Research Article
Open Access
Life Sciences, Medicine and Biomedicine

Comparative analysis of biofilm formation in clinical isolates of Group B Streptococcus

AbdulRahman Muthanna[1,2], Yusur Ramzi Hasan[3], Menagah Ezhumalai[1], Mazen M Jamil Al-Obaidi[4], Syafinaz Amin-Nordin[5], Mohammad Noor Amal Azmai[6] & Mohd Nasir Mohd Desa[1,*]

[1]*Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor.*
[2]*Office of Postgraduate Studies, UCSI University, 56000 Kuala Lumpur, Malaysia.*
[3]*Department of Medical Laboratory Techniques, College of Health and Medical Techniques, Al-Esraa University, 10069 Baghdad, Iraq.*
[4]*Biology Unit, Science Department, Rustaq College of Education, University of Technology and Applied Sciences, 329 Rustaq, Oman.*
[5]*Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor.*
[6]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor.*

**Correspondence:* mnasir@upm.edu.my

Received 8 December 2025; Revised 28 June 2026; Accepted 8 July 2026; Published online 26 November 2026

ABSTRACT

Biofilm-forming bacteria are notorious for their heightened resistance to antibiotics, complicating both treatment and the selection of appropriate antimicrobial agents. This study aimed to investigate biofilm formation in invasive and colonising Group B Streptococcus (GBS) isolates and its relationship with serotypes, virulence genes, and pili genes. A total of 51 GBS isolates were collected from three hospitals in Malaysia, comprising 23 invasive and 28 colonising strains. Isolates were re-identified, biofilm-forming capacity was assessed by crystal violet microtiter plate assay, and virulence and pili genes were detected by PCR. Of the 51 isolates, 28 (54.9%) were good biofilm formers, 19 (37.3%) were moderate biofilm formers, and 4 (7.8%) were poor biofilm formers. Colonising isolates showed a marginally higher proportion of good biofilm formers (57.1%) compared to invasive isolates (52.2%); however, this difference was not statistically significant (Fisher's exact test, $p > 0.05$). Notably, all 51 isolates carried all five virulence genes (*brp*, *psp*, *rib*, *bca*, and *spb1*) and all three pili genes (PI-1, PI-2a, and PI-2b), a finding that warrants further investigation given its rarity in the literature. Serotype distribution was similarly not significantly associated with biofilm-forming capacity (Fisher's exact test, $p > 0.05$). These findings indicate that biofilm-forming potential is a broadly conserved trait among GBS isolates regardless of clinical origin or serotype, with implications for understanding GBS persistence and pathogenicity in Malaysian clinical settings.

Keywords: *Group B streptococcus; biofilm; pili; virulence and serotype*

INTRODUCTION

Bacterial biofilm development is a complex, multi-stage process in which planktonic bacteria adhere to biotic or abiotic surfaces and become embedded in a polysaccharide-rich extracellular matrix, progressing through distinct phases of attachment, maturation, and dispersal (Sauer et al., 2022; Kumar et al., 2020). Biofilm-forming bacteria are strongly associated with both acute and chronic nosocomial infections and present significant clinical challenges due to their remarkable resistance to antibiotics, a resistance that stems from several intrinsic features of the biofilm lifestyle (Cangui-Panchi et al., 2022). Understanding biofilm formation is therefore critical for developing control strategies in clinical and industrial settings, where persistent biofilms contribute to refractory infections and equipment fouling (Crouzet et al., 2014; Rather, Gupta, & Mandal, 2021). Group B Streptococcus (GBS) is one organism for which biofilm formation is well documented as a virulence factor in bovine infections; however, its role in human GBS infections remains comparatively underexplored (Liu & Liu, 2022).

The pathogenicity of GBS is shaped by a constellation of virulence determinants. Among these, pili are hair-like

https://doi.org/10.28916/lsmb.10.1.2026.251

surface appendages first identified in Gram-positive bacteria in the 1960s and subsequently characterised in greater detail during the 1970s. They mediate bacterial adherence to host tissues, facilitating colonisation, microcolony formation, and ultimately biofilm development (Telford et al., 2006; Petrova & Sauer, 2012; Muhammad et al., 2020). Three pilus islands have been identified in GBS (PI-1, PI-2a, and PI-2b), each contributing differently to host-pathogen interactions; notably, strains carrying PI-2a demonstrate markedly superior biofilm-forming capacity compared with those carrying PI-1 or PI-2b (Springman et al., 2014; Rinaudo et al., 2010). Additional virulence genes, including *spb1* (immune evasion via IgA and factor H binding), *bca* (epithelial adherence and phagocytosis resistance), *rib* (phagocytosis resistance), *brp* (biofilm formation and persistent colonisation), and *psp* (stress response and host survival), further modulate GBS's ability to colonise, invade, and evade host defences (Dobrut & Brzychczy-Włoch, 2022; Maeland et al., 2015; Guo et al., 2019). GBS is currently classified into ten serotypes (Ia, Ib, II-IX) whose global distribution varies considerably, with serotype composition influencing pathogenic potential and complicating vaccine development (Bahez et al., 2021; Muthanna et al., 2024).

In the Malaysian clinical setting, GBS strains continue to show resistance to tetracycline, erythromycin, and clindamycin, and treatment is further complicated by their capacity to form biofilms (Eskandarian et al., 2015; Hayes, 2020). Despite the global recognition of biofilm formation as a key driver of antibiotic resistance and persistent infection, the specific factors, whether serotypic, genetic, or structural, that promote biofilm formation in Malaysian GBS isolates remain poorly defined (Petrova & Sauer, 2012; Verma et al., 2023). To address this gap, the present study employs a comparative design in which invasive and colonising GBS isolates from a Malaysian clinical context are characterised with respect to their serotype distributions, the prevalence of virulence genes (*spb1*, *bca*, *rib*, *brp*, *psp*), and the carriage of pilus islands (PI-1, PI-2a, PI-2b), enabling direct assessment of whether these factors differentially predict biofilm-forming capacity across the two clinical phenotypes. By linking isolate origin to molecular determinants and biofilm phenotype, this study aims to provide evidence that can inform more targeted infection-management strategies for GBS in Malaysia.

METHODOLOGY

Bacterial isolates

A total of 51 viable GBS isolates were available from our previous study (Suhaimi et al., 2017), which collected from three tertiary hospitals, with the majority originating from high vaginal swabs (51.0%), followed by blood (41.0%), placenta (4.0%), urine (2.0%), and tissue samples (2.0%). These isolates were categorized into invasive (n=23) and colonizing (n=28) strains based on their isolation sites.

GBS strains were identified using a standard phenotypic testing protocol that included colony morphology examination, β-haemolytic activity on 5% Columbia sheep blood agar, Gram staining, Catalase test and CAMP test (Muthanna et al., 2023a).

Biofilm Assay

Biofilm formation was assessed in GBS isolates using the crystal violet (CV) assay. Briefly, GBS isolates were cultured for 48 hours at 37°C on 5% sheep blood agar. The colonies were then transferred to 1 ml of tryptic soy broth (TSB) and diluted 1:200 in fresh broth, followed by overnight incubation at 37°C.

Approximately 200 µL of the diluted bacterial culture was then inoculated into a 96-well tissue culture plate (in triplicate). The plate was incubated overnight at 37°C in an atmosphere enriched with 5% CO_2.

After incubation, the plate was inverted and the contents of each well were carefully aspirated to remove unbound planktonic bacteria. The plate was then washed three times with phosphate-buffered saline (PBS, pH 7.4) by gentle inversion and aspiration, and residual liquid was removed by blotting the inverted plate onto absorbent tissue paper. Each well was then treated with 20 µL of 0.1% crystal violet solution and incubated at room temperature for 15 minutes. Excess dye was removed by three additional washes with PBS using the same gentle aspiration method, and the plate was air-dried completely before absorbance measurement (Stepanović et al., 2000).

Finally, the plate was air dried for 2 hours. Then a total volume of 230 µl of 95% ethanol was added to each well to dissolve the crystal violet. The absorbance was measured at 550 nm (A550) using a spectrophotometer.

Based on the absorbance values, the GBS isolates were categorized into good biofilm formers (A550 > 0.2), moderate biofilm formers (A550 = 0.081 - 0.2) and poor biofilm formers (A550 < 0.081), as previously described by El-kersh and Talat (2012) and Hayes (2020). The cut-off absorbance (ODc) was calculated as the mean OD of negative controls plus three standard deviations. Wells containing only media served as negative controls, while *Staphylococcus aureus* (ATCC 25923) was used as a positive control due to its well-established biofilm-forming ability, serving as an internal quality check for assay consistency (Lacasse et al., 2022).

Genomic DNA extraction

Genomic DNA was extracted from GBS using the GeneAll® Exgene™ Cell SV Mini kit (GeneAll, South Korea), a silica

membrane-based spin column system that isolates DNA through sequential cell lysis, protein digestion, selective adsorption, and elution, following the manufacturer's instructions. Briefly, pelleted cells were resuspended in lysozyme mixture (30 mg/mL) and incubated at 37°C for 30 minutes, followed by addition of Proteinase K and Buffer BL and further incubation at 56°C and 95°C for 30 and 15 minutes, respectively. After cooling, absolute ethanol was added, and the mixture was loaded onto the SV column and subjected to sequential washes with Buffers BW and TW before elution with Buffer TM. The extracted DNA was stored at -20°C until use. DNA integrity was assessed by gel electrophoresis on a 1.7% agarose gel (Vivantis), while concentration and purity were determined using a NanoPhotometer® (Implen, Munich, Germany); an A260/A280 ratio of 1.8 to 2.0 and a concentration of 20 to 5000 ng/µL were considered indicative of acceptable quality for downstream PCR applications.

Detection of Virulence and pili genes

PCR amplification was conducted to detect specific virulence genes (*spb1*, *bca*, *rib*, *brp*, and *psp*) using primer pairs from Smith et al. (2007) and for pilus islands (PI-1, PI-2a, and PI-2b) using primer pairs from Sukhnanand et al. (2005).

The amplification reaction mixture totalled 25 µL, comprising 12.5 µL of master mix (Bioline), 1.0 µL each of forward and reverse primers, 3.0 µL of DNA template, and 7.5 µL of nuclease-free water. Primers were synthesized at Apical Scientific Laboratory in Kuala Lumpur, Malaysia. Thermal cycling conditions: one cycle at 94°C (5 min), 35 cycles at 94°C (45 s), 54°C (45 s) and 72°C (1.30 min), with a final extension at 72°C (10 min). One tube of PCR reaction mix with DNA template from *Streptococcus agalactiae* (ATCC 13813) served as a positive control (PC), and a mixture without a DNA template but with a nuclease-free water was used as a negative control (NC). The amplified products were analysed via agarose gel electrophoresis on a 1.7% gel stained with GelRedTM (Biogenerica) for 100 min at 60 V. Visualization was done using a UV transilluminator (BioRad).

Capsular polysaccharides typing of GBS

The capsule serotypes of GBS isolates were previously determined by conventional serotyping (CS) via Latex Agglutination Kit (Statens Serum Institute, SSI, Sweden) and molecular serotyping (MS) by multiplex PCR (Suhaimi et al., 2017).

Data analysis

Data analysis was performed using dot plots, Chi-square and Fisher's exact tests (SPSS version 26.0). Chi-square test was used for categories with expected counts ≥5, while Fisher's exact test was applied when expected counts were <5. A p-value of <0.05 (two-tailed) was considered statistically significant.

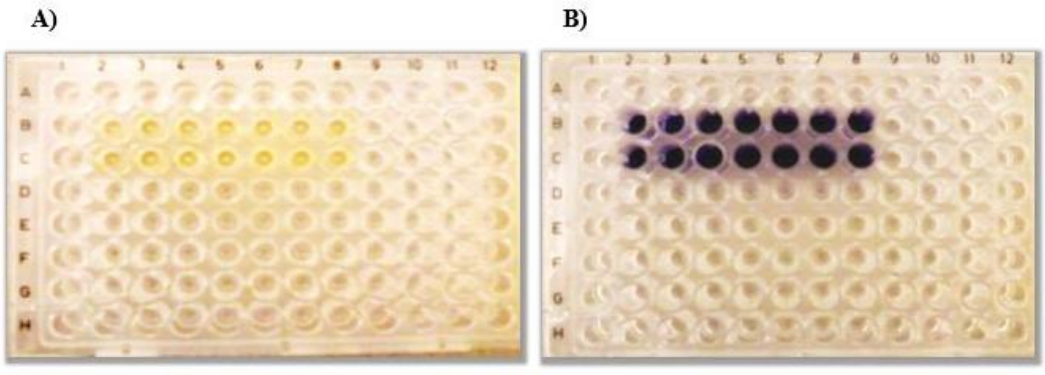
RESULTS

Biofilm assay using crystal violet

Biofilm production, assessed by absorbance at OD550nm in a microtiter plate (Figure 1), revealed that the majority of the 51 GBS isolates were capable of forming biofilms. Specifically, 28 isolates (54.9%) were classified as good biofilm formers (mean OD550 ± SD = 0.484 ± 0.163), 19 isolates (37.3%) as moderate biofilm formers (mean OD550 ± SD = 0.140 ± 0.029), and 4 isolates (7.8%) as poor biofilm formers (mean OD550 ± SD = 0.050 ± 0.025) (Figure 2).

Figure 1

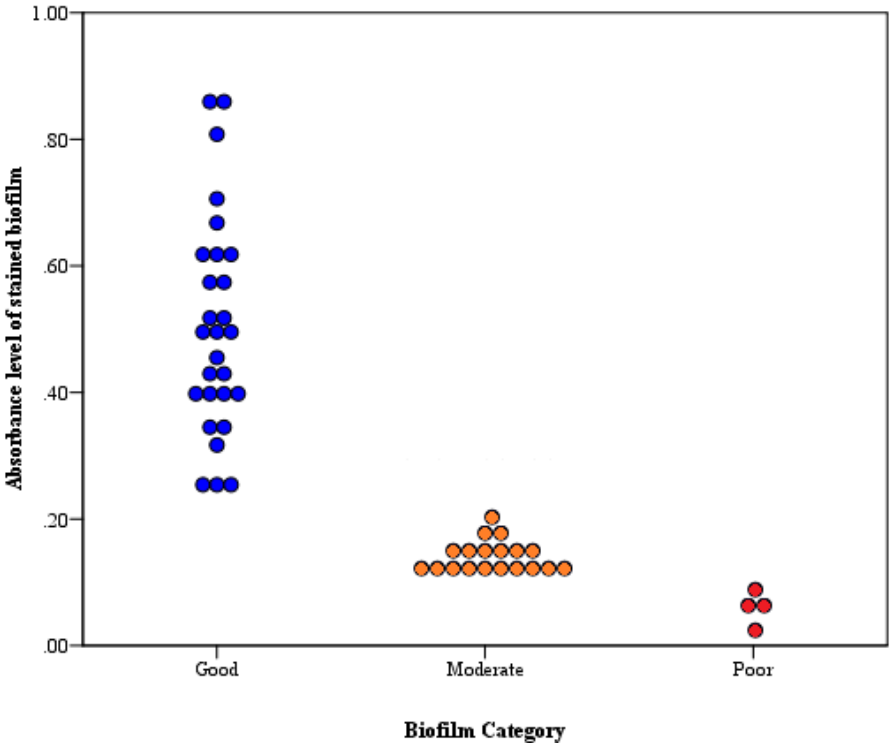
Biofilm assay using crystal violet



Note: A) Planktonic growth of GBS biofilm in 96 well tissue culture plate. B) GBS biofilm stained with crystal violet.

Figure 2

The biofilm formation index of all GBS isolates



Note: Good biofilm former (A550> 0.2), moderate (A550 = 0.081-0.2) and poor biofilm former (A550 < 0.081).

Distribution of biofilm formation and isolation site

Among the isolates from invasive sites, 12 (52.2%) have good biofilm formers, 10 (43.5%) have moderate biofilm formers, and 1 (4.3%) has poor biofilm formers. In colonising sites, 16 isolates (57.1%) formed good biofilms, followed by 9 isolates (32.1%) that formed moderate biofilms and 3 isolates (10.7%) that formed poor biofilms (Table 1).

Table 1

Biofilm former capacity by isolation sites.

Biofilm Former	**Invasive Site** **n (%)**	**Colonizing Site** **n (%)**	**P-value**
Good (*n*=28)	12 (52.2%)	16 (57.1%)	0.943
Moderate (*n*=19)	10 (43.5%)	9 (32.1%)	0.588
Poor (*n*=4)	1 (4.3%)	3 (10.7%)	0.750[a]
Total (n=51)	**n=23**	**n=28**	

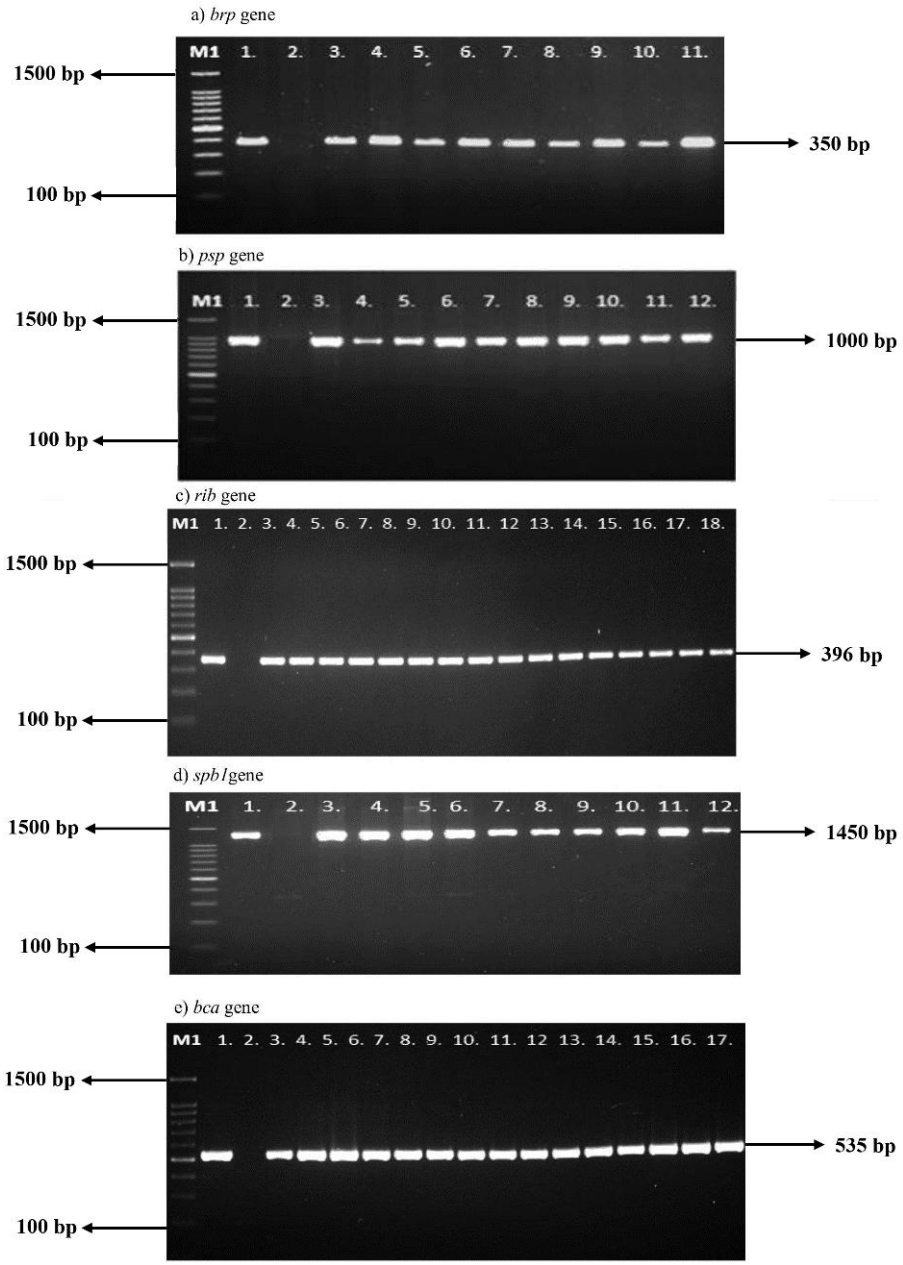
Note: p >0.05 was not significant, ([a]p) = Fisher's Exact Test

Distribution of virulence and the pili genes (PI-1, P2a and P2b)

The presence of all five targeted virulence genes (*brp*, *psp*, *rib*, *bca*, and *spb1*) (Figure 3) were detected in the 51 clinical GBS isolates. All 51 GBS isolates also carried all three pili genes (PI-1, PI-2a, and PI-2b) (Figure 4). The universal presence of all targeted virulence and pili genes in both invasive and colonizing isolates precludes any correlation analysis between these genetic determinants and biofilm-forming capacity or isolation site.

Figure 3

Representative result of the PCR assay for detection of virulence genes in GBS



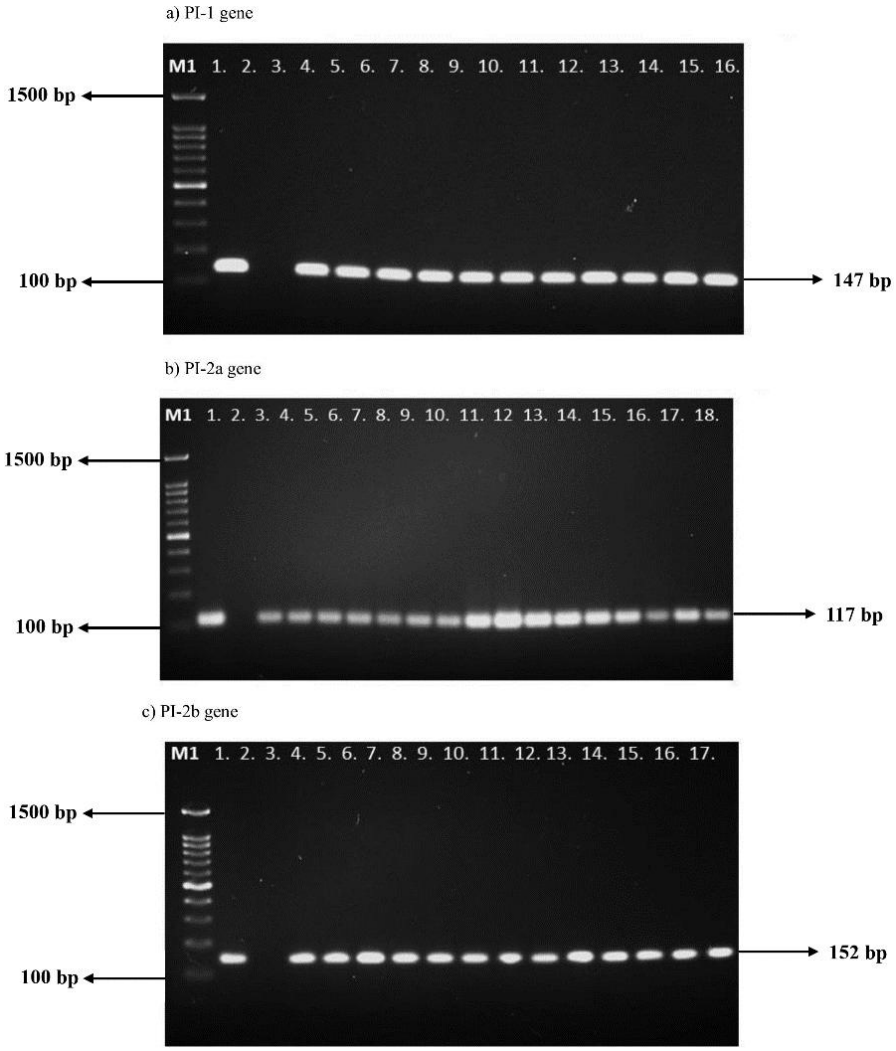
Note: a: brp (350bp), b: psp (1000bp), c: rib (369bp), d: bca (1450bp), e: spb1 (535bp). Lanes 1 is DNA ladders (100 bp); lane 2 represents a positive control; lane 3 represents negative control; and the remaining lanes represent positively amplified genes with the expected band size.

Serotypes distribution among the biofilm former isolates

Among the six serotypes detected, serotype Ia was the most prevalent overall and was represented across all three biofilm categories, with the highest proportion observed among moderate biofilm formers (57.9%), followed by poor (50.0%) and good (28.6%) formers. Serotype III isolates showed a tendency toward good biofilm formation, accounting for 25.0% of good formers, while being less represented among moderate formers (10.5%) and absent from poor formers. Serotype V was present only among good (17.9%) and poor (50.0%) biofilm formers, with no isolates classified as moderate formers. Serotypes II, VI, and VIII were detected exclusively among good and/or moderate biofilm formers, while the non-typeable isolate was found only in the moderate category. Despite these distributional patterns, Fisher's exact test revealed no statistically significant association between serotype and biofilm-forming capacity for any serotype (all $p > 0.05$), indicating that serotype distribution alone does not predict biofilm phenotype among the isolates studied (Table 2).

Figure 4

Representative result of the PCR assay for detection of pili genes in GBS



Note: a: PI-1 (147), b: PI2a (117), c: PI2b(152). Lanes 1 is DNA ladders (100 bp); lane 2 represents a positive control; lane 3 represents negative control; and the remaining lanes represent positively amplified genes with the expected band size.

Table 2

Biofilm former capacity by different types of serotypes

Serotypes*	Biofilm category			P-value[a]
	Good	Moderate	Poor	
Ia (n=21)	8 (28.6%)	11 (57.9%)	2 (50.0%)	0.086
II (n=3)	2 (7.1%)	1 (5.3%)	-	0.586
III (n=9)	7 (25.0%)	2 (10.5%)	-	0.115
V (n=7)	5 (17.9%)	-	2 (50.0%)	0.852
VI (n=8)	4 (14.3%)	4 (21.0%)	-	0.888
VIII (n=2)	2 (7.1%)	-	-	0.235
NT (n=1)	-	1 (5.3%)	-	0.460

*Note: p >0.05 was not significant, ([a]p) = Fisher's Exact Test; *(Suhaimi et al., 2017).*

DISCUSSION

Biofilm formation is a process in which a community of microbial cells attaches to a surface and is enclosed by a self-produced exopolysaccharide glycocalyx (Rinaudo et al., 2010). Research on GBS, particularly regarding its biofilm formation, has been limited despite numerous studies on its molecular characteristics (Rather, Gupta, Bardhan, et al., 2021; Verma et al., 2023). This study explored the relationship between biofilm formation, serotypes, virulence and pili genes, and isolation sites of GBS.

GBS strains in this study's collection exhibited variable biofilm-forming capacity, with the majority classified as good or moderate biofilm formers. When stratified by clinical origin, isolates from colonising sites showed a marginally higher proportion of good biofilm formers (57.1%) compared to those from invasive sites (52.2%); however, this difference was not statistically significant (Fisher's exact test, $p = 0.943$). This finding suggests that the ability to form biofilms is not exclusive to invasive GBS strains and is equally distributed among colonising isolates in the Malaysian clinical setting. The comparable biofilm-forming capacity between invasive and colonising isolates may reflect the capacity of colonising GBS strains to persist asymptomatically in the host through biofilm-mediated mechanisms, potentially serving as a reservoir for future invasive disease. These results are consistent with previous reports indicating that biofilm formation is a broadly conserved trait across GBS clinical phenotypes rather than a feature uniquely associated with invasive infection (Petrova & Sauer, 2012; Verma et al., 2023), underscoring the importance of monitoring biofilm-forming potential in both colonising and invasive GBS populations within Malaysian healthcare settings. In addition, Kaur et al. (2009) reported that GBS isolated from pregnant carriers and clinically infected individuals had the same biofilm properties. Recent studies have highlighted the significant ability of GBS isolates to produce biofilms, with a notable proportion classified as strong biofilm producers (Parker et al., 2016; Verma et al., 2023). Recent studies indicated that approximately 70% of GBS isolates are categorized as strong biofilm producers, while the remaining 30% are moderate producers. This was evidenced by absorbance values that often surpassed the threshold for high biofilm formation during assays (Lu et al., 2021; Verma et al., 2023).

One factor that may help explain the comparable biofilm-forming capacity observed between invasive and colonising GBS isolates in this study is the influence of environmental pH on biofilm development. Studies have demonstrated that GBS achieves maximum biofilm formation under neutral pH conditions (pH 7.0), while acidic conditions (pH 4.5) are less conducive to biofilm development (Dilrukshi et al., 2021). In the context of this study, both invasive and colonising isolates were cultured under standardised laboratory conditions at neutral pH, which may have provided equally permissive conditions for biofilm formation across both groups, potentially contributing to the absence of a statistically significant difference in biofilm-forming capacity between the two clinical phenotypes ($p = 0.943$). This observation is relevant to the study's objective of comparing biofilm formation between invasive and colonising GBS isolates, as it highlights that laboratory-assessed biofilm capacity reflects an intrinsic bacterial trait under controlled conditions rather than the variable in vivo environments encountered at colonising sites such as the vaginal tract, where acidic pH may naturally suppress biofilm development, or invasive sites such as the bloodstream, where neutral pH may favour it. Future studies incorporating pH-variable biofilm assays may therefore provide a more physiologically relevant comparison of biofilm-forming potential between invasive and colonising GBS isolates in the Malaysian clinical context.

Meanwhile, this study showed considerable variability in the ability to form biofilms between different GBS serotypes. Strong biofilm producers were identified mainly in serotypes Ia, III, VI, V, II, VIII and non-typeable strains. Conversely, the majority of weak biofilm producers belonged to serotypes Ia and V. These results are in contrast to a study by Kaur et al. (2009) which found that the strongest biofilm formers belonged predominantly to serotype Ia, followed by Ib, non-typeable strains, III and V. This suggests a different hierarchy of biofilm-forming potential between serotypes than in our study. Moreover, D'Urzo et al. (2014) reported that the most significant biofilm-forming strains belonged to serotype III. D'Urzo et al. (2014) also found that lowering the pH significantly increased the proportion of highly biofilm-forming strains within serotypes III and V, suggesting that environmental factors such as pH play a crucial role in the dynamics of biofilm formation. In particular, a subgroup of strains of serotype III, which were classified as hypervirulent (ST-17), showed increased biofilm formation in an acidic environment (D'Urzo et al., 2014). However, these findings contrast with Dilrukshi et al. (2021), who reported maximum biofilm formation at neutral pH (pH 7), with the majority of isolates showing non-biofilm-forming behaviour at pH 4.5, close to normal vaginal pH. The contrasting results of the various studies indicate that environmental factors, in particular pH, have a considerable influence on the distribution of serotypes among strong and weak biofilm formers. The decrease in strong biofilm-forming strains at lower pH values suggests that acidic conditions may selectively inhibit the ability of certain serotypes to effectively form biofilms. It should be noted that pH was not assessed in the present study; therefore, pH-related effects on biofilm formation remain speculative and warrant investigation in future studies. This emphasises the need to further investigate how different environmental conditions may influence the pathogenicity and persistence of GBS strains.

The results of this study revealed that all 51 GBS isolates carried all three pilus islands (PI-1, PI-2a, and PI-2b), a finding that is notably different from previously reported distributions and warrants careful interpretation. A study in Malaysia reported that PI-1, PI-2a, and PI-2b were detected in 86.7%, 70.8%, and 30.1% of human GBS isolates respectively (Muthanna et al., 2023b), and similar variability has been reported in other populations (Otaguiri et al.,

2013; Springman et al., 2014). The universal carriage observed in the present study may reflect genuine biological ubiquity within this specific cohort, potentially influenced by the limited geographic sampling from the Klang Valley; however, several technical explanations must also be considered. These include possible primer cross-reactivity between pilus island sequences, or insufficient primer specificity under the assay conditions employed. Notwithstanding these considerations, pilus islands are well established as critical virulence factors that facilitate GBS attachment to host cells and biofilm formation (Springman et al., 2014; Jiang et al., 2012; Rosini & Margarit, 2015), and the broader distribution of pili types across GBS populations likely reflects genetic adaptation under host immunological pressure, with differences between studies potentially attributable to genetic deletion events during GBS adaptation to various environments or hosts (Otaguiri et al., 2013). In the present study, however, the presence of all three pilus islands in both good and poor biofilm-forming isolates suggests that pilus island carriage alone may not be directly predictive of biofilm-forming capacity, and the limited sample size precluded the establishment of a statistically meaningful correlation between pilus island distribution and biofilm phenotype.

In this study, five different virulence genes (*brp*, *psp*, *rib*, *bca* and *spb1*) were detected in all GBS isolates. Several studies have found a prevalence of several virulence genes such as *spb1*, *bca* and *rib* in different populations of GBS isolates (Eskandarian et al., 2015; Hamad et al., 2023; Hernandez et al., 2022; Mudzana et al., 2021). A study in Malaysia by Muthanna et al. (2023b) reported that *spb1*, *bca* and *rib* genes were present in 99.1%, 83.2% and 77% of human GBS isolates, respectively. In another study, *bca* was found in 57.8% of isolates, while *rib* and *spb1* were also analysed, but their prevalence varied between different genotypes (Udo et al., 2013). Another study indicated that although several virulence genes were prevalent, specific genes such as *spb1* were not detected in all genotypes, suggesting variability in the presence of genes depending on the genetic background of the isolate (Chattopadhyay et al., 2011). According to Smith et al. (2007), the *rib* and *bca* genes are crucial for epithelial cell adhesion, invasion and resistance to phagocytosis in GBS. In addition, *rib* and *bca* genes are important candidates for GBS vaccine development due to their role in virulence and their potential to induce protective immunity (Bahez et al., 2021). The correlation between virulence genes, biofilm formation and serotypes is an important area in microbiology, especially for understanding pathogenic bacteria such as GBS. Previous research has shown that the presence of specific virulence genes often correlates with the ability to form biofilms and specific serotypes, which can be crucial for epidemiological studies and vaccine development (Eskandarian et al., 2015; Verma et al., 2023). However, in the current study, no significant correlation was found between biofilm-forming ability and serotypes or virulence and pili genes, as all GBS isolates tested, including invasives and colonizers, harbored all targeted virulence and pili genes.

This study has several limitations that should be considered when interpreting the findings. The relatively small sample size of 51 GBS isolates, drawn exclusively from the Klang Valley, may not adequately represent the broader Malaysian GBS population, limiting the generalisability of the observed associations between pilus islands, serotypes, and biofilm-forming capacity, as well as reducing the likelihood of capturing greater variability in the distribution of pilus genes and virulence genes that might emerge with a larger and more diverse isolate collection. Future studies should therefore incorporate larger, geographically diverse sample sizes spanning different regions of Malaysia and including isolates from asymptomatic pregnant women and neonates, to more accurately determine the distribution of GBS serotypes and virulence factors and to better inform vaccine development efforts. Additionally, expanding the scope of investigation to include pH-variable biofilm assays and a broader panel of pilus genes would provide a more comprehensive understanding of the environmental and molecular determinants driving biofilm-mediated pathogenesis in GBS.

CONCLUSION

This study demonstrated that GBS isolates from both colonising and invasive clinical sources are capable of forming biofilms, with the majority classified as good or moderate biofilm formers. Although colonising isolates showed a marginally higher proportion of good biofilm formers (57.1%) compared to invasive isolates (52.2%), this difference was not statistically significant ($p > 0.05$), indicating that biofilm-forming capacity is a broadly conserved trait across GBS clinical phenotypes rather than a feature exclusive to invasive strains. Similarly, no significant association was found between serotype distribution and biofilm category ($p > 0.05$), suggesting that serotype alone does not predict biofilm-forming potential. These findings collectively highlight that biofilm formation in GBS is likely multifactorial. Understanding the interplay between these factors is important for improving the management of GBS infections, particularly in maternal and neonatal health settings where persistent colonisation poses significant clinical risk.

AUTHOR CONTRIBUTIONS

AbdulRahman Muthanna was responsible for the conceptualisation of the study, contributed to the methodology, validation, formal analysis, and investigation, performed data curation, prepared the original draft, and participated in reviewing and editing the manuscript. Yusur Ramzi Hasan and Menagah Ezhumalai contributed to the methodology, validation, formal analysis, and investigation. They were also involved in data curation and in the preparation of the original draft of the manuscript. Mazen M Jamil Al-Obaidi and Mohammad Noor Amal Azmai contributed to the review

and editing of the manuscript and provided supervision for the study. Syafinaz Amin-Nordin and Mohd Nasir Mohd Desa contributed to the review and editing of the manuscript, provided supervision for the study, managed project administration, and secured funding for the research. All authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately addressed.

ETHICS APPROVAL

Acquisition of GBS isolates from hospital settings was approved by the Medical Research and Ethics Committee of the Ministry of Health Malaysia (National Medical Research Register approval no. NMRR 19-876-46665). The study utilised de-identified *Streptococcus agalactiae* isolates collected from the microbiology laboratories of participating hospitals. Informed consent was not required as this study did not meet the definition of research involving human subjects; only data pertaining to isolation site, age, gender, and ethnicity of patients associated with the isolates were gathered, and these data could not be traced back to individual patients. All methods were carried out in accordance with relevant guidelines and regulations.

FUNDING

This work was funded by the Ministry of Higher Education Malaysia under the Fundamental Research Grant Scheme (FRGS/1/2023/WAB04/UPM/01/4).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

ACKNOWLEDGEMENTS

We would like to thank laboratory staff at the Department of Biomedical Science and Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for assistance and support throughout the study. This work was funded by the Ministry of Higher Education under Fundamental Research Grant Scheme (FRGS/1/2023/WAB04/UPM/01/4).

REFERENCES

Bahez, A., Mahmud, M. I. A. M., Hamzah, H. A., & Wahid, H. H. (2021). Virulence-genes rib and bca in serotypes of group B streptococcus (GBS) isolated from symptomatic pregnant women in east coast Malaysia. *IIUM Medical Journal Malaysia*, *20*(3), 118–126. https://doi.org/10.31436/imjm.v20i3.1633

Cangui-Panchi, S. P., Ñacato-Toapanta, A. L., Enríquez-Martínez, L. J., Reyes, J., Garzon-Chavez, D., & Machado, A. (2022). Biofilm-forming microorganisms causing hospital-acquired infections from intravenous catheter: A systematic review. *Current Research in Microbial Sciences*, *3*, 100175. https://doi.org/10.1016/j.crmicr.2022.100175

Chattopadhyay, D., Carey, A. J., Caliot, E., Webb, R. I., Layton, J. R., Wang, Y., Bohnsack, J. F., Adderson, E. E., & Ulett, G. C. (2011). Phylogenetic lineage and pilus protein Spb1/SAN1518 affect opsonin-independent phagocytosis and intracellular survival of group B Streptococcus. *Microbes and Infection*, *13*(4), 369–382. https://doi.org/10.1016/j.micinf.2010.12.009

Crouzet, M., Le Senechal, C., Brözel, V. S., Costaglioli, P., Barthe, C., Bonneu, M., Garbay, B., & Vilain, S. (2014). Exploring early steps in biofilm formation: Set-up of an experimental system for molecular studies. *BMC Microbiology*, *14*(1), 1–12. https://doi.org/10.1186/s12866-014-0253-z

Dilrukshi, G. N., Kottahachchi, J., Dissanayake, T., Weerasekera, M., Peiris, M., Medis, S., & Fernando, N. (2021). In-vitro biofilm formation of vaginal isolates of Streptococcus agalactiae; effect of pH and culture media. *Brazilian Archives of Biology and Technology*, *64*, e21210151. https://doi.org/10.1590/1678-4324-2021210151

Dobrut, A., & Brzychczy-Włoch, M. (2022). Immunogenic proteins of group B streptococcus — potential antigens in immunodiagnostic assay for GBS detection. *Pathogens*, *11*(1), 1–20. https://doi.org/10.3390/pathogens11010043

D'Urzo, N., Martinelli, M., Pezzicoli, A., De Cesare, V., Pinto, V., Margarit, I., Telford, J. L., & Maione, D. (2014). Acidic pH strongly enhances in vitro biofilm formation by a subset of hypervirulent ST-17 Streptococcus agalactiae strains. *Applied and Environmental Microbiology*, *80*(7), 2176–2185. https://doi.org/10.1128/AEM.03627-13

El-Kersh, T. A., Neyazi, S. M., Al-Sheikh, Y. A. & Niazy, A. A. (2012). Phenotypic traits and comparative detection methods of vaginal carriage of group B streptococci and its associated micro-biota in term pregnant Saudi women. *African Journal of Microbiology Research, 6*(2):403–413. https://doi.org/10.5897/AJMR11.1156

Eskandarian, N., Ismail, Z., Neela, V. K., Van Belkum, A., Desa, M. N. M., & Nordin, S. A. (2015). Antimicrobial susceptibility profiles, serotype distribution and virulence determinants among invasive, non-invasive and colonizing Streptococcus agalactiae (group B streptococcus) from Malaysian patients. *European Journal of Clinical Microbiology & Infectious Diseases*, *34*(3), 579–584. https://doi.org/10.1007/s10096-014-2265-x

Guo, H., Fu, M., Peng, Q., Chen, Z., Liu, J., Qiu, Y., & Huang, Y. (2019). Antimicrobial resistance and molecular characterization of Streptococcus agalactiae from pregnant women in southern China. *Journal of Infection in Developing Countries*, *13*(9), 802–809. https://doi.org/10.3855/jidc.11395

Hamad, A. A., Farrag, A. A., El-Waseif, A. A., & Almawla, S. O. G. (2023). Novel molecular diagnosis of Cyl E, Spb 1 and bib A virulence genes of Streptococcus agalactiae from pregnant women. *Materials Today: Proceedings*, *80*, 2347–2352. https://doi.org/10.1016/j.matpr.2021.06.347

Hayes, K. M. (2020). *Group B Streptococcus: Molecular epidemiology, pathogenic profiling and control strategies* [Doctoral thesis, Munster Technological University]. https://sword.mtu.ie/allthe/18/

Hernandez, L., Cadona, J., Traverso, F., Altamiranda, S., Bustamante, A., & Sanso, A. (2022). Virulence profiles and antimicrobial resistance of Streptococcus agalactiae infective and colonizing strains from Argentina. *Current Microbiology*, *79*(12), 392. https://doi.org/10.1007/s00284-022-03050-w

Jiang, S., Park, S. E., Yadav, P., Paoletti, L. C., & Wessels, M. R. (2012). Regulation and function of pilus island 1 in group B Streptococcus. *Journal of Bacteriology*, *194*(10), 2479–2490. https://doi.org/10.1128/JB.00202-12

Kaur, H., Kumar, P., Ray, P., Kaur, J., & Chakraborti, A. (2009). Biofilm formation in clinical isolates of group B streptococci from north India. *Microbial Pathogenesis*, *46*(6), 321–327. https://doi.org/10.1016/j.micpath.2009.04.004

Kumar, J., Sharma, V. K., Parmar, S., Singh, P., & Singh, R. K. (2020). Biofilm: A microbial assemblage on the surface — A boon or bane? In *New and future developments in microbial biotechnology and bioengineering: Microbial biofilms* (pp. 139–150). Elsevier. https://doi.org/10.1016/B978-0-444-64279-0.00011-6

Lacasse, M., Valentin, A. S., Corvec, S., Bémer, P., Jolivet-Gougeon, A., Plouzeau, C., Tandé, D., Mereghetti, L., Bernard, L., & Lartigue, M. F. (2022). Genotypic characterization and biofilm production of group B Streptococcus strains isolated from bone and joint infections. *Microbiology Spectrum*, *10*(2), 1–8. https://doi.org/10.1128/spectrum.02329-21

Liu, Y., & Liu, J. (2022). Group B Streptococcus: Virulence factors and pathogenic mechanism. *Microorganisms*, *10*(12), 2483. https://doi.org/10.3390/microorganisms10122483

Lu, J., Guevara, M. A., Francis, J. D., Spicer, S. K., Moore, R. E., Chambers, S. A., & Gaddy, J. A. (2021). Analysis of susceptibility to the antimicrobial and anti-biofilm activity of human milk lactoferrin in clinical strains of Streptococcus agalactiae with diverse capsular and sequence types. *Frontiers in Cellular and Infection Microbiology*, *11*, 740872. https://doi.org/10.3389/fcimb.2021.740872

Maeland, J. A., Afset, J. E., Lyng, R. V., & Radtke, A. (2015). Survey of immunological features of the alpha-like proteins of Streptococcus agalactiae. *Clinical and Vaccine Immunology*, *22*(2), 153–159. https://doi.org/10.1128/CVI.00643-14

Mudzana, R., Mavenyengwa, R. T., & Gudza-Mugabe, M. (2021). Analysis of virulence factors and antibiotic resistance genes in group B streptococcus from clinical samples. *BMC Infectious Diseases*, *21*(1), 1–11. https://doi.org/10.1186/s12879-021-05820-6

Muhammad, M. H., Idris, A. L., Fan, X., Guo, Y., Yu, Y., Jin, X., Qiu, J., Guan, X., & Huang, T. (2020). Beyond risk: Bacterial biofilms and their regulating approaches. *Frontiers in Microbiology*, *11*, 928. https://doi.org/10.3389/fmicb.2020.00928

Muthanna, A., Baharin, N. H. Z., Desa, M. N. M., Dzaraly, N. D., Azmai, M. N. A., & Amin-Nordin, S. (2023a). Disease burden, antimicrobial resistance and molecular characterization of invasive group B Streptococcus among non-pregnant adults in Malaysia: A protocol study. *Asian Pacific Journal of Tropical Medicine*, *16*(11), 498–505. https://doi.org/10.4103/1995-7645.390165

Muthanna, A., Desa, M. N. M., Alsalemi, W., Liyana Abd Aziz, N. A., Dzaraly, N. D., Baharin, N. H. Z., Ali, M. M., Nor, L. A. M., Ismail, Z., Ahmad, N. H., Shan, C. H., Azmai, M. N. A., & Amin-Nordin, S. (2023b). Phenotypic and genotypic comparison of pathogenic group B Streptococcus isolated from human and cultured tilapia (Oreochromis species) in Malaysia. *Comparative Immunology, Microbiology and Infectious Diseases*, *97*, 101993. https://doi.org/10.1016/j.cimid.2023.101993

Muthanna, A., Desa, M. N. M., Rahman, N. A. A., Dzaraly, N. D., Baharin, N. H. Z., Aziz, N. A., Shan, C. H., Ismail, Z., Nor, L. A. M., Ali, M. M., Ahmad, N. H., Azmai, M. N. A., & Amin-Nordin, S. (2024). Epidemiological surveillance of invasive group B Streptococcus infections in six major hospitals in Peninsular Malaysia. *Asian Pacific Journal of Tropical Medicine*, *17*(9), 384–391. https://doi.org/10.4103/apjtm.apjtm_965_23

Otaguiri, E. S., Morguette, A. E. B., Tavares, E. R., Dos Santos, P. M. C., Morey, A. T., Cardoso, J. D., Perugini, M. R. E., Yamauchi, L. M., & Yamada-Ogatta, S. F. (2013). Commensal Streptococcus agalactiae isolated from patients seen at University Hospital of Londrina, Paraná, Brazil: Capsular types, genotyping, antimicrobial susceptibility and virulence determinants. *BMC Microbiology*, *13*(1), 297. https://doi.org/10.1186/1471-2180-13-297

Parker, R. E., Laut, C., Gaddy, J. A., Zadoks, R. N., Davies, H. D., & Manning, S. D. (2016). Association between genotypic diversity and biofilm production in group B Streptococcus. *BMC Microbiology*, *16*(1), 1–12. https://doi.org/10.1186/s12866-016-0704-9

Petrova, O. E., & Sauer, K. (2012). Sticky situations: Key components that control bacterial surface attachment. *Journal of Bacteriology*, *194*(10), 2413–2425. https://doi.org/10.1128/JB.00003-12

Rather, M. A., Gupta, K., & Mandal, M. (2021). Microbial biofilm: Formation, architecture, antibiotic resistance, and control strategies. *Brazilian Journal of Microbiology*, *52*(1), 1701–1718. https://doi.org/10.1007/s42770-021-00624-x

Rather, M. A., Gupta, K., Bardhan, P., Borah, M., Sarkar, A., Eldiehy, K. S. H., Bhuyan, S., & Mandal, M. (2021). Microbial biofilm: A matter of grave concern for human health and food industry. *Journal of Basic Microbiology*, *61*(5), 380–395. https://doi.org/10.1002/jobm.202000678

Rinaudo, C. D., Rosini, R., Galeotti, C. L., Berti, F., Necchi, F., Reguzzi, V., Ghezzo, C., Telford, J. L., Grandi, G., & Maione, D. (2010). Specific involvement of pilus type 2a in biofilm formation in group B Streptococcus. *PLoS ONE*, *5*(2), e9216. https://doi.org/10.1371/journal.pone.0009216

Rosini, R., & Margarit, I. (2015). Biofilm formation by Streptococcus agalactiae: Influence of environmental conditions and implicated virulence factors. *Frontiers in Cellular and Infection Microbiology*, *5*, 6. https://doi.org/10.3389/fcimb.2015.00006

Sauer, K., Stoodley, P., Goeres, D. M., Hall-Stoodley, L., Burmølle, M., Stewart, P. S., & Bjarnsholt, T. (2022). The biofilm life cycle: Expanding the conceptual model of biofilm formation. *Nature Reviews Microbiology*, *20*(10), 608–620. https://doi.org/10.1038/s41579-022-00767-0

Smith, T. C., Roehl, S. A., Pillai, P., Li, S., Marrs, C. F., & Foxman, B. (2007). Distribution of novel and previously investigated virulence genes in colonizing and invasive isolates of Streptococcus agalactiae. *Epidemiology and Infection*, *135*(6), 1046–1054. https://doi.org/10.1017/S0950268806007515

Springman, A. C., Lacher, D. W., Waymire, E. A., Wengert, S. L., Singh, P., Zadoks, R. N., Davies, H. D., & Manning, S. D. (2014). Pilus distribution among lineages of group B Streptococcus: An evolutionary and clinical perspective. *BMC Microbiology*, *14*(1), 159. https://doi.org/10.1186/1471-2180-14-159

Stepanović, S., Vuković, D., Dakić, I., Savić, B., & Švabić-Vlahović, M. (2000). A modified microtiter-plate test for quantification of staphylococcal biofilm formation. *Journal of Microbiological Methods*, *40*(2), 175–179. https://doi.org/10.1016/S0167-7012(00)00122-6

Suhaimi, M. E., Desa, M. N. M., Eskandarian, N., Pillay, S. G., Ismail, Z., Neela, V. K., Masri, S. N., & Nordin, S. A. (2017). Characterization of a group B Streptococcus infection based on the demographics, serotypes, antimicrobial susceptibility and genotypes of selected isolates from sterile and non-sterile isolation sites in three major hospitals in Malaysia. *Journal of Infection and Public Health*, *10*(1), 14–21. https://doi.org/10.1016/j.jiph.2016.01.009

Sukhnanand, S., Dogan, B., Ayodele, M. O., Zadoks, R. N., Craver, M. P. J., Dumas, N. B., ... & Wiedmann, M. (2005). Molecular subtyping and characterization of bovine and human Streptococcus agalactiae isolates. *Journal of Clinical Microbiology*, *43*(3), 1177-1186. https://doi.org/10.1128/jcm.43.3.1177-1186.2005

Telford, J. L., Barocchi, M. A., Margarit, I., Rappuoli, R., & Grandi, G. (2006). Pili in gram-positive pathogens. *Nature Reviews Microbiology*, *4*(7), 509–519. https://doi.org/10.1038/nrmicro1443

Udo, E. E., Boswihi, S. S., & Al-Sweih, N. (2013). Genotypes and virulence genes in group B Streptococcus isolated in the maternity hospital, Kuwait. *Medical Principles and Practice*, *22*(5), 453–457. https://doi.org/10.1159/000349932

Verma, S., Kumari, M., Pathak, A., Yadav, V., Johri, A. K., & Yadav, P. (2023). Antibiotic resistance, biofilm formation, and virulence genes of Streptococcus agalactiae serotypes of Indian origin. *BMC Microbiology*, *23*(1), 1–11. https://doi.org/10.1186/s12866-023-02877-y

Citation:

Muthanna, A., Hasan, Y. R., Ezhumalai, M., Al-Obaidi, M. M. J., Amin-Nordin, S., Amal Azmai, M. N., & Mohd Desa, M. N. (2026). Comparative analysis of biofilm formation in clinical isolates of Group B Streptococcus. *Life Sciences, Medicine and Biomedicine*, *10*(1).

https://doi.org/10.28916/lsmb.10.1.2026.251



Life Sciences, Medicine and Biomedicine
ISSN: 2600-7207

Copyright © 2026 by the Author(s). Life Sciences, Medicine and Biomedicine (ISSN: 2600-7207) Published by Biome Journals - Biome Scientia Sdn Bhd. Attribution 4.0 International (CC BY 4.0). This open access article is distributed based on the terms and conditions of the Creative Commons Attribution license https://creativecommons.org/licenses/by/4.0/