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Characterisation of two meningococcal group C isolates from a case and the contact of a case during the 1997 outbreak at Southampton University

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INTRODUCTION

During an outbreak of meningococcal disease at Southampton University in 1997, two *Neisseria meningitis* group C isolates were isolated from a person who died (Case) and a person who performed mouth-to-mouth resuscitation on the case (Carrier). Isolates were shown to have identical serological (PorA and PorB), MLST and PFGE profiles (**1**, **2**). In a later study whole genome comparison (**3**) demonstrated significant sequence homology with differences found in eight genes, four of which were adjacent to each other. However no further studies were undertaken at the time to investigate if the genetic differences resulted in phenotypic changes.

Here, we further interrogate differences in these isolates using comparative TMT® -proteomic and Serum Bactericidal Antibody assay (SBA) using mouse sera raised against Bexsero®. These differences could lead to a better understanding of why the outcomes of the carrier and case were so different.

MATERIALS AND METHODS

SBA was performed using mouse sera from female CD-1 immunised with six different lots of Bexsero® vaccines. Sera were tested from 1:4 to 1:32768 dilutions and bacteria were diluted 1:2500. Baby rabbit complement was used as complement source. Bactericidal MenC mAb 95/678 (NIBSC) was used as positive control.

TMT-Proteomic: Tandem-Mass-Tag (TMT®, Proteome Sciences) and isobaric tags (iTRAQ™; Applied Biosystems) were used for comparative proteomic analysis on three independent preparations of protein lysates.
RT-qPCR (Applied Biosystem 7500 Fast RT-PCR) was performed on extracted RNA using primers and TaqMan® probes (TAMRA™) from Invitrogen.
Western blots on whole cell bacterial lysates were performed using JAR4 and JAR5 monoclonal antibodies (1:1000, NIBSC) and secondary anti-mouse Cy5 (1:2500, Invitrogen). H44/76 was used as fHbp variant 1 positive control and 644 isolate (L93/4286 on PubMLST) expressing fHbp variant 2 as negative control.

RESULTS

Isolates in use

Case and Carrier isolates used in this study are shown in Table 1.

Isolate	PubMLST id	isolate	country	year	species	serogroup	ST	disease	Site of collection
Carrier	41785	2838	UK	1997	*N.meningitidis*	C	11	carrier	nasopharynx swab
Case	41784	2839						invasive	cerebrospinal fluid

Table 1. Carrier and Case isolates in use are N. meningitidis serogroup C ST-11.

TMT®-proteomic and RT-qPCR

Comparative TMT®-proteomic was performed revealing that **factor H binding protein (fHbp, NEIS0349)**, **citrate synthase (prpC, NEIS1732)**, **hypothetical integral membrane protein (imp, NEIS1147), fructose-1,6-bisphosphate aldolase (fba, NEIS0350)** and **aldehyde dehydrogenase A (aldA, NEIS1942)** are **up-regulated in the Case isolate** with fold changes (Case/Carrier) of 32.46, 2.99, 2.21, 1.81 and 1.50 respectively (Table 2). Type IV pilus-associated protein **pilC2 (NEIS0033)** is instead **downregulated in the Case isolate** with fold change of 0.05.

Protein ID	Fold change (Case/Carrier)	Status
Factor H binding protein (fHbp, NEIS0349)	32.46	Up-regulated Case/Carrier
Citrate synthase (prpC, NEIS1732)	2.99	
Hypothetical Integral membrane protein (imp, NEIS1147)	2.21	
Fructose-1,6-bisphosphate aldolase (fba, NEIS0350)	1.81	
Aldehyde dehydrogenase A (aldA, NEIS1942)	1.50	
Type IV pilus-associated protein (pilC2, NEIS0033)	0.05	Down-regulated Case/Carrier

Table 2. Comparative proteomic analysis between Case and Carrier isolates.

RT-qPCR confirmed up-regulated expression levels of prpC, imp, fba and aldA in the Case isolate showing relative transcript levels of 1.5, 1.7, 1.2 and 1.3 respectively (Figure 1).

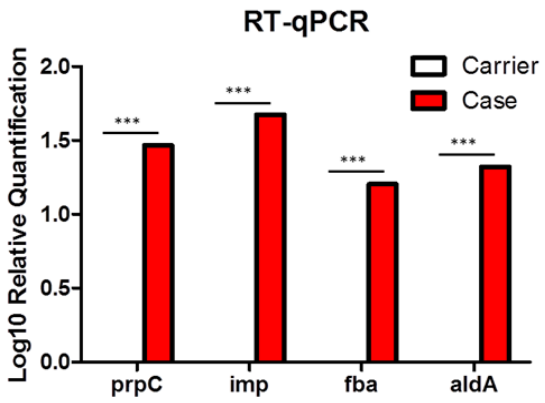

Figure 1. RT-qPCR on prpC, imp, fba and aldA confirmed their up-regulated expression levels in the Case isolate.

Factor H binding protein

Proteome data demonstrated 32.46 fold increase in expression of fHbp in the Case isolate. This is due to **lack of fHbp in the Carrier isolate resulting from a single base deletion (ΔT366)**. This mutation causes a frameshift in fHbp ORF leading to incomplete fHbp transcription and resulting in a **truncated protein**. No fHbp peptides were detected via Mass Spectrometry and Western Blotting using JAR4 and JAR5 monoclonal antibodies (mAb) in the Carrier indicating **absence of full length or truncated fHbp in the Carrier isolate** (Figure 2A,B).

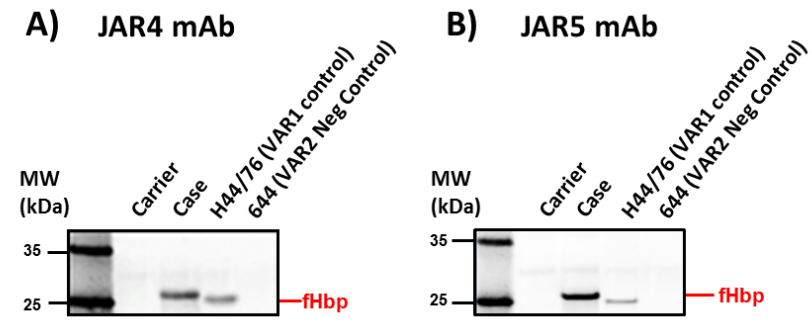

*Figure 2. Western Blots using JAR4 (**A**) and JAR5 (**B**) mAbs showed no fHbp in the Carrier isolate.*

Bexsero® efficacy

Efficacy of Bexsero® against both serogroup C Case and Carrier isolates was also evaluated. **Use of Bexsero® mouse sera in a Serum Bactericidal Antibody Assay (SBA) demonstrated killing against both serogroup C isolates** and has shown similar killing as a MenC mAb control. Increased titres were observed for the Case isolate compared to Carrier (p=0.005, Figure 3).

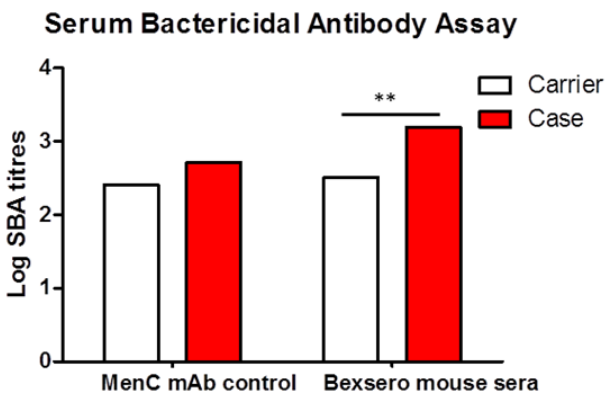

Figure 3. Log SBA titres for Case and Carrier isolates using Bexsero® mouse sera showed increased efficacy against the Case isolate. Results are shown as pool from all mice sera immunised with Bexsero®.

CONCLUSIONS

- **Comparative proteomic** revealed that **four proteins (prpC, imp, fba, aldA) are up-regulated in the Case isolate**, while one protein (pilC2) is down-regulated; **RT-qPCR confirmed expression levels of up-regulated proteins in the Case isolate**;
- **Factor H binding protein (fHbp)** resulted as major difference between Case and Carrier isolate.
- **Due to one base deletion (ΔT366), fHbp is not expressed in the Carrier isolate;**
- **Bexsero® MenB vaccine is effective against both serogroup C Case and Carrier isolates;**
- **Carrier isolate is killed by the action of Bexsero®'s antibodies likely targeting the other Bexsero® antigens,** confirming the **importance of a multi-component vaccine;**
- **Absence of fHbp in the Carrier isolate might explain its reduced virulence**.

Further studies will be undertaken to address the biological relevance of the highlighted differences.

REFERENCES and ACKNOWLEDGEMENTS

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(**1**) Gilmore, A., G. Jones, M. Barker, N. Soltanpoor, and J.M Stuart. 1999. Meningococcal disease at the University of Southampton: outbreak investigation. Epidemiol. Infect. 123:185-192
(**2**) Feavers, I. M., S. J. Gray, R. Urwin, J. E. Russell, J. A. Bygraves, E. B. Kaczmarski, and M. C. J. Maiden. 1999. Multilocus Sequence Typing and Antigen Gene Sequencing in the Investigation of a Meningococcal Disease Outbreak. J Clin Microbiol. 3883-3887
(**3**) Jolley, K.A, D. M. C. Hill, H.B. Bratcher, O.B. Harrison, I.M. Feavers, J. Parkhill, and M.C. J. Maiden. 2012. Resolution of a Meningococcal Disease Outbreak from Whole-Genome Sequence Data with Rapid Web-Based Analysis Methods. J Clin Microbiol. 3046–3053
(**4**) Van de Rijn, I. and R.E. Kessler. 1980. Growth characteristics of group A streptococci in a new chemically defined medium. Infect Immun. 27(2):444-8