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RESEARCH ARTICLE

Novel computational One Health analysis of fibrinogen-binding protein A in *Streptococcus agalactiae* isolated from fish and human cases

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ABSTRACT

Background and Aim: *Streptococcus agalactiae*, also known as Group B *Streptococcus* (GBS), is a zoonotic pathogen implicated in severe infections in humans and aquatic animals, particularly fish. Recent outbreaks of GBS sequence type 283 in Southeast Asia, associated with raw fish consumption, have raised public health concerns. Despite growing evidence of zoonotic transmission, comparative studies investigating virulence factor homology across hosts remain limited. This study focuses on the fibrinogen-binding protein A (FbsA), a critical virulence determinant in GBS pathogenesis. This study aimed to conduct a novel computational One Health analysis comparing the FbsA protein from fish- and human-derived GBS strains to elucidate their structural and functional similarities and explore their interaction with human fibrinogen (Fg).

Materials and Methods: Amino acid sequences of FbsA from fish and human GBS isolates were retrieved from the National Center for Biotechnology Information database. Sequence alignment was performed using the MUSCLE algorithm in Molecular Evolutionary Genetics Analysis (MEGA) software. Three-dimensional structures were predicted through ColabFold and validated using Ramachandran plots. Residue profiling and structural visualization were conducted in PyMOL. Molecular docking between FbsA and Aα (G), Bβ (H), and γ (I) chains of human Fg was performed using ClusPro, followed by interaction analysis using LigPlot+.

Results: Sequence alignment revealed 100% identity between fish and human-derived FbsA, indicating a conserved evolutionary relationship. The refined FbsA structure demonstrated α-helices and random coils with six repetitive regions. Molecular docking confirmed robust binding between FbsA and the D fragment of human Fg, involving key critical residues within the repetitive region (residues 45th–60th). Notably, interaction sites also included the $\beta_{119–129}$ regions, overlapping with the plasmin cleavage site, potentially contributing to endocarditis and septicemia pathogenesis.

Conclusion: The structural and functional equivalence of fish- and human-derived FbsA underscores the zoonotic risk of GBS transmission. These findings offer a compelling foundation for targeted vaccine development and preventative strategies to mitigate GBS infections across species boundaries.

Keywords: fibrinogen-binding protein A, fish-to-human transmission, *in silico* analysis, molecular docking, One Health, *Streptococcus agalactiae*, zoonosis.

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INTRODUCTION

Streptococcus agalactiae is a Gram-positive bacterium classified within the Group B *Streptococcus* (GBS) group [1]. In the 1930s, Rebecca Lancefield identified *S. agalactiae* as a distinct streptococcal species following its isolation from milk samples and cows affected by bovine mastitis [2]. GBS can be categorized into ten serotypes based on its capsular polysaccharide composition, namely Ia, Ib, and II to IX, with serotypes Ia, III, and V being most frequently associated with clinical disease. The multilocus sequence typing (MLST) method provides further resolution in classifying GBS, and currently, 134 sequence types (STs) have been identified based on this technique [3]. MLST analyzes allele profiles of seven conserved genes to assign GBS strains to specific STs [4].

GBS has emerged as a significant pathogen in both humans and animals, particularly in aquatic species such as fish. In neonates, GBS infection can result in pneumonia, meningitis, and septicemia during either the intrapartum or postpartum periods, as the bacterium commonly colonizes the gastrointestinal tract of pregnant women [5]. Wang *et al*. [6] have also noted GBS as an increasingly recognized cause of endocarditis. Comparable clinical manifestations, including endocarditis, meningoencephalitis, and septicemia, have been observed in fish infected with GBS [7, 8].

Recent investigations have highlighted the zoonotic potential of GBS, particularly through unprecedented outbreaks of the ST283 strain in Singapore (2015) and Malaysia (2023). These outbreaks, linked to the consumption of raw fish, emphasize the urgent need for integrative One Health approaches to elucidate cross-species transmission dynamics. The emergence of these cases has been attributed to the ingestion of GBS-contaminated animal-derived food, notably raw fish carrying the ST283 strain [9, 10]. This zoonosis represents a growing public health concern in Southeast Asia [11]. Rajendram *et al*. [9] further elucidate that the zoonotic transmission of ST283 is associated with genetic similarity in virulence factors between isolates from fish and humans. However, there remains a scarcity of One Health-focused studies assessing GBS virulence factors across hosts.

Fibrinogen-binding protein A (FbsA), a key virulence determinant of GBS, facilitates adhesion to host cells through its interaction with fibrinogen (Fg). In addition, FbsA impairs host immune defense by inhibiting complement factor C3b binding, thereby blocking activation of the alternative complement pathway [12–15]. This protein also poses a particular threat to fetuses, who depend exclusively on an underdeveloped, non-specific innate immune system for protection against postnatal infections, rendering them especially vulnerable [12, 16].

The emergence of GBS ST283 cases instigated a One Health investigation into the FbsA of *S. agalactiae* strains isolated from both fish and human cases. Biocomputational analysis was utilized to obtain relevant insights. This methodological approach offers advantages in reducing both time and cost, thereby enabling more rapid acquisition of essential information. As a result, preventive measures against zoonotic transmission can be implemented at earlier stages [17].

Despite increasing evidence of zoonotic transmission of *S. agalactiae* – particularly the ST283 – across fish and human hosts, there remains a paucity of integrative One Health studies that focus on the molecular and structural characterization of key virulence factors. In particular, the FbsA, which plays a pivotal role in host adhesion and immune evasion, has not been comprehensively investigated for its structural conservation and interaction dynamics between isolates derived from different host species. Most existing studies emphasize epidemiological or clinical perspectives, while molecular-level comparative analyses that could reveal potential zoonotic mechanisms are largely lacking. Furthermore, *in silico* methods have not been fully leveraged to elucidate the interaction between FbsA and human Fg, which may contribute to severe clinical outcomes such as endocarditis and septicemia.

The present study aims to conduct a novel computational One Health analysis of the FbsA from *S. agalactiae* strains isolated from both fish and human cases. By comparing amino acid sequences, predicting and validating three-dimensional (3D) protein structures, and performing molecular docking with human Fg, this study seeks to uncover structural similarities and interaction patterns that may facilitate cross-species transmission. The findings are expected to enhance understanding of the molecular basis of zoonotic potential in GBS ST283 and provide a foundation for the development of targeted preventative strategies, including vaccine design.

MATERIALS AND METHODS

Ethical approval

The study used completely open-source biocomputational databases that were publicly accessible and no human, experimental animals or other living organisms were used. Therefore, no ethical approval was necessary.

Study period and location

The study was conducted from January to March 2024 at the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Lampung, Bandar Lampung, Indonesia.

Sample collection and computational study tools

This study utilized a novel computational pipeline that integrated publicly available FbsA protein

sequences of *S. agalactiae* from both fish and human isolates [National Center for Biotechnology Information (NCBI) accession numbers ATZ89387 and AMQ16627, respectively] obtained from the National Center for Biotechnology Information (NCBI). These sequences were analyzed in conjunction with molecular docking simulations involving the crystal structure of human Fg (Protein Data Bank [PDB] ID: 3GHG) (The Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB), USA). The integration of molecular evolutionary genetic analysis (MEGA) (Pennsylvania State University, USA), ColabFold (Jupyter Notebook hosted by Google Colaboratory, California, USA), and ClusPro (Boston University, Massachusetts, USA) platforms enabled high-resolution analysis of protein interactions across species. All procedures in this study were conducted through a computational approach using a personal computer equipped with an Intel® Core™ i3-3227U (Intel Corporation, California, USA) Central Processing Unit operating at 1.90 GHz, 8.00 GB of Random Access Memory, a 512 GB Solid State Drive, a 64-bit architecture, and the Windows™ 10 operating system (Microsoft Corporation, Washington, USA).

Amino acid sequence alignment

The amino acid sequences of FbsA GBS derived from fish and human cases were aligned using the MUSCLE algorithm implemented in MEGA software version 11.0.13, with default parameters applied to assess sequence similarity [18]. The parameters used included a Gap Open value of −2.90, a Gap Extend of 0.00, and a hydrophobicity multiplier of 1.20. The resulting alignment was saved in FASTA format (.FASTA). The alignment output was subsequently visualized using the NCBI multiple sequence alignment (MSA) Viewer (version 1.25.0) (https://www.ncbi.nlm.nih.gov/projects/msaviewer/) (NCBI, Maryland, USA) [19]. If the alignment revealed 100% sequence similarity, the fish-derived FbsA GBS sequence was selected for further analysis. In contrast, if discrepancies were identified, both sequences would be subjected to additional comparative analysis.

Prediction of the 3D protein structure of FbsA GBS and validation using the Ramachandran plot

ColabFold version 1.5.5 (https://colab.research.google.com/github/sokrypton/ColabFold/blob/main/AlphaFold2.ipynb) (Jupyter Notebook) was utilized to predict the 3D structure of the FbsA protein from *S. agalactiae* GBS [20]. The modeling process employed a num_relax parameter value of 5, while all other settings remained at their default values. ColabFold generated five models, both relaxed and unrelaxed. The model with the highest-ranking post-relaxation was selected as the optimal structure. This selection was based on various computational metrics, including MSA depth, sequence diversity, and confidence indicators provided by AlphaFold2, specifically the predicted local distance difference test (pLDDT) and predicted alignment error (PAE) score [21]. The relaxed model was chosen due to its enhanced structural quality, achieved through automated energy minimization, thereby improving spatial and energetic conformity relative to the native protein structure [22].

To validate the selected 3D model, the Ramachandran plot was generated using the UCLA-DOE LAB-SAVES server version 6.0 (https://saves.mbi.ucla.edu/) (University of California, Los Angeles, California, USA) [23–26]. The evaluation was based on the distribution of residues across four defined regions: Most favored (quadrant I), additionally allowed (quadrant II), generously allowed (quadrant III), and disallowed (quadrant IV). A high-quality structure was defined by having more than 90% of residues in quadrant I, and no more than 15% of non-glycine residues in quadrant IV. A value approaching 0.0% in quadrant IV is indicative of a structurally sound model [24, 26–28]. If the Ramachandran plot yielded results below the 90% threshold in quadrant I and exceeded 15% in quadrant IV, the structure was subjected to refinement using the ModRefiner server (https://zhanggroup.org/ModRefiner/) (University of Michigan Medical School, Michigan, USA) [29].

Exploration residue profile of FbsA GBS

To explore the residue profile of FbsA GBS, PyMOL software (Schrödinger, Inc., New York, USA) was employed [30]. In this section, the types of amino acid residues constituting the FbsA GBS protein were examined. These residues were categorized into three groups: Hydrophobic residues (non-polar), uncharged hydrophilic residues (polar), and charged hydrophilic residues (polar) [31]. The identification and visualization of these residues followed the guidelines provided by PyMOLWiki (Harvard Medical School, Harvard University, Massachusetts, USA) [32], using the following selection commands:

1. Hydrophobic residues: Sele resn gly + ala + val + leu + ile + met + pro + phe + trp
2. Uncharged hydrophilic residues: Sele resn cys + ser + thr + tyr + asn + gln
3. Charged hydrophilic residues: Sele resn lys + arg + his + asp + glu.

Molecular docking

A molecular docking study was conducted to examine the interaction patterns between FbsA GBS and human Fg. Before docking, the human Fg molecule was prepared using BIOVIA Discovery Studio Visualizer version 21.1.0 (BIOVIA, California, USA) [33]. The crystal structure of Fg (PDB ID: 3GHG) contains two symmetrical sets of chains, designated as A–F and G–L. Each set comprises the Aα, Bβ, and γ chains. As the A–F chains exhibited greater residue loss, the G–L chains were selected for this study. Specifically, chains G and J represent Aα, chains H and K correspond to Bβ, and

chains I and L represent the γ chains, respectively [34]. Following the removal of the A–F chains, polar hydrogens were added, and native ligands along with water molecules were also removed.

Molecular docking was carried out using the ClusPro server version 2.0 (Boston University, Massachusetts, USA) (https://cluspro.org/login.php) [35–39]. The FbsA GBS structure was uploaded as the ligand in PDB format (.pdb), while the prepared human Fg structure was uploaded as the receptor, also in PDB format. The optimal docking model was selected based on the presence of interactions involving 16 residues located within the repetitive regions of FbsA GBS. Special consideration was given to a defined group of critical residues, as they play an essential role in Fg binding. The consensus sequence for these 16 residues is G-[N/S/T]-V-L-[A/E/M/Q]-R-R-X-[K/R/W]-[A/D/E/N/Q]-[A/F/I/L/V/Y]-X-X-[K/R]-X-X, where residues N2, V3, L4, R6, and R7 are identified as critical [40]. Selection criteria for the best docking model also included a balanced score and a root-mean-square deviation (RMSD) value of <2,000 Å [35–39, 41]. The selected docking model was subsequently visualized in three dimensions using PyMOL to investigate the binding site. Interaction analyses were conducted in terms of involved residues and functional groups, types of non-bonding interactions, hydrogen bonds, and hydrogen bond distances (in Å) using LigPlot+ version 2.2.8 through the Dimplot program (European Molecular Biology Laboratory - European Bioinformatics Institute (EMBL - EBI, Cambridgeshire, UK).

RESULTS

Two sets of FbsA GBS amino acid sequences, derived from fish and human cases, were aligned to evaluate their similarity. The alignment was conducted using the MUSCLE algorithm within MEGA software version 11.0.13. Subsequently, the alignment output in FASTA format was uploaded to the NCBI MSA Viewer version 1.25.0 for visualization. The computational analysis demonstrated a novel finding - 100% amino acid sequence identity between fish- and human-derived FbsA GBS strains (Figure 1). The absence of mutations, including substitutions or insertions/deletions, suggests the presence of a conserved evolutionary mechanism that may support zoonotic transmission.

In light of the observed 100% sequence similarity between FbsA GBS isolates from fish and human tissues, the fish-derived FbsA GBS was selected for subsequent analysis. This decision was consistent with the primary objective of the study: To investigate the infectivity of GBS in fish and its potential for transmission to humans, with a particular focus on the virulence factor FbsA.

The 3D protein structure of the fish-derived FbsA GBS was predicted using the ColabFold server version 1.5.5 (Jupyter Notebook). The server generated five structural models, both relaxed and unrelaxed. Based on the output from ColabFold, the relaxed model with the highest structural similarity to the native protein was selected for further evaluation (Figure 2). The quality of the selected model was validated using

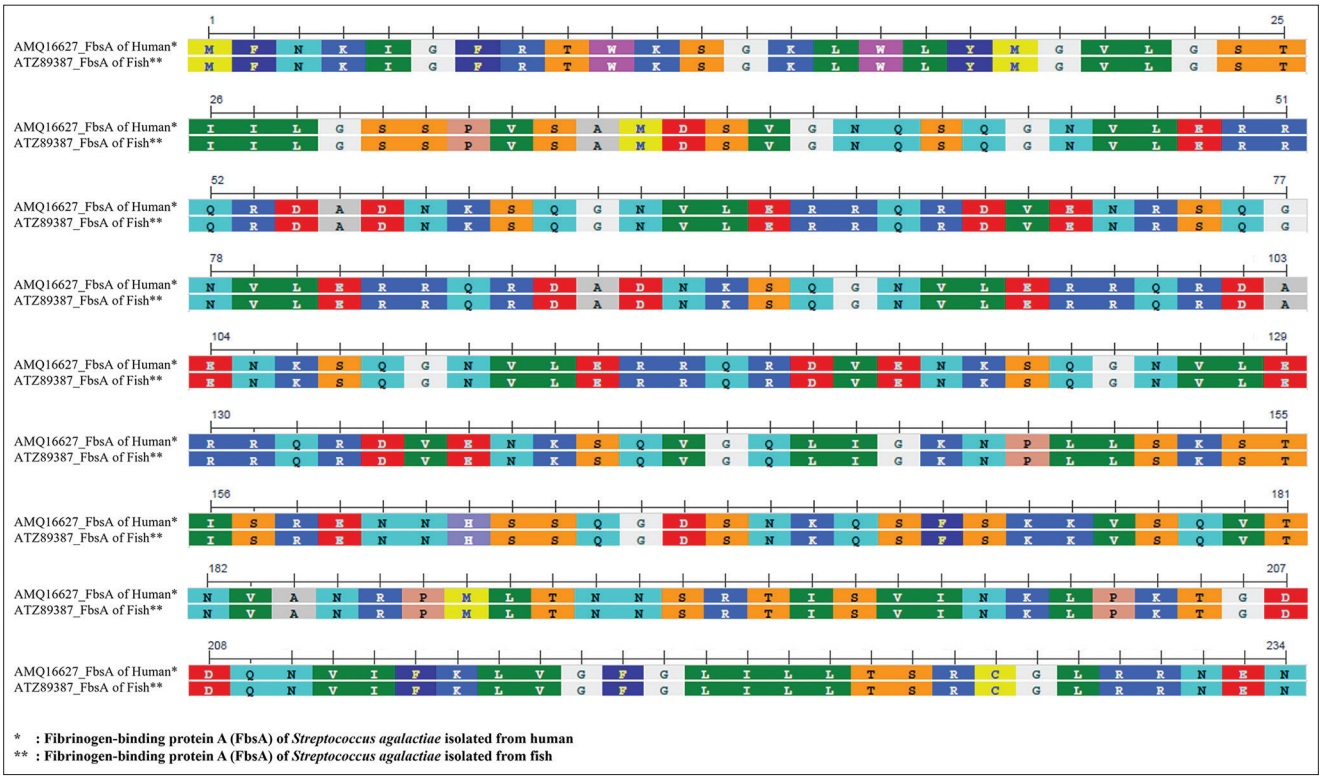

Figure 1: Amino acid sequence alignment of fibrinogen-binding protein A Group B *Streptococcus* derived from fish and human case. The results demonstrated that both possess a similarity of 100%. No mutation, such as substitution, insertion, or deletion.

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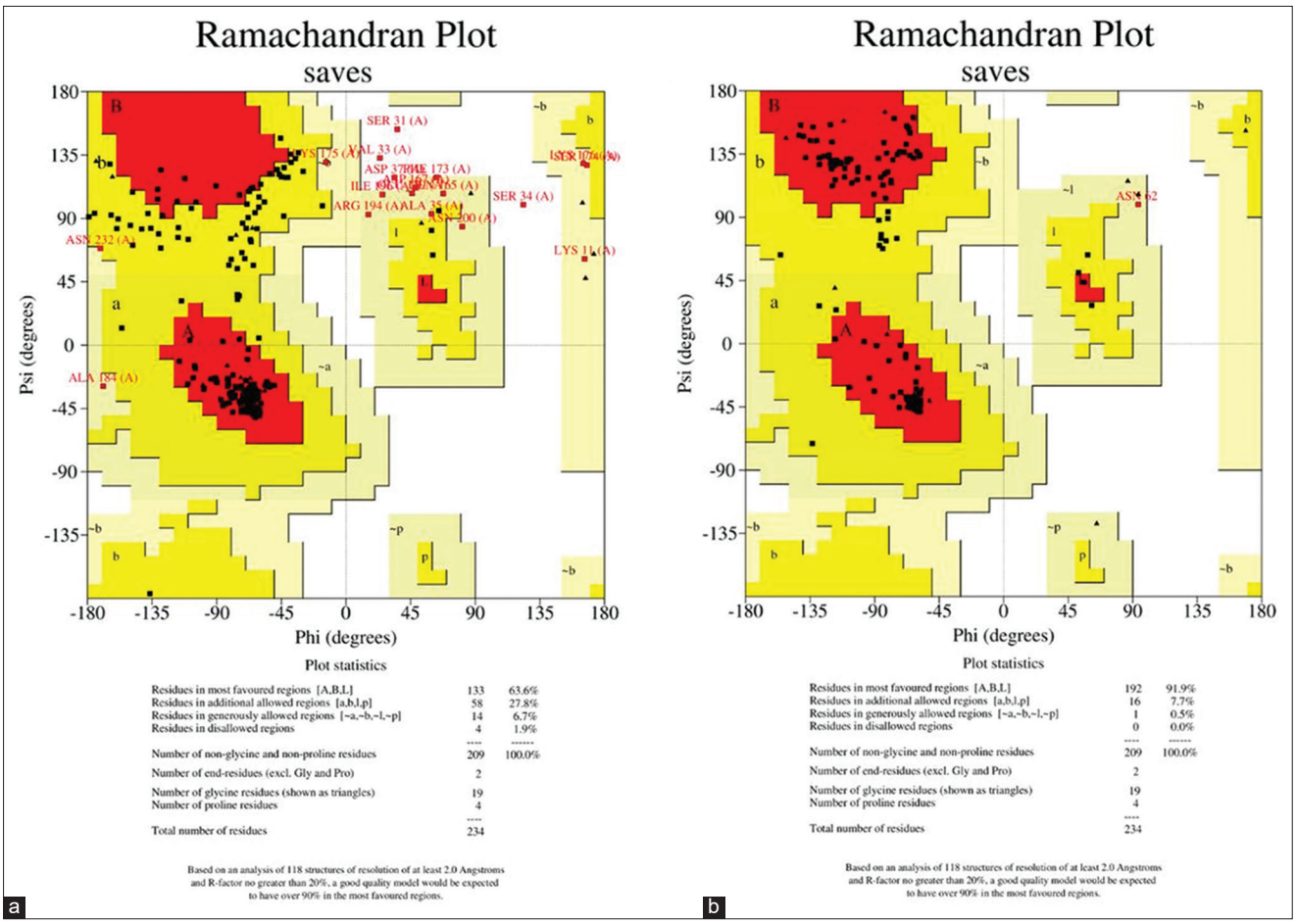

Figure 2: The result of Plot Ramachandran validation of fish-derived fibrinogen-binding protein A Group B *Streptococcus*. (a) Before protein structure repair. (b) After protein structure repair.

a Ramachandran plot. Figure 2a presents the results, showing that only 63.6% of the residues were located in the most favored regions (quadrant I), which falls below the 90% threshold required for a high-quality 3D protein model.

After refining the protein structure and revalidating it using the same validation server, the improved model showed a substantial increase in the percentage of residues located in the most favored region (quadrant I), rising from 63.6% to 91.9% (Figure 2b). In addition, the percentage of residues in the disallowed region (quadrant IV) dropped to 0.0%, indicating that the corrected model is structurally valid and suitable for subsequent analysis. The validated 3D structure was then visualized using PyMOL (Figure 3). As illustrated in Figure 3a, the tertiary structure of the fish-derived FbsA GBS consists exclusively of alpha-helices and random coils, with no beta-sheet structures observed. The residues forming this structure were predicted to span six repetitive regions, ranging from the 45th to the 140th amino acid position (Figure 1).

Figure 3b depicts the distribution of hydrophobic (nonpolar) residues, totaling 87 and shown in yellow. These include methionine (Met), phenylalanine (Phe), isoleucine (Ile), glycine (Gly), tryptophan (Trp), valine (Val), alanine (Ala), leucine (Leu), and proline (Pro) (Table 1). Figure 3c displays the 80 uncharged hydrophilic (polar) residues, shown in blue, comprising asparagine (Asn), threonine (Thr), serine (Ser), tyrosine (Tyr), glutamine (Gln), and cysteine (Cys) (Table 1). The charged hydrophilic (polar) residues are shown in Figure 3d and include lysine (Lys), arginine (Arg), histidine (His), aspartate (Asp), and glutamate (Glu), totaling 67 residues in the fish-derived FbsA GBS (Table 1).

A molecular docking study was then conducted using the ClusPro server, which generated 29 docking models. Each model was evaluated to identify the optimal interaction profile. The selection criteria focused on models that featured interactions involving the 16 residues within the repetitive regions of FbsA, particularly those classified as critical for binding to human Fg chains. In addition, the model with the lowest balanced energy score was preferred. Ultimately, the 25th model was selected as the best, exhibiting a balanced score of −1221.9 kcal/mol and an RMSD value of 1.649 Å (data not shown).

Figure 4 presents the 3D visualization of the interaction between fish-derived FbsA GBS and the human Fg chains, as visualized using PyMOL software. The fish-derived FbsA GBS was observed to interact with the G, H, and I chains of human Fg, which correspond

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Table 1: Profile of the types of residues containing fish-derived fibrinogen-binding protein A Group B *Streptococcus* (FbsA GBS).

Types of residues	Total	Information
Hydrophobic (non-polar)	87	Met1, Phe2, Ile5, Gly6, Phe7, Trp10, Gly13, Leu15, Trp16, Leu17, Met19, Gly20, Val21, Leu22, Gly23, Ile26, Ile27, Leu28, Gly29, Pro32, Val33, Ala35, Met36, Val39, Gly40, Gly45, Val47, Leu48, Ala55, Gly61, Val63, Leu64, Val71, Gly77, Val79, Leu80, Ala87, Gly93, Val95, Leu96, Ala103, Gly109, Val111, Leu112, Val119, Gly125, Val127, Leu128, Val135, Val141, Gly142, Leu144, Ile145, Gly146, Pro149, Leu 150, Leu151, Ile156, Gly166, Phe173, Val177, Val180, Val183, Ala184, Pro187, Met188, Leu189, Ile196, Val198, Ile199, Leu202, Pro203, Gly206, Val211, Ile212, Phe213, Leu215, Val216, Gly217, Phe218, Gly219, Leu220, Ile221, Leu222, Leu223, Gly228, Leu229
Uncharged hydrophilic (polar)	80	Asn3, Thr9, Ser12, Tyr18, Ser24, Thr25, Ser30, Ser31, Ser34, Ser38, Asn41, Gln42, Ser43, Gln44, Asn46, Gln52, Asn57, Ser59, Gln60, Asn62, Gln68, Asn73, Ser75, Gln76, Asn78, Gln84, Asn89, Ser91, Gln92, Asn94, Gln100, Asn105, Ser107, Gln108, Asn110, Gln116, Asn121, Ser123, Gln124, Ser126, Gln132, Asn137, Ser139, Gln140, Gln143, Asn148, Ser152, Ser154, Thr155, Ser157, Asn160, Asn161, Ser163, Ser164, Gln165, Ser168, Asn169, Gln171, Ser172, Ser174, Ser178, Gln179, Thr181, Asn182, Asn185, Thr190, Asn191, Asn192, Ser193, Thr195, Ser197, Asn200, Thr205, Gln209, Asn210, Thr224, Ser225, Cys227, Ser232, Ser234
Charged hydrophilic (polar)	67	Lys4, Arg8, Lys11, Lys14, Asp37, Glu49, Arg50, Arg51, Arg53, Asp54, Asp56, Lys58, Glu65, Arg66, Arg67, Arg69, Asp70, Glu72, Arg74, Glu81, Arg82, Arg83, Arg85, Asp86, Asp88, Lys90, Glu97, Arg98, Arg99, Arg101, Asp102, Glu104, Lys106, Glu113, Arg114, Arg115, Arg117, Asp118, Glu120, Lys122, Glu129, Arg130, Arg131, Arg133, Asp134, Glu136, Lys138, Lys147, Lys153, Arg158, Glu159, His162, Asp167, Lys170, Lys175, Lys176, Arg186, Arg194, Lys201, Lys204, Asp207, Asp208, Lys214, Arg226, Arg230, Arg231, Glu233

GBS=Group B *Streptococcus*, FbsA=Fibrinogen-binding protein A, Met=Methionine, Phe=Phenylalanine, Ile=Isoleucine, Gly=Glycine, Trp=Tryptophan, Val=Valine, Ala=Alanine, Leu=Leucine, Pro=Proline, Asn=Asparagine, Thr=Threonine, Ser=Serine, Tyr=Tyrosine, Gln=Glutamine, Cys=Cysteine, Lys=Lysine, Arg=Arginine, His=Histidine, Asp=Aspartate, Glu=Glutamate

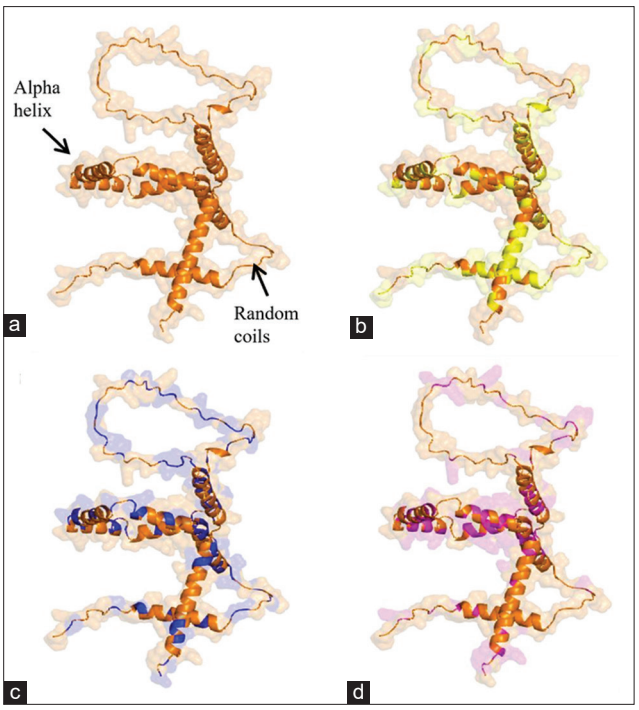

Figure 3: Three-dimensional structure of fish-derived fibrinogen-binding protein A Group B *Streptococcus*. (a) Structure including all residues. (b) Structure including hydrophobic residues (yellow). (c) Structure including uncharged hydrophilic residues (blue). (d) Structure including charged hydrophilic residues (magenta).

to the Aα, Bβ, and γ chains, respectively. Notably, an interaction was identified within the Bβ chain at residues 119–129 ($\beta_{119–129}$), which are highlighted in gray.

The interaction analysis also included a two-dimensional (2D) visualization, illustrating the specific residues and functional groups involved, types of interactions, and bond distances in the complex formed between FbsA GBS and the Aα, Bβ, and γ chains of human Fg (Figures 5-7, respectively). To support interpretation, these 2D interaction data were also provided in Tables 2-4. Based on the interacting residues, it was inferred that the fish-derived FbsA GBS binds to the D fragment of human Fg, as the majority of interacting residues were localized within this region – supporting previous findings by Pietrocola *et al*. [42].

In addition, three specific interaction profiles were analyzed: (1) fish-derived FbsA GBS with the G chain (Aα) of human Fg (FbsA-G chain), (2) with the H chain (Bβ) (FbsA-H chain), and (3) with the I chain (γ) (FbsA-I chain). In the FbsA-G chain interaction (Table 2), 13 hydrogen bonds were identified, along with several non-bonding interactions. Four residues from the repetitive region – Gly45, Asn46, Leu48, and Gln52 – were involved in this interaction, with Gly45, Asn46, and Leu48 classified as critical residues.

In the FbsA–H chain interaction (Table 3), 10 hydrogen bonds and one electrostatic interaction (salt bridge) were observed. Three residues from the repetitive region – Val47, Leu48, and Arg51 – participated in this binding, along with six residues from the $\beta_{119–129}$ region (Leu120, Leu121, Lys122, Trp125, Arg128, and Gln129), which were highlighted in blue.

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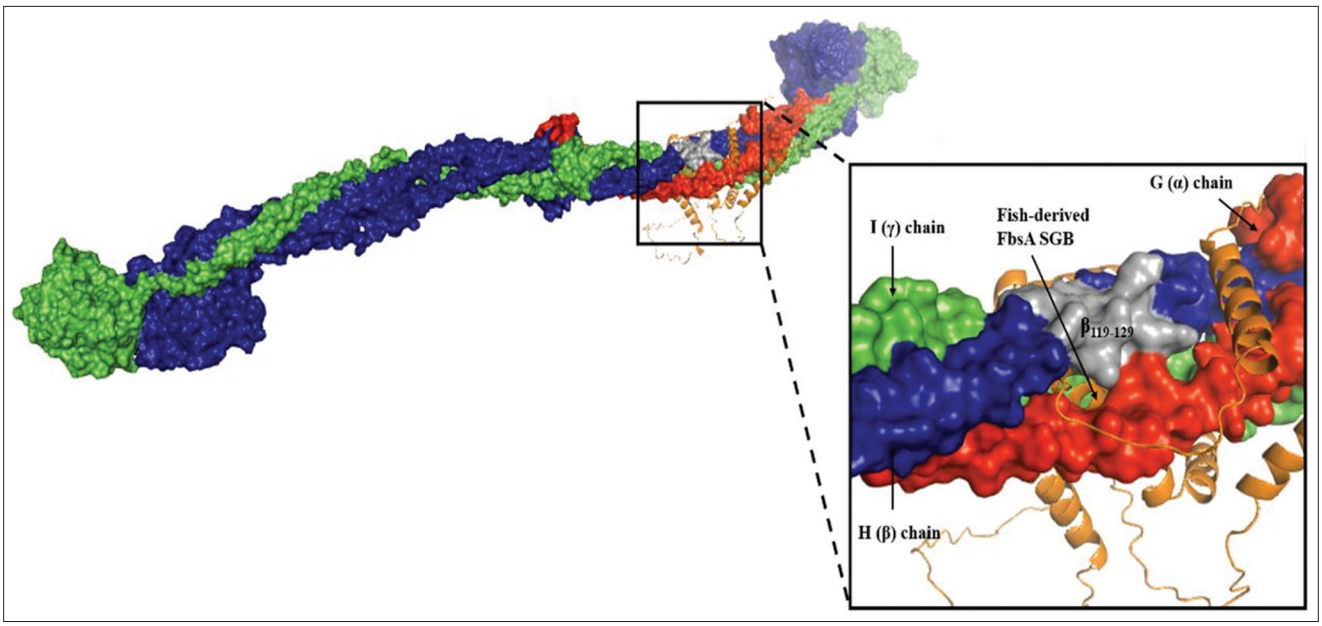

Figure 4: Three-dimensional visualization of the interaction between fish-derived fibrinogen-binding protein A Group B *Streptococcus* and human fibrinogen chains. The interaction sites predominantly involve the D fragment and the $\beta_{119\text{-}129}$ region, critical for plasmin binding.

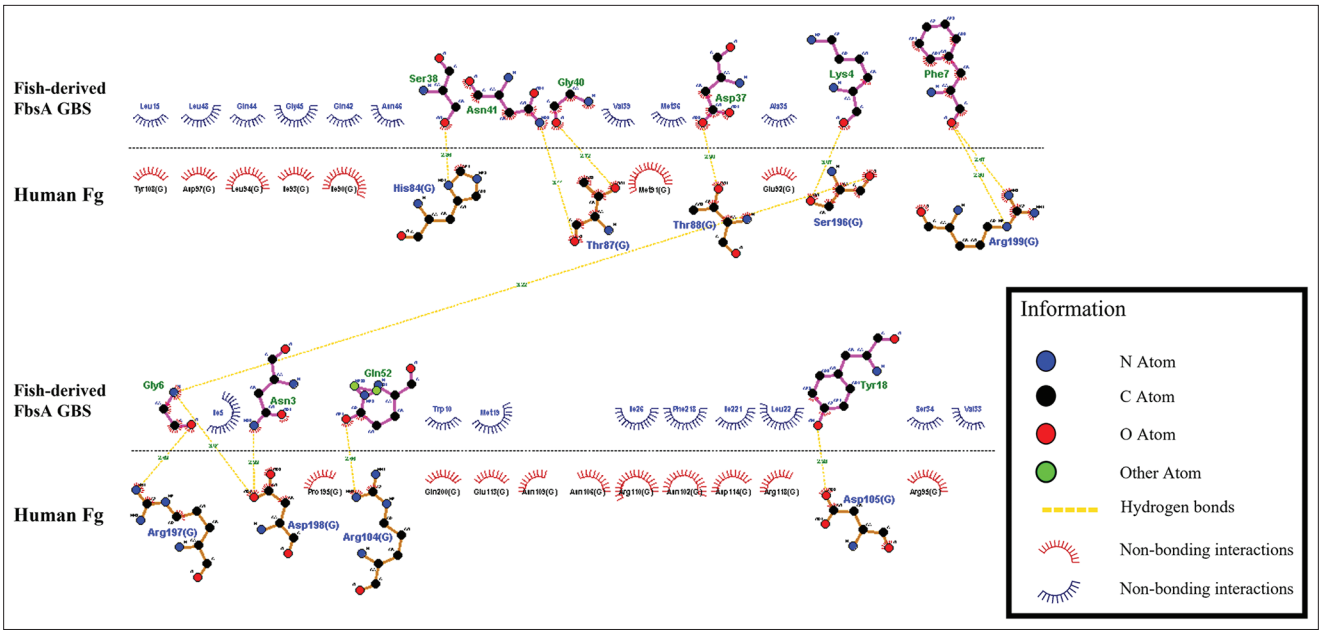

Figure 5: Two-dimensional visualization result of fibrinogen-binding protein A-G chain interaction. The results revealed that the interactions were stabilized by 13 hydrogen bonds (dotted yellow line) and non-bonding interactions.

Non-bonding interactions were also present.

The FbsA-I chain interaction (Table 4) revealed 13 hydrogen bonds and two salt bridges. Repetitive region residues involved included Asn46, Val47, Arg50, Arg51, Asp54, Lys58, Arg130, and Arg131, of which Asn46, Val47, Arg50, Arg51, Arg130, and Arg131 were identified as critical. Non-bonding interactions were likewise observed.

Collectively, these findings suggest that the repetitive regions of fish-derived FbsA GBS were predominantly composed of critical residues, most of which were localized between positions 45 and 60 of the protein sequence.

DISCUSSION

S. agalactiae, commonly referred to as GBS, is a significant pathogen affecting both humans and animals, particularly fish. In newborns, GBS can cause pneumonia, meningitis, and septicemia during the intrapartum and postpartum periods by colonizing the gastrointestinal tract of pregnant women [5]. In addition, this bacterium has been reported to cause endocarditis [6]. Fish infected with GBS exhibit similar clinical manifestations, including endocarditis, meningoencephalitis, and septicemia [7, 8]. Notable zoonotic outbreaks associated with raw fish consumption

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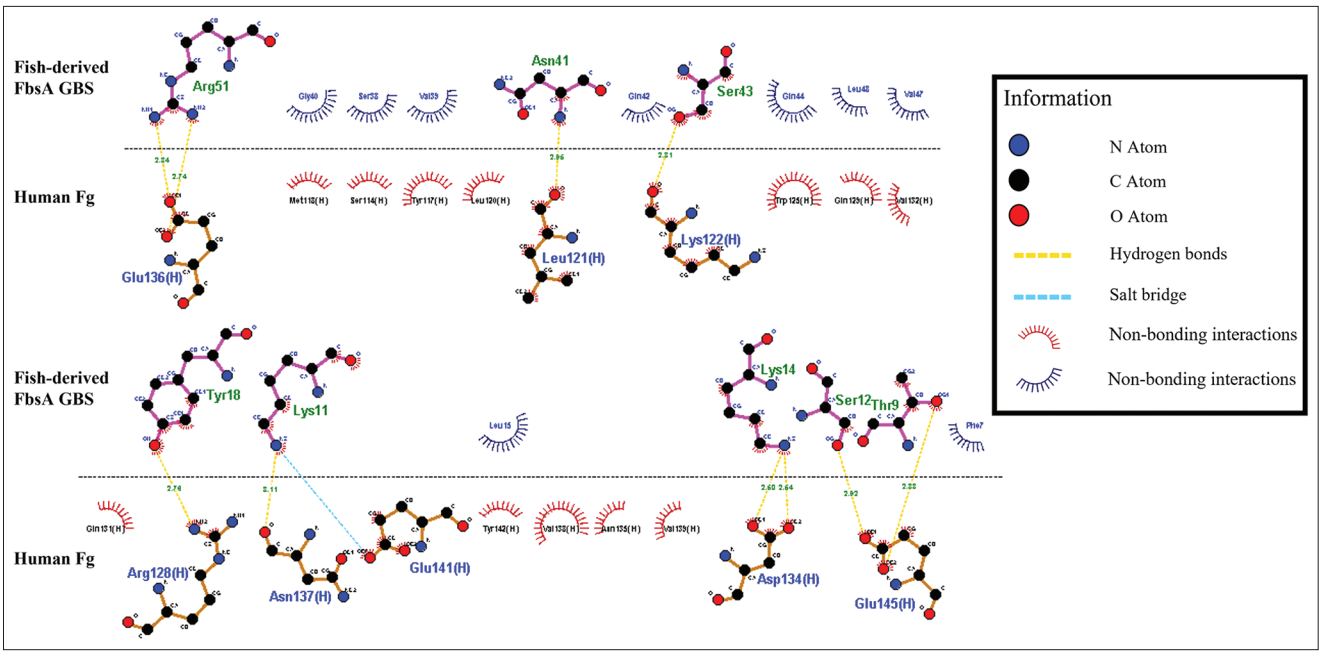

Figure 6: The two-dimensional visualization result of fibrinogen-binding protein A-H chain interaction. The results revealed that the interactions were stabilized by 10 hydrogen bonds (dotted yellow line), 1 salt bridge (dotted blue line), and non-bonding interactions.

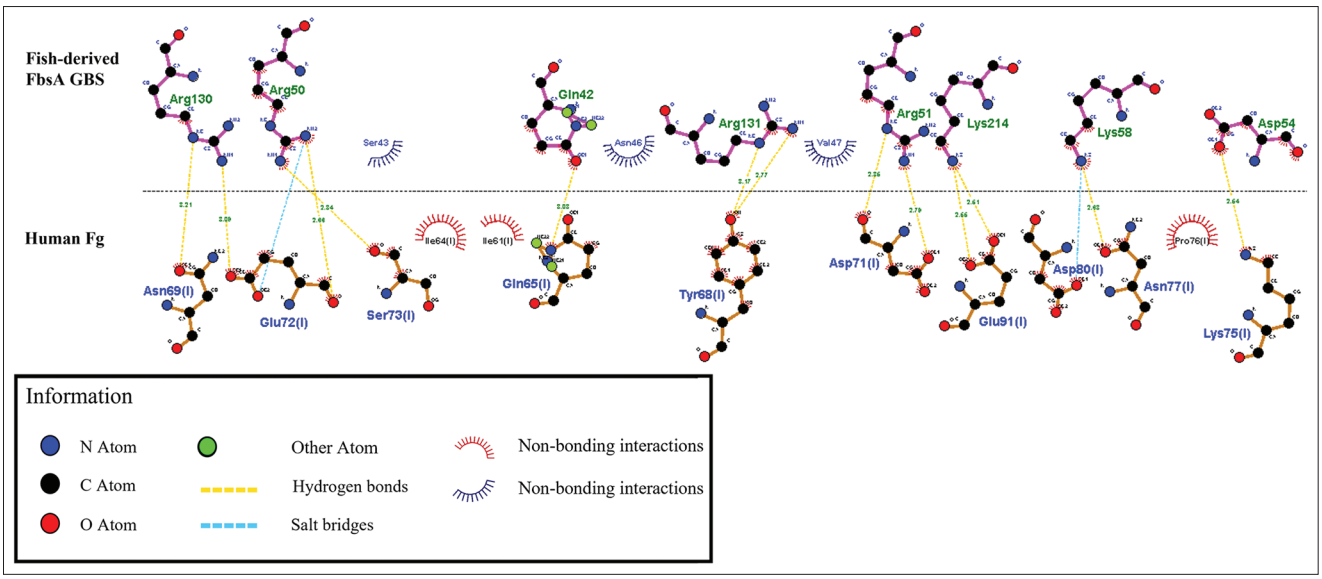

Figure 7: The two-dimensional visualization result of fibrinogen-binding protein A-I chain interaction. The results revealed that the interaction was stabilized by 13 hydrogen bonds (dotted yellow line), two salt bridges (dotted blue line), and non-bonding interactions.

have occurred in Singapore (2015) and Malaysia (2023), involving the ST283 strain [9, 10]. Rajendram *et al*. [9] identified a comparable virulence factor genetic profile in GBS strains infecting both fish and humans, thereby highlighting the urgent need for comprehensive One Health studies focused on virulence factors across different host species.

In this computational One Health study, we analyzed two amino acid sequences of the FbsA, derived from fish and human cases, obtained from the NCBI database. Sequence alignment using the MUSCLE program revealed 100% identity between the two sequences, supporting the findings reported by Rajendram *et al*. [9]. This complete sequence similarity helps explain how residents in Singapore could contract GBS from consuming raw fish. As the degree of amino acid sequence similarity between proteins increases, so too does the likelihood of structural similarity in their 3D conformations [43], potentially resulting in comparable interactions with other host proteins, such as human Fg.

The 3D structure of fish-derived FbsA GBS consists of alpha-helices and random coils, with no beta-sheet structures detected. A previous study by Ragunathan and Ponnuraj [40] reported that the secondary structure

Table 2: Residue interactions between fish-derived fibrinogen-binding protein A Group B *Streptococcus* (FbsA GBS) and the G chain of human fibrinogen (Fg).

Residues and binding functional groups	The bond distance (Å)	The bond types	Residues with non-bonding interactions	
			Fish-derived FbsA GBS	Human Fg
Ser38-His84(G) (O-N)	2.86	Hydrogen bond	Leu15; Leu48; Gln44; Gly45; Gln42; Asn46; Val39; Met36; Ala35; Ile5; Trp10; Met19; Ile26; Phe218; Ile221; Leu22; Ser34; Val33	Tyr108(G); Asp97(G); Leu94(G); Ile93(G); Ile90(G); Met91(G); Glu92(G); Pro195(G); Gln200(G); Glu113(G); Asn109(G); Asn106(G); Arg110(G); Asn102(G); Asp114(G); Arg118(G); Arg95(G)
Asn41-Thr87(G) (N -O)	3.11			
Gly40-Thr87(G) (O-O)	2.72			
Asp37-Thr88(G) (O-O)	2.90			
Lys4-Ser196(G) (O-O)	3.07			
Phe7-Arg199(G) (O-N)	2.80			
Phe7-Arg199(G) (O-N)	2.67			
Gly6-Ser196(G) (N-O)	3.22			
Gly6-Arg197(G) (O-N)	2.69			
Gly6-Asp198(G) (N-O)	3.01			
Asn3 Asp198(G) (N-O)	2.99			
Gln52-Arg104(G) (O-N)	2.66			
Tyr18 Asp105(G) (O-O)	2.98			

*Red and blue residues are residues in repetitive regions of fish-derived FbsA GBS. The colored blue residues indicated the critical residue group that interacted with the G chain of human Fg. GBS=Group B *Streptococcus*, Fg=Fibrinogen, FbsA=Fibrinogen-binding protein A, Met=Methionine, Phe=Phenylalanine, Ile=Isoleucine, Gly=Glycine, Trp=Tryptophan, Val=Valine, Ala=Alanine, Leu=Leucine, Pro=Proline, Asn=Asparagine, Thr=Threonine, Ser=Serine, Tyr=Tyrosine, Gln=Glutamine, Lys=Lysine, Arg=Arginine, His=Histidine, Asp=Aspartate, Glu=Glutamate

Table 3: Residue interactions between fish-derived fibrinogen-binding protein A Group B *Streptococcus* (FbsA GBS) and the H chain of human fibrinogen (Fg).

Residues and binding functional groups	The bond distance (Å)	The bond types	Residues with non-bonding interactions	
			Fish-derived FbsA GBS	Human Fg
Arg51-Glu136(H) (N-O)	2.84	Hydrogen bond	Gly40; Ser38; Val39; Gln42; Gln44; Leu48; Val47; Leu15; Phe7	Met118(H); Ser114(H); Tyr117(H); Leu120(H); Trp125(H); Gln129(H); Val132(H); Gln131(H); Tyr142(H); Val138(H); Asn135(H); Val139(H);
Arg51-Glu136(H) (N-O)	2.74			
Asn41-Leu121(H) (N-O)	2.95			
Ser43-Lys122(H) (O-O)	2.81			
Tyr18-Arg128(H) (O-N)	2.76			
Lys11-Asn137(H) (N-O)	3.11			
Lys14-Asp134(H) (N-O)	2.50			
Lys14-Asp134(H) (N-O)	2.54			
Ser12-Glu145(H) (O-O)	2.92			
Thr9-Glu145(H) (O-O)	2.83			
Lys11-Glu141(H) (N-O)	-	Salt bridge		

*Colored blue residues are residues in repetitive regions of fish-derived FbsA GBS as well as the critical residue group that interacted with the H chain of human Fg. Meanwhile, colored green residues revealed interacting residues in the $\beta_{119\text{-}129}$ site. GBS=Group B *Streptococcus*, Fg=Fibrinogen, FbsA=Fibrinogen-binding protein A, Met=Methionine, Phe=Phenylalanine, Gly=Glycine, Val=Valine, Leu=Leucine, Asn=Asparagine, Thr=Threonine, Ser=Serine, Tyr=Tyrosine, Gln=Glutamine, Lys=Lysine, Arg=Arginine, Asp=Aspartate, Glu=Glutamate

of the FbsA protein from the GBS strain NEM316 was predominantly composed of alpha-helices, followed by random coils. The authors suggested that this structural pattern may be linked to the presence of repetitive regions in FbsA and the absence of Pro residues within these regions. They further noted that peptides containing the sequence VLERRQRDAE contribute to alpha-helix formation while remaining residues form random coils. In the present study, the fish-derived FbsA GBS was predicted to contain approximately six repetitive regions, spanning residues 45–140. In contrast, Ragunathan and Ponnuraj [40] reported the presence of only three to four repetitive regions.

GBS infection in host cells is mediated by various virulence factors, including FbsA, which facilitates adhesion to host Fg molecules [12]. Our molecular docking analysis demonstrated that FbsA specifically binds to the G, H, and I chains of human Fg – corresponding to the Aα, Bβ, and γ chains, respectively – particularly within the D fragment, consistent with the Fg region classification described by Litvinov *et al*. [44]. This finding is in agreement with a previous study by Pietrocola *et al*. [42] showing FbsA's adherence to the D fragment of Fg. FbsA contains repetitive regions, each composed of 16 amino acids, that are essential for Fg binding, with certain critical residues playing a particularly important role in this interaction [40]. Our findings indicate that these critical residues, especially those located between positions 45 and 60, are central to the formation of the FbsA-Fg complex. This study offers novel insights into the significance of residues within this region for mediating Fg binding, thereby contributing to a better understanding of the molecular mechanisms underlying

Table 4: Residue interactions between fish-derived fibrinogen-binding protein A Group B *Streptococcus* (FbsA GBS) and the I chain of human fibrinogen (Fg).

Residues and binding functional groups	The bond distance (Å)	The bond types	Residues with non-bonding interactions	
			Fish-derived FbsA GBS	Human Fg
Arg130-Asn69(I) (N-O)	3.21	Hydrogen bond	Ser43; Asn46; Val47	Ile64(I); Ile61(I); Pro76(I)
Arg130-Glu72(I) (N-O)	3.09			
Arg50-Glu72(I) (N-O)	2.66			
Arg50-Ser73(I) (N-O)	2.84			
Gln42-Gln65(I) (O-N)	3.08			
Arg131-Tyr68(I) (N-O)	3.17			
Arg131-Tyr68(I) (N-O)	2.77			
Arg51-Asp71(I) (N-O)	2.85			
Arg51-Asp71(I) (N-O)	2.79			
Lys214-Glu91(I) (N-O)	2.55			
Lys214-Glu91(I) (N-O)	2.51			
Lys58-Asn77(I) (N-O)	2.63			
Asp54-Lys75(I) (O-N)	2.54			
Lys58-Asp80(I) (N-O)	-	Salt bridge		
Arg50-Glu72(I) (N-O)	-			

*Red and blue residues are residues in repetitive regions of fish-derived FbsA GBS. The colored blue residues indicate the critical residue group that interacted with the I chain of human Fg. GBS=Group B *Streptococcus*, Fg=Fibrinogen, FbsA=Fibrinogen-binding protein A, Ile=Isoleucine, Val=Valine, Pro=Proline, Asn=Asparagine, Ser=Serine, Tyr=Tyrosine, Gln=Glutamine, Lys=Lysine, Arg=Arginine, Asp=Aspartate, Glu=Glutamate

the zoonotic transmission and pathogenesis of GBS. Previous *in vitro* study by Schubert *et al*. [12] has shown that even a single repetitive region is sufficient to facilitate binding to Fg.

Following the formation of the FbsA–Fg complex, FbsA promotes host cell infection, which may lead to the development of clinical symptoms. Notably, our results indicate that this complex formation involves residues within the $\beta_{119–129}$ region of human Fg, a known binding site for plasmin - a fibrinolytic enzyme that degrades fibrin clots [34, 45]. The manifestation of endocarditis in systemic GBS infections suggests that FbsA binding at the $\beta_{119–129}$ site may initiate thrombus formation through interactions with platelets and other blood components. This aggregation could form a protective matrix that shields the bacterium from immune responses, thereby enabling colonization of the endocardium and contributing to the development of endocarditis [46]. These results expand our understanding of how FbsA may influence fibrin clot formation triggered by thrombin activity [42]. Moreover, the resulting immune response to GBS infection raises concern about septicemia, a severe systemic condition caused by infection-induced immune dysregulation [47].

In addition, previous studies by Lee *et al*. [15] and Merle *et al*. [48] have demonstrated that the Fg-binding protein of *Staphylococcus aureus* can bind not only to Fg but also to complement protein C3b, which is critical for activation of the complement immune system. This interaction blocks the activation of the complement system by inhibiting alternative pathway activity [13, 14, 15], representing an immune evasion strategy that facilitates successful infection. Such mechanisms are particularly hazardous to neonates born to GBS-colonized mothers, as they are especially vulnerable due to their immature complement systems. These infants rely heavily on the non-specific innate immune response to combat postnatal infections [12, 16].

CONCLUSION

This study presents a novel *in silico* One Health investigation into the FbsA of *S. agalactiae* (GBS) derived from fish and human sources. The complete amino acid sequence identity (100%) between fish- and human-derived FbsA highlights a conserved structural and functional profile, reinforcing the zoonotic potential of the ST283 strain. Structural modeling confirmed that FbsA predominantly comprises alpha-helices and random coils, with six repetitive regions extending from residues 45th to 140th. Molecular docking analyses further revealed specific interactions between fish-derived FbsA and the Aα, Bβ, and γ chains of human Fg, particularly within the D fragment and the $\beta_{119–129}$ region – both of which are critical sites for fibrin binding and plasmin activity. These interactions were largely mediated by critical residues located within the 45th to 60th positions, underscoring their essential role in pathogenesis.

The strength of this study lies in its comprehensive computational approach, which effectively integrates sequence alignment, structural modeling, residue profiling, and docking simulations to elucidate cross-species protein interactions relevant to zoonotic transmission. In addition, the application of high-precision bioinformatic tools (MEGA, ColabFold, ClusPro, PyMOL, and LigPlot+) ensures robustness and reproducibility in the analysis pipeline.

However, the primary limitation of this study is its reliance on *in silico* predictions. While these

findings provide critical preliminary insights, they lack experimental validation. The absence of *in vitro* or *in vivo* studies restricts the interpretation of biological functionality and limits the direct clinical applicability of the data.

Future *in silico* investigations should examine the interaction between fish-derived FbsA GBS and the human C3b protein to further elucidate the pathophysiological basis of GBS infections. A key limitation of the current study is that the presented data are restricted to computational predictions of potential protein–protein interactions. Therefore, further research involving both *in vitro* and *in vivo* approaches is necessary to validate these *in silico* findings and fully understand the mechanisms underlying GBS pathogenicity.

In summary, this study offers valuable evidence supporting the structural and functional equivalence of fish- and human-derived FbsA GBS, laying a foundation for targeted preventive strategies, including vaccine development, to mitigate the risk of zoonotic transmission.

AUTHORS' CONTRIBUTIONS

RIY: Sample and data collection, biocomputational analysis, literature review, and wrote the manuscript. ELW, WAS, and FRL: Supervised the study and provided guidance for the study. RR: Conceptualized the study, supervised biocomputational analysis, guided the concept of zoonotic disease from aquatic animals, and supervised manuscript writing. All authors have read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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