https://doi.org/10.1099/00221287-58-2-145.

Lin, T.L., Yang, F.L., Ren, C.T., Pan, Y.J., Liao, K.S., Tu, I.F., Chang, Y.P., Cheng, Y.Y., Wu, C.Y., Wu, S.H., Wang, J.T., 2022. Development of *Klebsiella pneumoniae* capsule polysaccharide-conjugated vaccine candidates using phage depolymerases. Front. Immunol. 13, 843183 https://doi.org/10.3389/fimmu.2022.843183.

Liu, B., Guo, X., Wang, J., Wu, P., Li, S., Feng, L., Wang, L., 2021. Development of a molecular serotyping scheme for *Morganella morganii*. Front. Microbiol. 12, 791165. https://doi.org/10.3389/fmicb.2021.791165.

Lopez, P., Bridel, S., Saulnier, D., David, R., Magariños, B., Torres, B.S., Bernardet, J.F., Duchaud, E., 2022a. Genomic characterization of *Tenacibaculum maritimum* O-antigen gene cluster and development of a multiplex PCR-based serotyping scheme. Transbound. Emerg. Dis. 69, e2876–e2888. https://doi.org/10.1111/tbed.14637.

Lopez, P., Saulnier, D., Swarup-Gaucher, S., David, R., Lau, C., Taputuarai, R., Belliard, C., Basset, C., Labrune, V., Marie, A., Bernardet, J.F., Duchaud, E., 2022b. First isolation of virulent *Tenacibaculum maritimum* isolates from diseased orbicular batfish (*Platax orbicularis*) farmed in Tahiti Island. Pathogens (Basel) 11, 131. https://doi.org/10.3390/pathogens11020131.

Mabrok, M., Machado, M., Serra, C.R., Afonso, A., Valente, L.M.P., Costas, B., 2016. Tenacibaculosis induction in the Senegalese sole (*Solea senegalensis*) and studies of *Tenacibaculum maritimum* survival against host mucus and plasma. J. Fish Dis. 39, 1445–1455. https://doi.org/10.1111/jfd.12483.

Magi, G., Avendano-Herrera, R., Magarinos, B., Toranzo, A., Romalde, J., 2007. First reports of flexibacteriosis in farmed tub gurnard (*Chelidonichthys lucernus L.*) and wild turbot (*Scophthalmus maximus*) in Italy. Bulletin-European Association of Fish Pathologists 27, 177.

Masumura, K., Wakabayashi, H., 1977. An outbreak of gliding bacterial disease in hatchery-born red seabream (*Pagrus major*) and gilthead (*Acanthopagrus schlegeli*) fry in Hiroshima. Fish Pathology 12, 171–177. https://doi.org/10.3147/jsfp.12.171.

Mcvicar, A.H., White, P.G., 1982. The prevention and cure of an infectious disease in cultivated juvenile Dover sole, *Solea solea* (L.). Aquaculture 26, 213–222. https://doi.org/10.1016/0044-8486(82)90157-0.

Miccoli, A., Saraceni, P.R., Scapigliati, G., 2019. Vaccines and immune protection of principal Mediterranean marine fish species. Fish Shellfish Immunol. 94, 800–809. https://doi.org/10.1016/j.fsi.2019.09.065.

Ministry for Primary Industry, 2020. Biosecurity New Zealand: Salmon Response [Online]. Available: https://www.mpi.govt.nz/biosecurity/about-biosecurity-in-new-zealand/salmon-response/ [Accessed 19 June 2022].

New Zealand King Salmon, 2016. NZKS operations report [Online]. Available. https://www.mpi.govt.nz/dmsdocument/16102-New-Zealand-King-Salmon-Operations-report [Accessed 19/11/2022].

New Zealand Salmon Farmers Association, 2022. New Zealand Salmon Farming [Online]. Available: http://www.salmon.org.nz/new-zealand-salmon-farming/production/ [Accessed 12/11/2022].

New Zealand Veterinary Association, 2019. Antimicrobial Resistance (AMR) [Online]. New Zealand. Available: https://nzva.org.nz/resource/general/amr/#:%E2%88%BC:text=Our%20goal%3A%20By%202030%20Newof%20animal%20health%20and%20wellness [Accessed 18 November 2022].

Niwa, 2022. Climate Change and Possible Impacts for New Zealand [Online]. Available: https://niwa.co.nz/education-and-training/schools/students/climate-change/impacts-for-NZ#species [Accessed 20 November 2022].

Norman, R., Brosnahan, C., Fischer, J., Frazer, J., Johnston, C., Jones, J.B., Keeling, S., Pande, A., Pulford, D., Rawdon, T., Williams, R., New Zealand. Ministry for Primary, I, 2013. Salmon mortality investigation: REW-1017 Pelorus Sound [Online]. Ministry for Primary Industries, Wellington. Available: https://www.mpi.govt.nz/dmsdocument/4094-Salmon-Mortality-Investigation [Accessed Book, Whole no. 2013/19.;2013/19;, p.Book, Whole.].

Nowlan, J.P., Lumsden, J.S., Russell, S., 2020. Advancements in characterizing *Tenacibaculum* infections in Canada. Pathogens (Basel) 9, 1029. https://doi.org/10.3390/pathogens9121029.

Nowlan, J.P., Britney, S.R., Lumsden, J.S., Russell, S., 2021. Application of quantitative-PCR to monitor netpen sites in British Columbia (Canada) for *Tenacibaculum* species. Pathogens (Basel) 10, 414. https://doi.org/10.3390/pathogens10040414.

Orskov, I., Orskov, F., Jann, B., Jann, K., 1977. Serology, chemistry, and genetics of O and K antigens of *Escherichia coli*. Bacteriology Reviews 41, 667–710. https://doi.org/10.1128/br.41.3.667-710.1977.

Ostland, V., Morrison, D., Ferguson, H., 1999. *Flexibacter maritimus* associated with a bacterial stomatitis in Atlantic salmon smolts reared in net-pens in British Columbia. J. Aquat. Anim. Health 11, 35–44. https://doi.org/10.1577/1548-8667(1999)011<0035:FMAWAB>2.0.CO;2.

Pacific Salmon Foundation, 2022. New Research Indicates Pathogen Levels are Twelve Times Higher Near Salmon Farms [Online]. Available: https://psf.ca/news-media/new-salmon-research-indicates-pathogen-levels-are-twelve-times-higher-near-farms/#:%E2%88%BC:text=This%20newly%20published%20research%20directly,farms%20in%20the%20Discovery%20Islands [Accessed 18 May 2022].

Pazos, F., Santos, Y., Nunez, S., Ae, Toranzo, 1993. Increasing occurrence of Flexibacter maritimus in the marine aquaculture of Spain [Online]. Available: https://www.observatorio-acuicultura.es/recursos/bases-de-datos/publicaciones/increasing-occurrence-flexibacter-maritimus-rhe-marine.

Perera, S.R., Sokaribo, A.S., White, A.P., 2021. Polysaccharide vaccines: a perspective on non-typhoidal salmonella. Polysaccharides 2, 691–714. https://doi.org/10.3390/polysaccharides2030042.

Pérez-Pascual, D., Lunazzi, A., Magdelenat, G., Rouy, Z., Roulet, A., Lopez-Roques, C., Larocque, R., Barbeyron, T., Gobet, A., Michel, G., Bernardet, J.-F., Duchaud, E., 2017. The complete genome sequence of the fish pathogen *Tenacibaculum maritimum* provides insights into virulence mechanisms. Front. Microbiol. 8, 1542. https://doi.org/10.3389/fmicb.2017.01542.

Pettis, G.S., Mukerji, A.S., 2020. Structure, function, and regulation of the essential virulence factor capsular polysaccharide of *Vibrio vulnificus*. Int. J. Mol. Sci. 21, 3259. https://doi.org/10.3390/ijms21093259.

Piñeiro-Vidal, M., Centeno-Sestelo, G., Riaza, A., Santos, Y., 2007. Isolation of pathogenic *Tenacibaculum maritimum*-related organisms from diseased turbot and sole cultured in the Northwest of Spain. Bulletin-European Association of Fish pathologists 27, 29.

Piñeiro-Vidal, M., Gijón, D., Zarza, C., Santos, Y., 2012. *Tenacibaculum dicentrarchi* sp. nov., a marine bacterium of the family Flavobacteriaceae isolated from European sea bass. Int. J. Syst. Evol. Microbiol. 62, 425–429. https://doi.org/10.1099/ijs.0.025122-0.

Preena, P.G., Swaminathan, T.R., Kumar, V.J.R., Singh, I.S.B., 2020. Antimicrobial resistance in aquaculture: a crisis for concern. Biologia 75, 1497–1517. https://doi.org/10.2478/s11756-020-00456-4.

Rafidah, N., Seng, J., Seng, E., 2015. Isolation and Characterization of *Tenacibaculum maritimum* for Autogenous Vaccine Development.

Rahman, T., Suga, K., Kanai, K., Sugihara, Y., 2014. Biological and serological characterization of a non-gliding strain of *Tenacibaculum maritimum* isolated from a diseased puffer fish *Takifugu rubripes*. 魚病研究 49, 121–129. https://doi.org/10.3147/jsfp.49.121.

Rahman, T., Suga, K., Kanai, K., Sugihara, Y., 2015. Infection kinetics of *Tenacibaculum maritimum* on the abraded skin of Japanese flounder *Paralichthys olivaceus*. 魚病研究 50, 44–52. https://doi.org/10.3147/jsfp.50.44.

Reverter, M., Sarter, S., Caruso, D., Avarre, J.-C., Combe, M., Pepey, E., Pouyaud, L., Vega-Heredía, S., De Verdal, H., Gozlan, R.E., 2020. Aquaculture at the crossroads of global warming and antimicrobial resistance. Nat. Commun. 11, 1870. https://doi.org/10.1038/s41467-020-15735-6.

Rochat, T., Fujiwara-Nagata, E., Calvez, S., Dalsgaard, I., Madsen, L., Calteau, A., Lunazzi, A., Nicolas, P., Wiklund, T., Bernardet, J.F., Duchaud, E., 2017. Genomic characterization of *Flavobacterium psychrophilum* serotypes and development of a multiplex PCR-based serotyping scheme. Front. Microbiol. 8, 1752. https://doi.org/10.3389/fmicb.2017.01752.

Romalde, J.L., Ravelo, C., López-Romalde, S., Avendaño-Herrera, R., Magariños, B., Toranzo, A.E., 2005. Vaccination strategies to prevent emerging diseases for Spanish aquaculture. Dev. Biol. 121, 85–95.

Sanford, 2022. King salmon farming [Online]. Available. https://www.sanford.co.nz/operations/aquaculture/salmon-farming/ [Accessed 19 November 2022].

Shirajum Monir, M., Yusoff, S.M., Mohamad, A., Ina-Salwany, M.Y., 2020. Vaccination of tilapia against motile Aeromonas septicemia: A review. J. Aquat. Anim. Health 32, 65–76. https://doi.org/10.1002/aah.10099.

Sloan, A., Wang, G., Cheng, K., 2017. Traditional approaches versus mass spectrometry in bacterial identification and typing. Clin. Chim. Acta 473, 180–185. https://doi.org/10.1016/j.cca.2017.08.035.

Småge, S.B., Frisch, K., Brevik, Ø.J., Watanabe, K., Nylund, A., 2016. First isolation, identification and characterisation of *Tenacibaculum maritimum* in Norway, isolated from diseased farmed sea lice cleaner fish *Cyclopterus lumpus L.* Aquaculture 464, 178–184. https://doi.org/10.1016/j.aquaculture.2016.06.030.

Stenutz, R., Weintraub, A., Widmalm, G., 2006. The structures of *Escherichia coli* O-polysaccharide antigens. FEMS Microbiol. Rev. 30, 382–403. https://doi.org/10.1111/j.1574-6976.2006.00016.x.

Suzuki, M., Nakagawa, Y., Harayama, S., Yamamoto, S., 2001. Phylogenetic analysis and taxonomic study of marine Cytophaga-like bacteria: proposal for *Tenacibaculum* gen. nov. with *Tenacibaculum maritimum* comb. nov. and *Tenacibaculum ovolyticum* comb. nov., and description of *Tenacibaculum mesophilum* sp. nov. and *Tenacibaculum amylolyticum* sp. nov. Int. J. Syst. Evol. Microbiol. 51, 1639–1652. https://doi.org/10.1099/00207713-51-5-1639.

Tinsley, J.W., Lyndon, A.R., Austin, B., 2011. Antigenic and cross-protection studies of biotype 1 and biotype 2 isolates of *Yersinia ruckeri* in rainbow trout, *Oncorhynchus mykiss* (Walbaum). J. Appl. Microbiol. 111, 8–16. https://doi.org/10.1111/j.1365-2672.2011.05020.x.

Valdes, S., Irgang, R., Barros, M.C., Ilardi, P., Saldarriaga-Córdoba, M., Rivera-Bohle, J., Madrid, E., Gajardo-Córdova, J., Avendaño-Herrera, R., 2021. First report and characterization of *Tenacibaculum maritimum* isolates recovered from rainbow trout (*Oncorhynchus mykiss*) farmed in Chile. J. Fish Dis. 44, 1481–1490. https://doi.org/10.1111/jfd.13466.

Van Gelderen, R., Carson, J., Gudkovs, N., Nowak, B., 2010. Physical characterisation of *Tenacibaculum maritimum* for vaccine development. J. Appl. Microbiol. 109, 1668–1676. https://doi.org/10.1111/j.1365-2672.2010.04795.x.

Vilar, P., Faílde, L.D., Bermúdez, R., Vigliano, F., Riaza, A., Silva, R., Santos, Y., Quiroga, M.I., 2012. Morphopathological features of a severe ulcerative disease outbreak associated with *Tenacibaculum maritimum* in cultivated sole, *Solea senegalensis* (L.). J. Fish Dis. 35, 437–445. https://doi.org/10.1111/j.1365-2761.2012.01360.x.

Wakabayashi, H., Hikida, M., Masumura, K., 1986. *Flexibacter maritimus* sp. nov., a pathogen of marine fishes. Int. J. Syst. Bacteriol. 36, 396–398. https://doi.org/10.1099/00207713-36-3-396.

Wang, J.-T., Chou, Y.-J., Chou, J.-H., Chen, C.A., Chen, W.-M., 2008. *Tenacibaculum aiptasiae* sp. nov., isolated from a sea anemone *Aiptasia pulchella*. Int. J. Syst. Evol. Microbiol. 58, 761–766. https://doi.org/10.1099/ijs.0.65437-0.

Whitfield, C., Roberts, I.S., 1999. Structure, assembly and regulation of expression of capsules in *Escherichia coli*. Mol. Microbiol. 31, 1307–1319. https://doi.org/10.1046/j.1365-2958.1999.01276.x.

Whitfield, C., Trent, M.S., 2014. Biosynthesis and export of bacterial lipopolysaccharides. Annu. Rev. Biochem. 83, 99–128. https://doi.org/10.1146/annurev-biochem-060713-035600.

Whitfield, C., Williams, D.M., Kelly, S.D., 2020. Lipopolysaccharide O-antigens-bacterial glycans made to measure. J. Biol. Chem. 295, 10593–10609. https://doi.org/10.1074/jbc.REV120.009402.

Wilson, T.K., Douglas, M., Dunn, V., 2019. First identification in Tasmania of fish pathogens *Tenacibaculum dicentrarchi* and *T. soleae* and multiplex PCR for these organisms and *T. maritimum*. Dis. Aquat. Organ. 136, 219–226. https://doi.org/10.3354/dao03407.

Wyres, K.L., Wick, R.R., Gorrie, C., Jenney, A., Follador, R., Thomson, N.R., Holt, K.E., 2016. Identification of *Klebsiella* capsule synthesis loci from whole genome data. Microbial Genomics 2, e000102. https://doi.org/10.1099/mgen.0.000102.

Yardımcı, R.E., Timur, G., 2015. Isolation and identification of *Tenacibaculum maritimum*, the causative agent of Tenacibaculosis in farmed sea bass (*Dicentrarchus labrax*) on the Aegean Sea coast of Turkey. Israeli Journal of Aquaculture-Bamidgeh 67. https://doi.org/10.46989/001c.20708.

Yardimci, R., Timur, G., 2016. Antigenic characterisation of *Tenacibaculum maritimum* isolates from sea bass (*Dicentrarchus labrax, L.*) farmed on the Aegean Sea coasts of Turkey. J. Aquac. Res. Dev. 7, 1–4. https://doi.org/10.4172/2155-9546.1000408.

Zhang, D., Ke, X., Liu, Z., Cao, J., Su, Y., Lu, M., Gao, F., Wang, M., Yi, M., Qin, F., 2019. Capsular polysaccharide of *Streptococcus agalactiae* is an essential virulence factor for infection in Nile tilapia (*Oreochromis niloticus Linn.*). J. Fish Dis. 42, 293–302. https://doi.org/10.1111/jfd.12935.

Zhao, Y., Yang, Q.E., Zhou, X., Wang, F.-H., Muurinen, J., Virta, M.P., Brandt, K.K., Zhu, Y.-G., 2021. Antibiotic resistome in the livestock and aquaculture industries: status and solutions. Crit. Rev. Environ. Sci. Technol. 51, 2159–2196. https://doi.org/10.1080/10643389.2020.1777815.