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NIBSC

Factor H binding protein (fHbp) mediates differential complement resistance of a serogroup C *Neisseria meningitidis* isolate from CSF of a patient with invasive meningococcal disease

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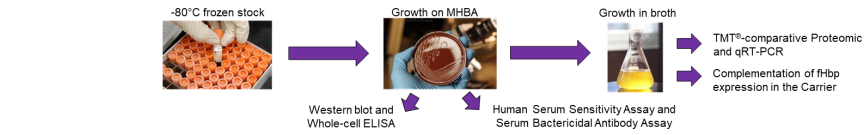
INTRODUCTION

During an outbreak of invasive meningococcal disease at University of Southampton in 1997, two *Neisseria meningitis (Nme)* group C isolates were retrieved from a person who died (Case) and a person (Carrier) who performed mouth-to-mouth resuscitation on the case without contracting the disease. Isolate were shown to have identical serological (PorA and PorB), MLST and PFGE profiles[1, 2] and whole genome comparison revealed differences in only eight genes[3].

Here we expand on previous studies to investigate phenotypic differences which could explain their contrasting clinical outcomes.

MATERIALS AND METHODS



TMT®-MS comparative proteomic (Proteome Sciences) and isobaric tags (Applied Biosystems) were performed on three independent protein lysates.

qRT-PCR was performed using custom primers and probes (Thermo Fisher Scientific) in MicroAmp Fast Optical 96-Well using ~50ng of RNA. Expressions were normalised using housekeeping gene *gdh* then further normalised against the Carrier calibrator strain.

fHbp expression in the Case but absence of expression in the Carrier was demonstrated **by Western Blot** using anti-fHbp mAb JAR5 (1:1,000, NIBSC) and secondary anti-mouse Cy5 (1:2,500, Invitrogen). H44/76 was used as fHbp variant 1 (V1) positive control while isolate 644 (L93/4286, PubMLST) was used as fHbp variant 1 negative control as expresses variant 2. RmpM polyclonal mouse sera (1:1,000, NIBSC) was used as loading control.

fHbp (V1 from MC58) in pGCC4 (kindly donated by Prof. Tang, University of Oxford) **was re-introduced in the Carrier via molecular cloning** and homologous recombination at *lctP* and *aspC* loci. Expression was achieved by overnight incubation in broth with 1mM IPTG (Sigma).

Human Serum Survival Assay to measure isolates' ability to survive in human serum was performed by incubating 1:2,500 of bacteria with heat (HI) or non-heat inactivated human CDC1992 serum (NIBSC 99/706) for 1 hour at 37°C, 5% CO_2.

Whole-cell ELISA using 1:2,000 Bexsero (1/160 of human dose), NadA or NHBA mouse sera (NIBSC) and secondary antibody anti-mouse IgG (1:2,500, Sigma) was performed on both isolates.

Serum Bactericidal Antibody Assay was performed incubating serial dilutions of pooled CD1 mouse sera immunised with Bexsero, 1:2,500 of bacteria and baby rabbit complement (Pel-Freez Biologicals) for 1 hour at 37°C.

RESULTS

Isolates

***Table 1**. Isolates used in this study are N. meningitidis serogroup C cc11.*

Isolate	PubMLST id	country	year	species	Sero-group	ST	outcome	Site of collection
Carrier	41785	UK	1997	*Neisseria meningitidis*	C	11	Carrier	nasopharynx
Case	41784						Disease	CSF

Isolates' proteomes show a high degree of homology

Comparative proteomics revealed that the isolates' proteomes are almost identical. Four proteins (prpC, imp, fba, aldA) are upregulated in the Case isolate while one protein (pilC2) is downregulated. Expression levels for *prpC*, *imp*, *fba* and *aldA* genes were quantified via qRT-PCR and shown to be upregulated in the Case isolate (**Table 2**).

***Table 2**. Comparative proteomics results.*

Locus	Protein ID	RQ of relative gene expression (Case vs. Carrier)	Protein fold change (Case/Carrier)	Functions	Status
NEIS1732	Citrate synthase (**PrpC**)	29.27	2.99	transferase activity	Upregulated in the Case isolate
NEIS1147	Hypothetical Integral membrane protein (**Imp**)	47.1	2.21	hypothetical membrane protein	
NEIS0350	Fructose-1,6-bisphosphate aldolase (**Fba**)	15.94	1.81	metabolism; binding to human plasminogen; additional "moonlight" functions	
NEIS1942	Aldehyde dehydrogenase A (**AldA**)	20.91	1.5	oxidoreductase activity	
NEIS0033	Type IV pilus-associated protein (**PilC2**)	not measured	0.05	adhesion	Downregulated in the Case isolate

Factor H binding protein (fHbp) is not expressed by the Carrier

Peptides from Factor H binding protein (fHbp) were not detected in the Carrier isolate in mass spectrometric analysis.

The lack of fHbp was further confirmed by the presence of a one base deletion (ΔT366) causing a frameshift mutation in the Carrier isolate.

Furthermore Western blot analysis on the isolates detected fHbp in Case but not in the Carrier (**Fig. 1**).

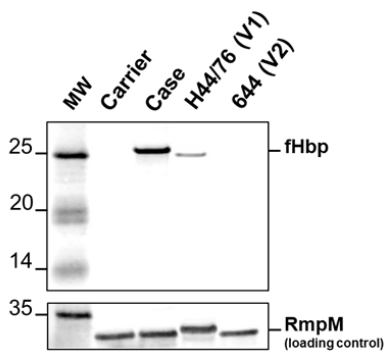
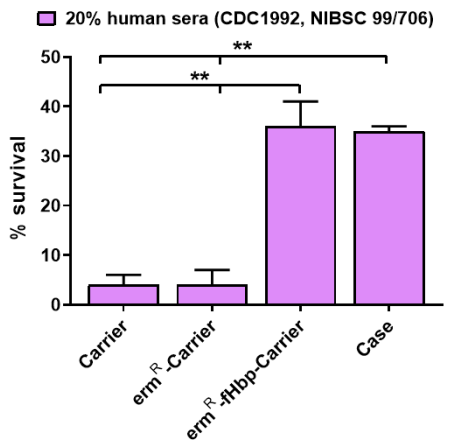


***Figure 1**. Western Blot using JAR5 anti-fHbp mAb showed no fHbp expression in the Carrier. Full-length fHbp (variant 1, V1) is expressed by the Case. RmpM was used as loading control.*

Absence of fHbp expression accounts for reduced complement resistance of the Carrier isolate

Increased survival in human serum for the Case compared to the Carrier isolate was observed in a serum survival assay.



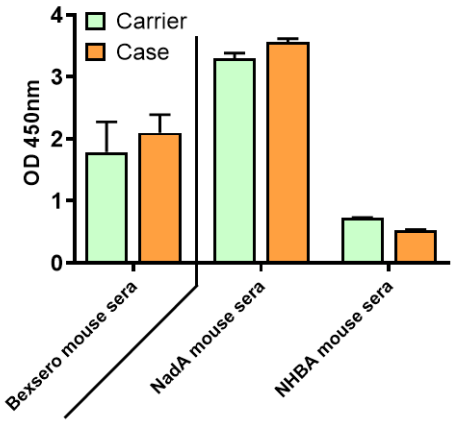
To test whether this could be attributed to fHbp, fHbp expression was complemented in the Carrier strain (erm^R-fHbp-Carrier), resulting in a significant increase in serum survival for this isolate.
This indicates an important role played by fHbp in complement resistance (**Fig. 2**).

***Figure 2**. Serum survival assay showed an increased survival in serum for the Case compared to the Carrier. Complementation of fHbp expression in the Carrier increases its survival by 31%.*

Bexsero induces bactericidal antibodies against both isolates

Bexsero, a vaccine designed to target MenB strains, can be effective in preventing disease caused by other serogroups, if these isolates express one or more of its antigens.

Sera from mice vaccinated with Bexsero or NadA strongly reacted against both isolates in whole-cell ELISA, suggesting that isolates express at least one Bexsero antigen (**Fig. 3**).



Moreover, bactericidal killing of both isolates was demonstrated in Serum Bactericidal Antibody assays (SBA). Increased titres were observed for the Case compared to the Carrier (killing at reciprocal dilution of 50% with titres of 2048 and 512 respectively), likely due to the presence of anti-fHbp antibodies in Bexsero serum.

***Figure 3**. Whole-cell ELISA using Bexsero, NadA and NHBA mouse sera on Carrier and Case showed strong reactivity with Bexsero and NadA sera for both isolates.*

CONCLUSIONS

- Isolates genomes and proteomes are mostly identical;
- **factor H binding protein (fHbp) is not expressed by the Carrier,** while it is expressed by the Case isolate;
- **fHbp expression by the Case isolate enhanced its survival in human serum. This might have played a role in the virulence of this isolate** in the deceased student;
- **Re-introduction of fHbp expresssion in the Carrier isolate increases Carrier's survival in human serum** indicating an important role played by fHbp in complement resistance;
- **SBA killing of group C Case and Carrier isolates was observed with murine serum raised against Bexsero.**

REFERENCES and ACKNOWLEDGEMENTS

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