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THE DIFFERENTIAL EXTRACTION METHOD OF *NEISSERIA MENINGITIDIS* AND NON-PATHOGENIC SPECIES FOR PROTEIN PROFILING BY SDS-PAGE

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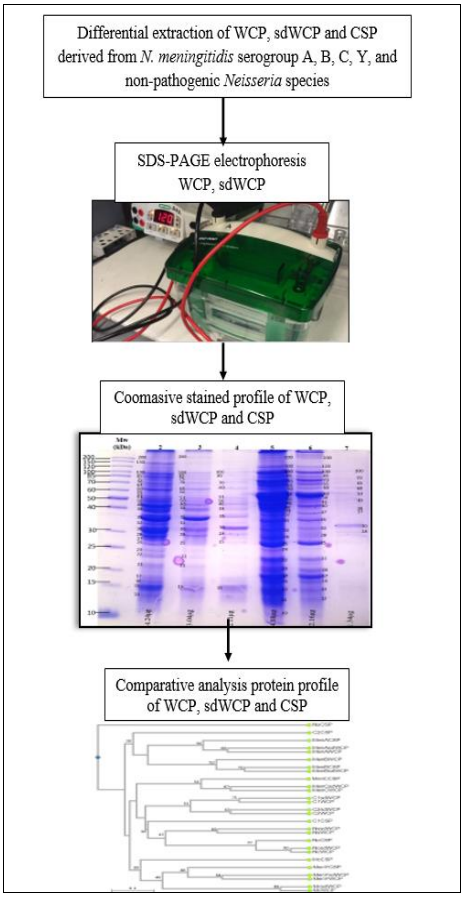
Graphical abstract



Abstract

Invasive meningococcal disease (IMD) is an acute, severe, and potentially fatal infection caused by *Neisseria meningitidis* and is a global public health burden. Given the high fatality rate linked to acute bacterial meningitis, it is imperative to initiate treatment and diagnosis concurrently in most cases. While a culture demonstrating the growth of *N. meningitidis* from blood or cerebrospinal fluid (CSF) remains the gold standard, however, its sensitivity diminishes following antibiotic administration. Therefore, in this study, differentially extracted whole cell protein (WCP), surface depleted whole cell protein (sdWCP), and cell surface protein (CSP) profiles were analysed by using SDS-PAGE to characterise and classify pathogenic *N. meningitidis and* non-pathogenic *species*. This study provides clear evidence that SDS-PAGE yields distinct protein patterns across Men A, B, C, and Y, clinical isolates 1 and 2, *N. sicca*, *N. cinerea*, and *M. catarrhalis*. It effectively demonstrates that SDS-PAGE protein profiling can serve as a reliable method for characterising and separating bacterial proteins at both the species and strain levels. Despite the availability of more advanced technologies, SDS-PAGE remains a valuable tool, particularly in settings where advanced equipment and knowledgeable personnel are lacking. Our approach employs various extraction techniques, has successfully defined and characterised *N. meningitidis*, non-pathogenic *Neisseria* species, and *M. catarrhalis*.

Keywords: Invasive meningococcal disease, whole cell protein, surface depleted, whole cell protein, cell surface protein, differential extraction

Abstrak

Penyakit meningokokal invasif ialah jangkitan akut, teruk dan berpotensi membawa maut yang disebabkan oleh *Neisseria meningitidis* dan merupakan beban kesihatan awam global. Memandangkan kadar kematian yang tinggi dikaitkan dengan meningitis bakteria akut, adalah penting untuk memulakan rawatan dan diagnosis serentak dalam kebanyakan kes. Walaupun kultur yang menunjukkan pertumbuhan *N. meningitidis* dari darah atau cecair saraf tunjang masih dianggap sebagai piawaian emas, bagaimanapun sensitivitinya berkurangan selepas pemberian antibiotik. Oleh itu, dalam kajian ini, protein sel keseluruhan yang diekstrak (WCP), protein sel keseluruhan habis permukaan (sdWCP), dan protein permukaan sel (CSP) dianalisis dengan menggunakan SDS-PAGE

untuk mencirikan dan mengklasifikasikan *N. meningitidis* patogenik dan spesies bukan patogenik. Kajian ini memberikan bukti jelas bahawa SDS-PAGE menghasilkan corak protein yang berbeza terhadap Men A, B, C dan Y, *N. sicca*, *N. cinerea* dan *M. catarrhalis*. Ianya berkesan menunjukkan bahawa pemprofilan protein SDS-PAGE berfungsi sebagai kaedah yang boleh dipercayai untuk mencirikan dan mengasingkan protein bakteria pada kedua-dua peringkat spesies dan strain. Walaupun terdapat teknologi yang lebih maju, SDS-PAGE kekal sebagai alat yang bernilai, terutamanya pada tempat yang kekurangan peralatan canggih dan kakitangan yang berpengetahuan. Pendekatan kami menggunakan pelbagai teknik pengekstrakan, telah berjaya mentakrifkan dan mencirikan *N. meningitidis*, spesies *Neisseria* bukan patogenik dan *M. catarrhalis*.

Kata kunci: Penyakit meningokokol invasif, protein sel keseluruhan, protein sel keseluruhan habis permukaan, protein permukaan sel, pengekstrakan berbeza

1.0 INTRODUCTION

Invasive meningococcal disease (IMD) caused by the Gram-negative bacterium *Neisseria meningitidis* is a global public health burden [1], [2]. IMD is an acute, severe, and potentially fatal infection characterised by meningitis, sepsis or, less commonly, pneumonia, arthritis, pericarditis, and abdominal disorders. The disease's severity, rapid progression, and nonspecific syndromes continue to cause morbidity and mortality. In 2019, the World Health Organisation (WHO) estimated 250,000 deaths, with at least one in five people experiencing severe long-term sequelae [2]. Therefore, it is crucial to diagnose and treat meningococcal disease rapidly. Early and appropriate intravenous antimicrobial therapy is the most important therapy for patients with suspected severe meningococcal infection. Patients are usually treated with third-generation cephalosporins (e.g., cefotaxime or ceftriaxone) [3]. When *N. meningitidis* is identified, and the antibiogram shows complete sensitivity, antibiotic treatment can be continued with penicillin or ampicillin. If the patients were allergic to beta-lactams, the alternative would be chloramphenicol, aztreonam or fluoroquinolone such as moxifloxacin [4]. Antimicrobial chemoprophylaxis is given to prevent sporadic meningococcal disease in cases of close contact with infected persons. Although IMD can be treated with antibiotics, antibiotic-resistant has become a worldwide problem, making it challenging to treat and control the spread of the disease.

While meningococci and gonococci have been extensively studied, other species of Neisseria have not received as much research focus. These species are typically non-pathogenic and are considered commensal members of the normal microbiota in both human and animal nasopharynx. However, there have been instances where they have caused invasive opportunistic infections in individuals with compromised immune systems [5]. It is unclear whether this commensal population aids human health or affects bacterial pathogen colonisation and disease. According to Kim *et al*. (2019), commensal Neisseria can exhibit antagonistic behaviour towards their pathogenic counterparts, which may have a negative impact on colonization [7]. In addition, they are capable of causing a range of infections, many of which may be secondary to primary infections elsewhere.

Most people infected with meningococcal disease present with acute illness, and their symptoms are often nonspecific, including fever, headache and malaise, making early detection of a meningococcal infection challenging. One textbook describes the 'profound dread' this disease causes among clinicians due to its difficult diagnosis, rapid progression and high mortality [8]. Due to the high fatality rate associated with acute bacterial meningitis, treatment and diagnosis should be initiated simultaneously in most cases. Conventionally, a culture that shows the growth of the bacterium *N. meningitidis* from blood or cerebrospinal fluid (CSF) still stands as gold-standard or microscopy of Gram-negative diplococci in the CSF is used for diagnosis. The sensitivity of CSF cultures prior to treatment is relatively high (>80 – 90%) compared to blood cultures (40 – 80%)[4], [9]. However, after antibiotics were given, the CSF cultures were negative within 24 to 36 hours, possibly within 2 hours. At the same time, blood culture sensitivity reduces by 20% after antibiotic treatment [4]. Rapid direct bacterial antigen (capsular polysaccharide) detection methods in CSF, serum and urine are readily commercially available kits based on antibody-coated latex agglutination, but their sensitivity and specificity are low. Several polymerase chain reaction (PCR) tests for detecting serogroup-specific *N. meningitidis* have been developed, which are rapid, specific, extremely sensitive, do not require live bacteria and may allow direct typing of the bacterium but are costly. Among the molecular techniques available for typing are restriction fragment length polymorphism (RFLP), rRNA

probe technology (ribotyping), PCR amplification restriction endonuclease analysis of chromosomal dhps (dihydropteroate synthase), *pil A*, *pil B* and *por A N. meningitidis* genes, repetitive sequence-based PCR, and pulsed-field gel electrophoresis (PFGE). Recent advances in rapid diagnosis using mass spectrophotometry and multilocus sequence typing (MLST) to target conserved regions of bacterial ribosomal DNA genes have allowed for identifying multiple respiratory microorganisms [10].

Nowadays, there are several different technologies available for identifying and categorizing bacteria, such as whole genome sequencing. However, in situations where this method is unavailable, or there are no trained personnel, whole-cell protein profiling using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) can be a useful alternative method for bacterial identification, strain typing, and epidemiology [11]. Over the past decade, the SDS-PAGE method for analysing bacterial protein profiles has been utilised for bacterial classification, identification, and comparative research [12]. The electrophoretic pattern of bacterial cell proteins produced by SDS-PAGE extracted by various methods, either whole proteins or membrane components, has proved valuable for taxonomical and epidemiological studies [13], [14]. Since these cell proteins are genetically directed, their patterns reveal genetic relationships between microorganisms [15]. This approach is highly reproducible when performed under standardised settings and has been found to provide results that exhibit an excellent correlation with methods for species identification, such as DNA-DNA hybridisation [11], [16]. According to several researchers, SDS-PAGE whole cell protein profiling is a reliable method for identifying bacteria [17]–[19]. However, several studies found this method ineffective in characterising specific bacterial species [11]. Although it has been shown to be useful as a supplementary method for bacterial identification and typing, its practical applications still require additional evaluation [11]. Therefore, in this study, differentially extracted whole cell protein (WCP), surface depleted whole cell protein (sdWCP) and cell surface protein (CSP) profiles were analysed by using SDS-PAGE. Comparative protein pattern analysis was carried out to characterise and classify pathogenic *N. meningitidis* (serogroup A, B, C, and Y), including clinical isolates *N. meningitidis*, closely related *Neisseria species (N. sicca and N. cinerea)*, and *Moraxella catarrhalis.*

2.0 METHODOLOGY

2.1 Bacteria Strains

The Microbiology and Parasitology Laboratory in Kubang Kerian, Kelantan provided two clinical isolates from meningitis cases in Hospital Universiti Sains Malaysia (HUSM). Additionally, a stock culture of Neisseria cinerea was obtained from the same laboratory at HUSM. Four reference strains of bacteria were also used in the study, including *Neisseria meningitidis* serogroup A (ATCC13077), B (ATCC13090), C (ATCC13102), Y (ATCC35561), Moraxella catarrhalis (ATCC25238) and N. sicca (ATCC 9913), which were commercially purchased from ThermoScientific, US. Colony morphology and Gram staining confirmed the identification of all isolates. The cultures were incubated at 37°C on sheep blood agar (SBA) medium for 48 hours in a 5% CO2 environment at Proteomic Laboratory 1, Advanced Medical and Dental Institute (AMDI). After incubation, the cultures were preserved in brain heart infusion (BHI) broth containing 5% blood and 20% glycerol at -80°C for future use.

2.2 Preparation and Extraction of Whole Cell Protein (WCP)

The WCP were extracted using the Horvath and Riezman [20] approach with some modifications. All cultures were cultivated for 48 hours on sheep blood agar (SBA) medium at 37°C in 5% CO_2. After 48 hours, the colonies were collected and suspended in a tube with 5 mL sample preparation buffer (SPB) containing Tris, 2% sodium dodecyl sulphate (SDS), and glycerol with a pH of 6.8. ß-mercaptoethanol was added to reach 10% v/v concentration prior to extraction. The suspension was heated to 100°C for 5 minutes, then centrifuged at 10,000 x g for 10 minutes at 4°C. The supernatant was collected, and ice-cold ethanol was added for overnight precipitation at -20 °C. The precipitated proteins were pelleted at 10,000 x g for 10 minutes at 4°C. The protein pellet was dissolved in 10mM Tris pH 7.4 containing the PMSF (phenylmethanesulfonylfluoride) as the protease inhibitor and stored at -20 °C.

2.3 Preparation and Extraction of Cell Surface Protein (CSP)

In the CSP preparation, we followed the method proposed by Sheik Abdul Kader, Z. [21] with some modification. The bacterial cultures were harvested after 48 hours and treated with glycine-HCL at pH 2.0 for 15 minutes at room temperature. After 10-minute centrifugation at 10,000 x g at 4°C, a supernatant was collected, while the pellet was saved for further extraction. The supernatant's pH was adjusted to 7.4, and ice-cold ethanol was added for protein precipitation for overnight at -20 °C, followed by centrifugation for 10 minutes at 4°C at 10,000 x g. The obtained pellet was dissolved in Tris-PMSF and stored at -20°C.

2.4 Preparation and Extraction of surface depleted Whole Cell Protein (sdWCP)

The pellet obtained from the preparation of CSP was dissolved in 5 mL of SPB containing Tris, 2% sodium

dodecyl sulphate (SDS), glycerol and ß-mercaptoethanol. The solubilized pellet was then subjected to boiling for 5 minutes and centrifuged at 10,000 x g for 10 minutes at a temperature of 4°C. The resulting supernatant was collected, and the addition of ice-cold ethanol was done to precipitate the sdWCP protein, followed by centrifugation. This precipitated protein was subsequently dissolved in Tris-PMSF buffer and stored at a temperature of -20°C for future use.

2.5 Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Protein concentration was quantified at 595nm using a protein assay kit (Bio-Rad, USA) with an Agilent Cary 60 UV-Vis spectrophotometer. Protein samples (ranging from 2.5 to four micrograms) were combined with an equal volume (2.5 µL, 4 µL, and 4 µL) of sample preparation buffer (SPB), containing 63 mM Tris-HCL (pH 6.8), 10% glycerol, 2% SDS, and bromophenol blue. This mixture was then heated at 100°C for 5 minutes. The protein samples were separated by SDS-PAGE using a 10% polyacrylamide separating gel and a 4% stacking gel [22]. Electrophoresis was conducted in an electrophoretic cell (Mini Protein II; Bio-Rad, USA) at a constant current (25 mA per gel) for one hour using a running buffer of 25 mM Tris, 192 mM glycine, and 0.1% SDS. Each gel included unstained SDS-PAGE standards (Broad Range; ThermoScientific) as controls. Subsequently, the gels were stained with Coomassie Brilliant Blue R-250, and protein band images were captured using a VersaDoc 4000 MP Imaging System (Bio-Rad). Analysis of the SDS-PAGE protein bands were performed using Quantity One Software (Bio-Rad, USA).

2.6 Data Analysis

The image of the SDS-PAGE gels was analysed with Quantity One software (Bio-Rad, USA), and each clearly identifiable differentially extracted protein band was taken into consideration. The polymorphic bands were scored visually as present (1) or absent (0). Hierarchical cluster analysis (HCA), similarity matrix, and distance matrix were computed using DendroUPGMA (http://genomes.urv.cat/UPGMA/) with Jaccard's coefficient and standard settings [23].

All of the identified protein bands were presented them in Table 1, allowing for a comprehensive visualisation of the protein profile patterns. The presence of well-defined bands using SDS-PAGE gel densitometry readings, and notable bands were denoted in the table with "+" to indicate their significance. In contrast, the absence of bands was indicated with "_". This method allowed for a clear and concise representation of the observed protein banding patterns, which aided in further analysis and interpretation.

3.0 RESULTS AND DISCUSSION

The field of microbiology has seen significant advances in recent years, and various techniques have emerged for bacterial characterisation and classification. Among these, whole genome sequencing stands out as a prominent tool. However, in scenarios where either resources or expertise in genomic-based molecular typing are limited, protein profiling via sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) offers a valuable alternative. This method enables the analysis of the protein composition of the cell in its entirety, thereby facilitating the differentiation and comparison of bacterial strains [11]. Moreover, the analysis of whole-cell proteins through SDS-PAGE can achieve discrimination levels comparable to those of DNA fingerprinting [24].

As shown in Figure 1, the results of SDS-PAGE of WCP of *N. meningitidis* serogroup A (Men A), B (Men B), and C (Men C) observed in lanes 2, 5 and lane 8 show clear protein bands within the molecular weight (Mw) range from 13 – 120 kDa, 13 – 132 kDa and 13 – 142 kDa respectively. In Figure 2, the WCP of *N. meningitidis* serogroup Y (Men Y) and *M. catarrhalis,* as in lanes 2 and 5, show protein bands ranging from 13 to 200 kDa and 10 to 200 kDa. Furthermore, Figures 3 and 4 show the protein bands of clinical isolates 1, 2 and non-pathogenic Neisseria spp. (*N. sicca* and *N. cinerea*) as in lanes 2 and 5 are within the range of 13 - 200 kDa, 13 - 140 kDa, 13 – 140 kDa and 13 – 186 kDa, respectively. The SDS-PAGE protein profiles for sdWCP and CSP derived from *N. meningitidis* strains, as well as *Neisseria* spp. and *M. catarrhalis* exhibit comparable protein bands to those observed in WCP of *N. meningitidis* and non-pathogenic spp. However, sdWCP and CSP protein profiles generally displayed a reduced number of bands and staining intensity compared with WCP. This could be related to the extraction method used, which could have resulted in the loss and appearance of some bands in sdWCP and CSP during the SDS-PAGE analysis.

The results of both WCP and sdWCP (Figure 2 to 4) extracted from *Neisseria* strains and species displayed distinguishable SDS-PAGE protein profile patterns, featuring prominent bands with molecular masses ranging from 25 to 40 kDa, with the exception *M. catarrhalis*. Notably, protein bands of *M. catarrhalis* exhibited heavy staining within the 12 to 100 kDa range, with diminished staining intensity and bands observed in sdWCP. Additionally, a decrease in staining intensity was also evident in the sdWCP and CSP of *Neisseria* strains and species. This observation aligns with the findings of McKenzie *et al.* [25], who characterised *M. catarrhalis* whole cell proteins and identified heavily stained bands with molecular masses between 48 and 60 kDa. Furthermore, Mocca and Frasch [26] highlight the presence of protein bands as in WCP and sdWCP of *Neisseria* strains and species. Their study reported the

presence of major proteins in most *Neisseria* strains with molecular weights ranging between 38 and 46 kDa, which exhibited a high degree of stability. Conversely, the lower molecular weight proteins, falling within the range of 30 kDa and below, displayed greater variability in molecular weight among strains and were absent in certain strains. Our study noticed differences in the protein bands of differently extracted proteins, particularly those with molecular weights greater than 46 kDa. This variation shows a wide range of protein profiles among different strains and species.

When comparing the protein profiles of WCP derived from the two clinical isolates, a remarkable similarity becomes apparent. Additional protein bands are present in clinical isolate 1's WCP, distributed across molecular weights of 200, 80, 56, 53, and 26 kDa, whereas clinical isolate 2 exhibits such bands at molecular weights of 130, 80, 77, 58, 55, 54, 28, 25, and 19 kDa. While the number of detectable protein bands differed between sdWCP and CSP, however, the staining intensity remained uniform. Furthermore, clinical isolate 1 has more bands in its CSP than clinical isolate 2. However, distinct differences were evident between the protein profiles of clinical isolates and other *Neisseria* strains and species, except for Men C. Men C displayed a similar relationship with the clinical isolates, which was further confirmed by the dendrogram, placing them within the same cluster group. Furthermore, the similarity between the two clinical isolates suggests they might belong to the same species or strains. Their close relationship is evident from the dendrogram (Figure 5), where their similarity level of 51% underscores their significant relatedness. Furthermore, their similarity relationship with Men C was found to be 30%. Nonetheless, further laboratory testing of these isolates is imperative to precisely determine their species and strains.

On the other hand, the protein profiles of closely related *Neisseria* spp. vary significantly. Many strongly stained protein bands in WCP and sdWCP were observed in 25 to 100 kDa for *N. sicca* and 30 to 100 kDa for *N. cinerea*. Whole cell protein (WCP) and surface depleted whole cell protein (sdWCP) extracted from *N. sicca* produced more separation and clear protein bands than *N. cinerea*. However, a reduced number of protein bands and staining were detected in CSP of *N. sicca*, in contrast with *N. cinerea*, the number of bands present in sdWCP and CSP and staining was almost similar with WCP. Dendrogram results in Figure 5 showed that *N. sicca* shared 47% of its similarities *N. cinerea*. Knapp *et al*. [27] reported on Hoke and Vedros study using the thermal renaturation method and found that there was 60% relative homology between *N. gonorrhoeae* and *N. cinerea*, *N. sicca, N. subflava*, and *N. flavescens*. Although no previous DNA-DNA homology studies using the S1 endonuclease procedure have been reported between *N. gonorrhoeae* and *N. cinerea*, the relative homology of 44% between these species suggests that *N. cinerea* and *N. gonorrhoeae* may be closely related than are other commensal *Neisseria* species.

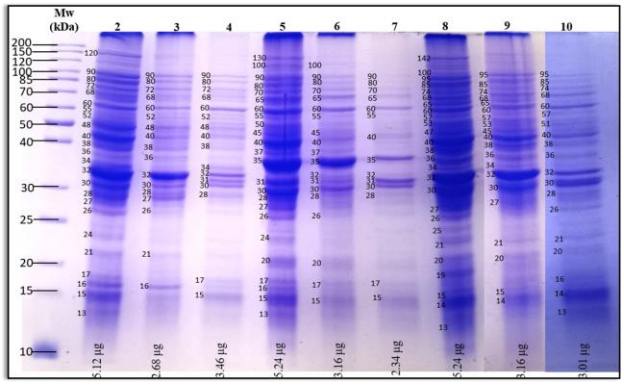


Figure 1 Coomassie brilliant blue (CBB) stained profile of WCP, sdWCP and CSP separated by 10% SDS-PAGE. Lane 1 shows the molecular weight (MW) in kDA. Lane 2 WCP *N. meningitidis* A (Men A), Lane 3 sdWCP Men A, Lane 4 Csp Men A, Lane 5 WCP Men B, Lane 6 sdWCP Men B, Lane 7 Csp Men B, Lane 8 WCP Men C, Lane 9 sdWCP Men C, and Lane 10 Csp Men C

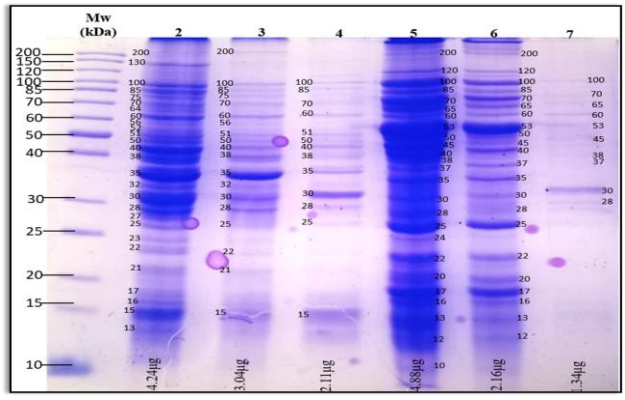


Figure 2 CBB stained profile of WCP, sdWCP and CSP separated by 10% SDS-PAGE. Lane 1 shows the molecular weight (MW) in kDA. Lane 2 WCP Men Y, Lane 3 sdWCP Men Y, Lane 4 Csp Men Y, Lane 5 WCP M. catarrhalis (Mc), Lane 6 sdWCP Mc, and Lane 7 Csp Mc

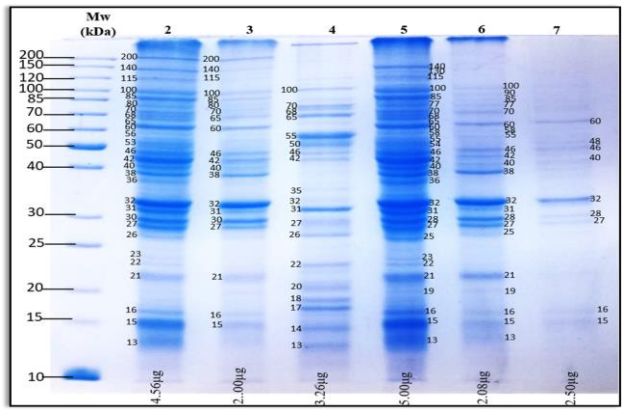


Figure 3 CBB stained profile of WCP, sdWCP and CSP separated by 10% SDS-PAGE. Lane 1 shows the molecular weight (MW) in kDa. Lane 2 WCP Clinical isolated (C1), Lane 3 sdWCP C1, Lane 4 Csp C1, Lane 5 WCP C2, Lane 6 sdWCP C2, and Lane 7 Csp C3

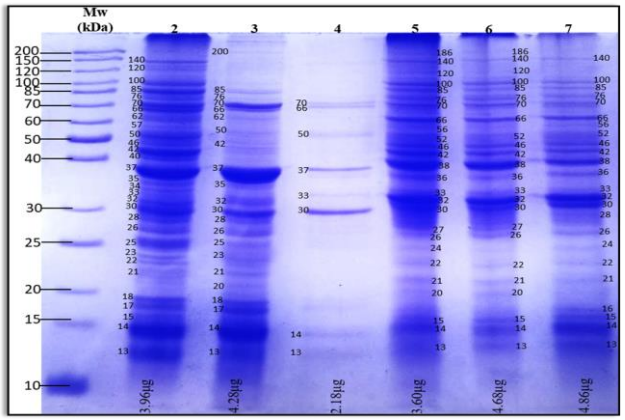


Figure 4 CBB stained profile of WCP, sdWCP and CSP separated by 10% SDS-PAGE. Lane 1 shows the molecular weight (MW) in kDa. Lane 2 WCP *N. sicca (Ns),* Lane 3 sdWCP *Ns*, Lane 4 Csp *Ns*, Lane 5 WCP *N. cinerea (Nc)*, Lane 6 sdWCP *Nc*, and Lane 7 Csp *Nc*

As shown in Table 1 and Table 2, the protein bands vary with the extraction method, however, there are six, three and zero conserved protein bands on SDS-PAGE that can be observed for pathogenic *N. meningitidis* of differentially extracted WCP, sdWCP and CSP, respectively. Conversely, for non-pathogenic *Neisseria* spp., seven, five and two conserved protein bands can be detected. The conserved protein bands of pathogenic *N. meningitidis* (Men A, B, C, Y, clinical isolate 1 and 2) are 13, 15, 27, 30, 40 and 60 kDa in WCP, while 32, 40 and 60 kDa in sdWCP. However, no conserved protein bands are seen in CSP. For *N. sicca*, *N. cinerea* and *M. catarrhalis*, the conserved proteins detected are 13, 22, 30, 70, 85, 100 and 120 kDa within WCP, and 13, 20, 30, 70, and 85 kDa within sdWCP. Moreover, CSP exhibits conserved bands of only 30 and 70 kDa.

According Derrrick *et al.* [28] in their book, they reported on Frash and colleague study that meningococcal outer membranes typically composed two to five major proteins when examined using SDS-PAGE. Frasch *et al.* [29] further identified different protein serotypes display distinct outer membrane protein profiles on SDS-PAGE. The major outer membrane proteins can be classified into five structural classes, approximately ranging from 46,000 ± 1,000, 41,000 ± 1,000, 38,000 ± 1,000, 33,000 ± 1,000, and 28,000 ± 1,000, respectively, based on their apparent molecular weights on Laemmli gels,. These classes are designated as Class 1 through Class 5, respectively. Therefore, the detection of protein bands at 27, 30, and 40 kDa in WCP, and 32 and 40 kDa in sdWCP in thid study, may correspond to the major outer membrane proteins proposed by the earlier study. Furthermore, Masforrol et al. [30] conducted an SDS-PAGE analysis of outer membrane vesicles (OMV) of Men B, revealing the presence of five major membrane proteins identified as FetA, Por A, Por B, RmpM, and Opc. The results of our SD-PAGe protein profile anlaysis align with their finding, as the presence of these five major proteins in their study could correspond to our results, given the similarity in the range of detected molecular weights (Mw).

The finding in Table 3 revealed that protein patterns obtained from WCP were found to be highly complex, yielding between 23 to 30 more protein bands than sdWCP and CSP. On the other hand, sdWCP had a slightly lower number of protein bands (17 – 24) than WCP, while CSP had significantly fewer protein bands, ranging from 9 to 19, than both WCP and sdWCP. This study's findings are consistent with Towner and Cockayne [31], who also pointed out that whole cell lysates of microorganisms can contain multiple proteins, resulting in complex profiles that frequently necessitate computer-assisted recording and analysis. Typing schemes typically focus on a small subset of the organism's major proteins to facilitate visual differentiation. In addition, profiles can be simplified by employing sub-cellular fractions, such as membrane extracts or specific protein groups. This statement is consistent with many studies done by researchers who use outer membrane protein and lipopolysaccharides for typing *N. meningitidis* and *Neisseria* spp. rather than whole cell protein has proven helpful in epidemiological studies [26], [29], [32]. Similarly, Berber et al. [33] also confirmed that whole cell protein profiles were more complex and difficult to identify from extracellular protein profiles, where they found both procedures could discriminate *S. aureus* strains from other *Staphylococcus* strains at the species or subspecies levels. In our study, we employed three different extraction methods to analyse the protein profiles of both *N. meningitidis* and non-pathogenic *Neisseria*, aiming to enhance the characterisation and differentiation of various *Neisseria* strains and species.

Table 1 Summary of differently extracted WCP, sdWCP and CSP coomassie stained protein profiles of *N. meningitidis* and non-pathogenic *spp.*

		Species specific																			Closely Related species						Distantly related		
	kDa	Wcp Men A	sdWcp Men A	Csp Men A	Wcp Men B	sdWcp Men B	Csp Men B	Wcp Men C	sdWcp Men C	Csp Men C	Wcp Men Y	sdWcp Men Y	Csp Men Y	Wcp C1	SdtWcp C1	Csp C1	Wcp C2	sdWcp C2	Csp C2	Wcp Ns	sdWcp Ns	Csp Ns	Wcp Nc	sdWcp Nc	Csp Nc	Wcp Mc	sdWcp Mc	Csp Mc
		Lane																											
		Figure 2.5									Figure 2.6									Figure 2.7								
No.	MW	2	3	4	5	6	7	8	9	10	2	3	4	2	3	4	5	6	7	2	3	4	5	6	7	5	6	7
1.	200	-	-	-	-	-	-	-	-	-	+	+	-	+	+	-	-	-	-	-	+	-	-	-	-	+	+	-
2.	186	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
3.	140	-	-	-	-	-	-	+	-	-	-	-	-	+	+	-	+	-	-	+	-	-	+	+	+	-	-	-
4.	130	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
5.	120	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	+	+	-
6.	115	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	-
7.	100	-	-	-	+	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+
8.	95	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9.	90	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
10.	85	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	-
11.	80	+	+	+	+	+	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-
12.	77	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
13.	76	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	+	+	-	-	-
14.	75	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15.	74	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16.	72	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
17.	70	-	-	-	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18.	68	+	+	+	-	-	-	+	+	+	-	-	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-
19.	66	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-
20.	65	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	+
21.	64	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22.	62	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-
23.	60	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	+	+	+
24.	58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
25.	57	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-
26.	56	-	-	-	-	-	-	-	-	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-
27.	55	+	-	-	+	+	+	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
28.	54	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-	-	-
29.	53	-	-	-	-	-	-	+	+	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	+	+
30.	52	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	+	-	+	-	-	-
31.	51	-	-	-	-	-	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
32.	50	-	-	-	+	-	-	-	-	-	+	+	+	-	-	+	-	-	-	+	+	+	-	-	-	+	+	-
33.	48	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
34.	47	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
35.	46	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	-	-	-
36.	45	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
37.	42	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-
38.	40	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	-	-	-	+	+	-
39.	38	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	+	+	+	+	-	+
40.	37	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	+	+	+
41.	36	+	+	-	-	-	-	+	+	+	-	-	-	+	-	-	+	-	-	-	-	-	+	+	+	-	-	-
42.	35	-	-	-	+	+	+	-	-	-	+	+	+	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-
43.	34	+	-	+	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
44.	33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	+	+	-	-	-
45.	32	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-
46.	31	-	-	+	+	+	+	-	-	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-
47.	30	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	+	+
48.	28	+	+	+	+	+	+	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	+
49.	27	+	+	-	+	-	-	+	+	-	+	-	-	+	+	+	+	+	+	-	-	-	+	+	+	-	-	-
50.	26	+	+	-	+	+	-	+	-	+	-	-	-	+	-	+	-	-	-	+	+	-	+	+	+	-	-	-
51.	25	-	-	-	-	-	-	+	-	-	+	+	+	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-
52.	24	+	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	+	+	-	-
53.	23	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+	+	-	-	-	-	-	-	-
54.	22	-	-	-	-	-	-	-	-	-	+	+	-	+	-	+	+	-	+	+	-	-	+	+	+	+	+	-
55.	21	+	+	-	-	-	-	+	+	+	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	-	-	-
56.	20	-	-	-	+	+	-	+	+	+	-	-	-	-	-	+	-	-	-	-	+	-	+	+	-	+	+	-
57.	19	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	-
58.	18	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	+	-	-	-	-	-	-	-
59.	17	+	-	+	+	+	+	-	-	-	+	-	-	-	-	+	-	-	-	+	+	-	-	-	-	+	+	-
60.	16	+	+	-	+	-	-	-	-	+	+	-	-	+	+	-	+	+	+	-	-	-	-	-	+	+	+	-
61.	15	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-	-	+	+	+	-	-	-
62.	14	-	-	-	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-	-	-
63.	13	+	-	-	+	-	-	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-
64.	12	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
65.	10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-

Abbreviation used in the table : +/ red = presence of distinct band; -/yellow = absence of the band

Table 2 Molecular weight (MW) in kDa of protein bands present in WCP, sdWCP and CSP of *N. meningitidis* and non-pathogenic *spp*

	Molecular weight (MW) kDa		
	WCP	sdWCP	CSP
N. meningitidis A (Men A)	120, 90, 80, 72, 68, 60, 55, 52, 48, 40, 38, 36, 34, 32, 30, 28, 27, 26, 24, 21, 17, 16, 15, 13	90, 80, 72, 68, 60, 52, 48, 40, 38, 36, 32, 30, 28, 27, 26, 21, 16	90, 80, 72, 68, 60, 52, 48, 38, 34, 32, 31, 30, 28, 17, 15
N. meningitidis B (Men B)	130, 100, 90, 80, 70, 65, 60, 55, 50, 45, 40, 37, 35, 31, 30, 28, 27, 26, 24, 20, 17, 16, 15, 13	100, 90, 80, 70, 65, 60, 55, 45, 40, 37, 35, 32, 31, 30, 28, 26, 20, 17, 15	90, 80, 70, 65, 60, 55, 40, 35, 32, 31, 30, 28, 17, 15
N. meningitidis C (Men C)	140, 100, 95, 85, 74, 68, 65, 60, 57, 53, 47, 40, 38, 36, 34, 32, 30, 27, 26, 25, 21, 20, 19, 15, 14, 13	95, 85, 74, 68, 65, 60, 57, 53, 45, 40, 38, 36, 34, 32, 30, 27, 21, 20, 15, 14	95, 85, 74, 68, 60, 57, 51, 40, 38, 36, 32, 30, 28, 26, 21, 20, 16, 14, 13
N. meningitidis Y (Men Y)	200, 130, 100, 85, 75, 70, 64, 60, 56, 52, 51, 50, 40, 38, 35, 32, 30, 28, 27, 25, 23, 22, 21, 17, 16, 15, 13	200, 100, 85, 75, 70, 60, 56, 51, 50, 40, 38, 35, 32, 30, 28, 25, 22, 21, 15	100, 85, 70, 60, 51, 50, 40, 38, 35, 30, 28, 25, 15
Clinical isolated 1 (C1)	200, 140, 115, 100, 85, 80, 70, 68, 65, 60, 56, 53, 46, 42, 40, 38, 36, 32, 31, 30, 27, 26, 23, 22, 21, 16, 15, 13	200, 140, 115, 100, 85, 80, 70, 60, 46, 42, 40, 38, 32, 31, 30, 27, 21, 16, 15	100, 70, 68, 65, 55, 50, 46, 42, 40, 35, 32, 31, 27, 26, 22, 20, 18, 17, 14, 13
Clinical isolated 2 (C2)	140, 130, 115, 100, 85, 77, 70, 68, 65, 60, 58, 55, 54, 46, 42, 40, 38, 36, 32, 31, 30, 28, 27, 25, 23, 22, 21, 19, 16, 15, 13	100, 90, 85, 77, 70, 60, 58, 55, 46, 42, 40, 38, 32, 31, 28, 27, 25, 21, 19, 16, 15, 13	60, 48, 46, 40, 38, 32, 28, 27, 16, 15
N. sicca (Ns)	140, 120, 100, 85, 76, 70, 66, 62, 57, 50, 46, 42, 40, 37, 35, 34, 33, 32, 30, 28, 27, 26, 25, 23, 22, 21, 18, 17, 15, 14, 13	200, 85, 76, 70, 66, 62, 50, 42, 40, 37, 35, 32, 30, 28, 27, 26, 25, 23, 21, 20, 18, 17, 15, 14, 13	200, 85, 76, 70, 66, 62, 50, 42, 40, 37, 35, 32, 30, 28, 26, 25, 23, 21, 20, 18, 17, 15, 14, 13
N. cinerea (Nc)	186, 140, 120, 100, 85, 76, 70. 66, 62, 52, 46, 42, 38, 36, 33, 32, 30, 27, 26, 24, 22, 21, 20, 15, 14,	186, 140, 120, 100, 85, 76, 70, 66, 52, 46, 42, 38, 36, 33, 32, 30, 27, 26, 24, 22, 21, 20, 15, 14, 13	186, 140, 120, 100, 85, 70, 66, 62, 52, 46, 42, 38, 36, 33, 32, 30, 27, 26, 24, 22, 21, 20, 15, 14, 13
M. catarrhalis (Mc)	200, 120, 100, 85, 70, 65, 60, 53, 50, 45, 40, 38, 37, 35, 30, 28, 25, 24, 22, 20, 17, 16, 13, 12, 10	200, 120, 100, 85, 70, 65, 60, 53, 50, 45, 40, 37, 35, 30, 28, 25, 22, 20, 17, 16, 13, 12	100, 70, 65, 60, 53, 45, 38, 37, 30, 28

Table 3 Total number of protein band present in differentially extracted *N. meningitidis* serogroup A (Men A), B (Men B), C (Men C), and Y (Men Y), *M. catarrhalis (M c)*, *N. sicca (Ns)*, *N. cinerea (Nc)* and clinically isolated *(C1 and C2)*

Total number of bands									
	Men A	Men B	Men C	Men Y	Mc	Ns	Nc	C1	C2
WCP	23	24	26	27	25	30	26	28	30
sdWCP	17	19	20	19	22	22	24	20	22
CSP	16	14	19	13	10	8	24	19	9

The cluster dendrogram analysis produced by numerical analysis of *N. meningitidis* and non-pathogenic spp. of WCP, sWCP and CSP is displayed in Figure 5. The analysis employs the unweighted pair group method (UPGMA) with an arithmetic average algorithm, computed using the Jaccard coefficient. The cut off level was set at 28% which resulting dendrogram revealed three groups, including one simplicifolious (single-leafed) *N. sicca* of CSP. Among these, Men A and Men B of WCP, sdWCP, and CSP, as well as CSP of clinical isolate 2 constitute the first group. Men B of sdWCP and CSP were discovered to be most similar within this group, with a 72% similarity, while Men B of WCP showed a similarity of 63%. The similarity range between Men A of WCP, sdWCP, and CSP is between 56 - 66%, whereas Men A and Men B had a similarity range of 37%. Finally, CSP of clinical isolate 2 formed with 28% similarity to Men A and Men B.

The second group of bacteria consists of Men C, *N. sicca*, and *N. cinerea*, and both of clinical isolates. *N. cinerea* of WCP and sdWCP displayed the highest similarity of 92%, while the CSP of *N. cinerea* showed 77% similarity with *N. cinerea* of WCP and sdWCP. In terms of comparing Men C of WCP, sdWCP, and CSP, the similarities ranged from 53% to 67%. The similarity between clinical isolate 1 of WCP and sdWCP is 71%, whereas the similarity between clinical isolate 2 of WCP and sdWCP is 67%. As a result, the similarities between clinical isolates 1 and 2 are 51%. N. sicca of WCP and sdWCP had a similarity of 62%, and the similarities between both closely related species in this study are 47%. Clinical isolate 1 of CSP exhibited similarities with *N. sicca* and *N. cinerea* of about 36%. The similarity relatedness between clinical isolates and closely related species Neisseria is 34%, whereas with Men C is at 30%.

The third group comprises Men Y with *M. catarrhalis*, where the *M. catarrhalis* of WCP and sdWCP displayed an 88% similarity. Men Y of WCP, sdWCP, and CSP possess similarities ranging between 48% and 64%. Men Y and *M. catarrhalis* exhibit a 40% similarity. However, the similarity between the *M. catarrhalis* of CSP and the WCP, sdWCP of *M. catarrhalis*, and Men Y was only 33%. The dendrogram analysis revealed CSP of *N. sicca* as a single-leafed, indicating that the similarity is far from other differentially extracted *Neisseria* strains, species, and also *M. catarrhalis*. It should be noted that the extracted protein does not share any resemblance with the WCP and sdWCP of *N. sicca*. This may be due to the extraction process that resulted in the loss of some of the primary protein bands that would have shown similarity with the *N. sicca*.

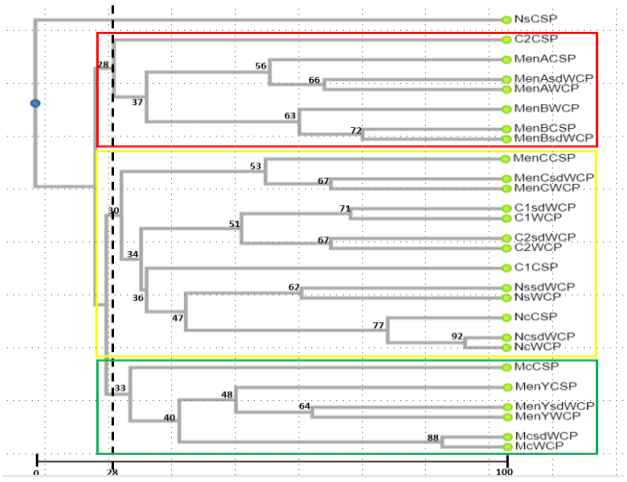


Figure 5 A UPGMA dendrogram analysis was conducted to show the relationship between different extracted strains of *N. meningitidis* and non-pathogenic *Neisseria* spp. The similarity matrix was computed using the Jaccard coefficient. The cut-off level was set at 28%, which revealed three clusters. The first cluster, marked in red, the second marked in yellow, and the third marked in green. The numbers at each joining indicate the percentage of similarity

Therefore, this study demonstrates that the protein patterns revealed by SDS-PAGE are significantly different for Men A, B, C, Y, clinical isolates 1 and 2, *N. sicca*, *N. cinerea* and *M. catarrhalis*. The results indicate that applying differential extraction methods yields diverse protein bands with a heterogeneous number of protein bands between the samples. As noted by Robertson *et al.* [34] and Abdul Lateef Khan *et al.* [13], these distinct protein patterns resulting from differential extraction provide valuable insights into species differentiation, which may aid in bacterial taxonomic classification. The differential extraction technique is employed to segregate and isolate specific protein types according to their unique properties. This process is utilised to obtain targeted protein fractions or to segregate proteins for subsequent analysis, including protein identification. Through this differential extraction, certain protein bands that exhibit heavy staining in whole cell protein (WCP) become more distinct in surface depleted whole cell protein (sdWCP) and cell surface protein (CSP). Conversely, some bands that are not visible in WCP become apparent in sdWCP and CSP. However, it's important to note that a few bands may also be eliminated during the extraction process. In a study conducted by N. Du Rand and M.D Laing [35], a comparison was made between whole cell preparation and crude extract procedures to reduce the likelihood of missing bands in SDS-PAGE analysis caused by the extraction procedure. Their study revealed a number of absent bands between crude protein extraction and whole-cell preparation. Their study indicated that the crude procedure eliminated certain proteins, retaining only those present in insecticidal crystal proteins (ICPs) as opposed to the whole cell content. In agreement with their findings, we thought a similar occurrence between the protein bands of WCP, sdWCP, and CSP for each strain and species in our research. Moreover, the dendrogram cluster analysis of differentially extracted protein for each *Neisseria* strain, species and *M. catarrhalis* shows a high degree of similarities between each own strain and species except CSP of *N. sicca*.

4.0 CONCLUSION

This study has successfully demonstrated that protein profiling via SDS-PAGE proves to be a valuable alternative, offering a comprehensive analysis of the protein composition of bacterial cells. The results presented in this study demonstrate the efficacy of SDS-PAGE in discriminating bacterial strains at both species and strain levels. The comparison of protein profiles between whole cell proteins (WCP), surface depleted whole cell proteins (sdWCP), and cell surface proteins (CSP) intricate patterns are unveiled, providing insights into the heterogeneity of protein bands among different strains and species. The dendrogram cluster analysis deepens our understanding of the relationships and similarities among the studied strains, revealing distinct clusters and highlighting the potential relatedness between certain clinical isolates and known strains.

The limitation of our study is the need for the identification of specific detectable proteins, which presents an avenue for future research. Furthermore, to expand the scope of this study, it would be beneficial to include other pathogenic *Neisseria* strains, such as W and X, along with *Neisseria gonorrhoeae*, in addition to the strains already examined. A study conducted by Rohani *et al.* [36] investigated the *Neisseria* carriage rate and serogroup distribution in an army recruit training camp in Malaysia. The findings revealed the presence of various serogroups, including A (3.3%) and W135 (4.7%), as well as other serogroups like X, Y, and Z (81%). Incorporating such additional strains could provide a more comprehensive understanding of the protein profiles and further enrich the results of this research.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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References

[1] S. Bosis, A. Mayer, and S. Esposito. 2015. Meningococcal Disease in Childhood: Epidemiology, Clinical Features and Prevention. *J. Prev. Med. Hyg.* 56(3): E121-E124.

[2] U. Thisyakorn *et al.* 2022. Invasive Meningococcal Disease in Malaysia, Philippines, Thailand, and Vietnam: An Asia-Pacific Expert Group Perspective on Current Epidemiology and Vaccination Policies. *Hum. Vaccines Immunother.* Doi: 10.1080/21645515.2022.2110759.

[3] P. A. Campsall, K. B. Laupland, and D. J. Niven. 2013. Severe Meningococcal Infection. A Review of Epidemiology, Diagnosis, and Management. *Crit. Care Clin.* 29(3): 393-409. Doi: 10.1016/j.ccc.2013.03.001.

[4] V. L. Strelow and J. E. Vidal. 2013. Invasive Meningococcal Disease. *Arq. Neuropsiquiatr. 71*(9B): 653-658. Doi: 10.1590/0004-282X20130144.

[5] R. B. Dorey, A. A. Theodosiou, R. C. Read, C. E. Jones, and J. C. D. R. T. A. Read, R. C. 2019. The Nonpathogenic Commensal Neisseria: Friends and Foes in Infectious Disease. *Curr. Opin. Infect. Dis.* 32(5): 490-496. Doi: 10.1097/QCO.0000000000000585.

[6] W. J. Kim *et al.* 2019. Commensal Neisseria Kill Neisseria gonorrhoeae through a DNA-dependent mechanism. *Cell Host Microbe*. 26(2): 228-239.e8. Doi: 10.1016/j.chom.2019.07.003.

[7] M. V. Humbert and M. Christodoulides. 2020. Atypical, yet not Infrequent, Infections with Neisseria Species. *Pathogens*. 9(1). Doi: 10.3390/pathogens9010010.
[8] T. W. Bourke, D. J. Fairley, and M. D. Shields. 2010. Rapid Diagnosis of Meningococcal Disease. *Expert Rev. Anti. Infect. Ther.* 8(12): 1321-1323. Doi: 10.1586/eri.10.132.
[9] M. C. Brouwer, G. E. Thwaites, A. R. Tunkel, and D. Van De Beek. 2012. Dilemmas in the Diagnosis of Acute Community-acquired Bacterial Meningitis. *Lancet*. 380(9854): 1684-1692. Doi: 10.1016/S0140-6736(12)61185-4.
[10] V. Manchanda, S. Gupta, and P. Bhalla. 2006. Meningococcal Disease: History, Epidemiology, Pathogenesis, Clinical Manifestations, Diagnosis, Antimicrobial Susceptibility and Prevention. *Indian J. Med. Microbiol.* 24(1): 7-19. Doi: 10.4103/0255-0857.19888.
[11] N. Singhal, A. K. Maurya, and J. S. Virdi. 2019. Bacterial Whole Cell Protein Profiling: Methodology, Applications and Constraints. *Curr. Proteomics*. 16(2): 102-109. Doi: 10.2174/1570164615666180905102253.
[12] I. Kustos, B. Kocsis, I. Kerepesi, and F. Kilár. 1998. Protein Profile Characterization of Bacterial Lysates by Capillary Electrophoresis. *Electrophoresis*. 19(13): 2317-2323. Doi: 10.1002/elps.1150191311.
[13] K. Abdul Lateef Khan and Z. Sheik Abdul Kader. 2023. Comparative Protein Profile Analysis of Differentially Extracted Whole Cell Bacterial Protein Derived from Salmonella Typhi and Invasive Non-typhoidal Salmonella. *J. Teknol. 85*(4): 189-197. Doi: 10.11113/jurnalteknologi.v85.19827.
[14] A. Arzese, G. A. Botta, G. P. Gesu, and G. Schito. 1988. Evaluation of a Computer-assisted Method of Analysing SDS—PAGE Protein Profiles in Tracing a Hospital Outbreak of Serratia Marcescens. *J. Infect.* 17(1): 35-42. Doi: 10.1016/S0163-4453(88)92284-0.
[15] J. Nicolet, P. Paroz, and M. Krawinkler. 1980. Polyacrylamide Gel Electrophoresis of Whole-cell Proteins of Porcine Strains of Haemophilus. *Int. J. Syst. Bacteriol.* 30(1): 69-76. Doi: 10.1099/00207713-30-1-69.
[16] P. Vandamme, B. Pot, M. Gillis, P. De Vos, K. Kersters, and J. Swings. 1996. Polyphasic Taxonomy, a Consensus Approach to Bacterial Systematics. *Microbiol. Rev.* 60(2): 407-438. Doi: 10.1128/mmbr.60.2.407-438.1996.
[17] O. Dos Santos, M. C. C. de Resende, A. L. de Mello, A. P. G. Frazzon, and P. A. D'Azevedo. 2012. The Use of Whole-cell Protein Profile Analysis by SDS-PAGE as an Accurate Tool to Identify Species and Subspecies of Coagulase-negative Staphylococci. *Apmis*. 120(1): 39-46. Doi: 10.1111/j.1600-0463.2011.02809.x.
[18] T. J. Biol. 2000. Availibility of Use of Total Extracellular Proteins in SDS-PAGE for Characterization of Gram-positive Cocci. *Turkish J. Biol.* 24(4): 817-824.
[19] H. Körkoca and B. Boynukara. 2003. The Characterization of Protein Profiles of the Aeromonas Hydrophila and a. Caviae Strains Isolated from Gull and Rainbow Trout Feces by SDS-PAGE. *Turkish J. Vet. Anim. Sci.* 27(5): 1173-1177.
[20] A. Horvath and H. Riezman. 1994. Rapid Protein Extraction from Saccharomyces Cerevisiae. *Yeast*. 10(10). 1305-1310. Doi: 10.1002/yea.320101007.
[21] Z. Sheik Abdul Kader. 2006. The Role of the Cell Surface Proteins of Burkholderia Pseudomallei in the Serodiagnosis of Melioidosis and Comparative Serological Proteomic Analysis of the Humoral Immune Response in Melioi. Universiti Sains Malaysia.
[22] U. K. Laemmli. 1970. Cleavage of Structural Proteins during the Assembly of the Head of Bacteriophage T4. *Nature*. 227: 680-685.
[23] S. Garcia-Vallve, A. Romeu, and J. Palau. 2000. Horizontal Gene Transfer in Bacterial and Archaeal Complete Genomes. *Genome Res.* 10(11): 1719-1725. Doi: 10.1101/gr.130000.
[24] M. Mahendrakumar and M. Asrar Sheriff. 2015. Whole Cell Protein Profiling of Human Pathogenic Bacteria Isolated from Clinical Samples. *Asian J. Sci. Res.* 8(3): 374-380. Doi: 10.3923/ajsr.2015.374.380.
[25] H. McKenzie, M. G. Morgan, J. Z. Jordens, M. C. Enright, and M. Bain. 1992. Characterisation of Hospital Isolates of Moraxella (Branhamella) catarrhalis by SDS-PAGE of Whole-cell proteins, Immunoblotting and Restriction-Endonuclease Analysis. *J. Med. Microbiol.* 37(1): 70-76. doi: 10.1099/00222615-37-1-70.
[26] L. F. Mocca and C. E. Frasch. 1982. Sodium Dodecyl Sulfate-Polyacrylamide Gel Typing System for Characterization of Neisseria Meningitidis Isolates. *J. Clin. Microbiol.* 16(2): 240-244. Doi: 10.1128/jcm.16.2.240-244.1982.
[27] J. S. Knapp, P. A. Totten, M. H. Mulks, and B. H. Minshew. 1984. Characterization of Neisseria Cinerea, a Nonpathogenic Species Isolated on Martin-Lewis Medium Selective for Pathogenic Neisseria spp. *J. Clin. Microbiol.* 19(1): 63-67. Doi: 10.1128/jcm.19.1.63-67.1984.
[28] J. Derrick, J. E. Heckels, and M. Virji. 2006. *Major Outer Membrane Proteins of Meningococci*. Wiley-VCH Verlag.
[29] C. E. Frasch, W. D. Zollinger, and J. T. Poolman. 1985. Serotype Antigens of Neisseria Meningitidis and a Proposed Scbemefor Designation of Serotypes. *Rev. Infect. Dis.* 7(4): 504-510. Doi: 10.1093/clinids/7.4.504.
[30] Y. Masforrol *et al.* 2017. A Deeper Mining on the Protein Composition of VA-MENGOC-BC®: An OMV-based Vaccine against N. Meningitidis Serogroup B and C. *Hum. Vaccines Immunother.* 13(11): 2548-2560. Doi: 10.1080/21645515.2017.1356961.
[31] K. J. Towner and A. Cockayne. 1993. *Molecular Methods for Microbial Identification and Typing*. Springer.
[32] D. A. Caugant, L. F. Mocca, C. E. Frasch, L. O. Frøholm, W. D. Zollinger, and R. K. Selander. 1987. Genetic Structure of Neisseria Meningitidis Populations in Relation to Serogroup, Serotype, and Outer Membrane Protein Pattern. *J. Bacteriol. 169*(6): 2781-2792. Doi: 10.1128/jb.169.6.2781-2792.1987.
[33] I. Berber, C. Cokmus, and E. Atalan. 2003. Characterization of Staphylococcus Species by SDS-PAGE of Whole-cell and Extracellular Proteins. *Microbiology*. 72(1): 42-47. Doi: 10.1023/A:1022221905449.
[34] D. Robertson, G. P. Mitchell, J. S. Gilroy, C. Gerrish, G. P. Bolwell, and A. R. Slabas. 1997. Differential Extraction and Protein Sequencing Reveals Major Differences in Patterns of Primary Cell Wall Proteins from Plants. *J. Biol. Chem.* 272(25): 15841-15848. Doi: 10.1074/jbc.272.25.15841.
[35] N. Du Rand and M. D Laing. 2011. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Crude Extracted Insecticidal Crystal Proteins of Bacillus Thuringiensis and Brevibacillus Laterosporus. *African J. Biotechnol.* 10(66): 15094-15099. Doi: 10.5897/AJB11.026.
[36] M. Y. Rohani *et al.* (2007). Serogroups and Antibiotic Susceptibility Patterns of Neisseria Meningitidis Isolated from Army Recruits in a Training Camp. *Malays. J. Pathol.*, 29(2): 91-94.