Characterisation of Non-Meningococcal/Gonococcal Neisseria Strains From Invasive Cases in England

Lloyd Walsh [1], Stephen A Clark [1], Jay Lucidarme [1], Aiswarya Lekshmi [1], Jeremy P Derrick [2], Ray Borrow [1]

[1] Meningococcal Reference Unit, UK Health Security Agency, Manchester, United Kingdom

[2] School of Biological Sciences, Faculty of Biology, Medicine, and Health, University of Manchester, Manchester, United Kingdom.

INTRODUCTION

- Many *Neisseria* species are commensal to the human oro-nasopharynx in the same manner as *N. meningitidis,* but with a much reduced association with disease.
- On rare occasions, many of these species cause a range of invasive diseases including meningitis, septicaemia and endocarditis. Patients may be predisposed to these infections by surgery and immunosuppression but they can occur in seemingly healthy patients. The exact incidence of disease caused by these organisms is unknown, but there are many published case reports.
- *Neisseria* species uptake DNA primarily through type IV pili and undergo frequent horizontal gene transfer (HGT). This includes genes encoding for vaccine antigens and genes encoding antibiotic resistance.
- The UKHSA Meningococcal Reference Unit (MRU) received 35 non-meningococcal/gonococcal *Neisseria* isolates from cases in England between 2010 and 2021.

METHODS

Neisseria from invasive sites (expected to be sterile in a healthy patient) isolated between 2010 and 2021 were characterized visually, biochemically, and with antimicrobial susceptibility testing.

DNA was extracted from the isolates, whole-genome sequencing performed and sequences uploaded to the Neisseria BIGSDb database (PubMLST.org) to determine species, antigenic profile and genetic similarity.

RESULTS

- Of the 35 isolates characterised (Table 1), *N. subflava* was the most commonly-isolated species (n=11), followed by *N. mucosa* (n=9), *N. polysaccharea* (n=4), *N. oralis* (n=4), *N. cinerea* (n=3), *N. bergeri* (n=2) and *N. elongata* (n=2).
- Almost all (33/35, 94.3%) of isolates were isolated from blood culture, indicating bacteraemia/septicaemia. The two remaining strains were grown from CSF confirming clinically-suspected meningitis. Four isolates were grown from blood but the clinical notes suggest concurrent meningitis.
- Interestingly, eight cases (23%) were in suspected or confirmed cancer patients; this comprised 100% of *N. polysaccharea* infections and 50% of *N. bergeri* and *N. oralis* cases. The majority of cancer patients (63%) had leukaemia and 36% had lymphoma.
- In 14% of cases, other bacteria or viruses were detected, possibly indicative of coinfection.
- Two cases, an *N. mucosa* infection in a possible cancer patient and an *N. cinerea* and *Parainfluenza* virus coinfection were fatal

Table 1 : Strains isolated from invasive sites with age and clinical presentation

Isolate	Species	Age Group	Sample Site	Clinical Presentation
M12 240875	***N. bergeri***	45-64	Blood	Bacteraemia
M16 240279	***N. bergeri***	12-16	Blood	Bacteraemia, Cancer patient
M11 240331	***N. cinerea***	0	Blood	Bacteraemia
M12 240348	***N. cinerea***	0	Blood	Bacteraemia, *Parainfluenza* virus coinfection, Fatal
M16 240038	***N. cinerea***	0	Blood	Bacteraemia
M13 240630A	***N. elongata***	45-64	Blood	Bacteraemia, *A. faecalis* and *M. osloensis* coinfection
M14 240009	***N. elongata***	1-4	Blood	Bacteraemia
M11 240095	***N. mucosa***	1-4	Blood	Bacteraemia
M11 240746	***N. mucosa***	1-4	Blood	Meningitis, Bacteraemia, Enterovirus coinfection
M13 240010	***N. mucosa***	0	Blood	Bacteraemia
M13 240156	***N. mucosa***	0	CSF	Meningitis
M13 240159	***N. mucosa***	1-4	Blood	Bacteraemia, *Measles morbillivirus* coinfection
M14 240642	***N. mucosa***	65+	Blood	Bacteraemia, *Moraxella* species coinfection
M16 240658	***N. mucosa***	65+	Blood	Bacteraemia, Suspected cancer, Fatal
M16 240664	***N. mucosa***	0	Blood	Bacteraemia
M19 240195	***N. mucosa***	65+	Blood	Bacteraemia
M12 240690	***N. oralis***	25-44	Blood	Bacteraemia, Cancer patient
M13 240387	***N. oralis***	0	CSF	Meningitis
M16 240505	***N. oralis***	65+	Blood	Bacteraemia, Cancer patient
M19 240699	***N. oralis***	45-64	Bone	Bone necrosis
M15 240827	***N. polysaccharea***	65+	Blood	Bacteraemia, Cancer patient
M17 240155	***N. polysaccharea***	1-4	Blood	Bacteraemia, Cancer patient
M20 240201	***N. polysaccharea***	1-4	Blood	Bacteraemia, Cancer patient
M21 240071	***N. polysaccharea***	5-11	Blood	Meningitis, Bacteraemia, Cancer patient
M10 240439	***N. subflava***	1-4	Blood	Bacteraemia
M11 240194	***N. subflava***	65+	Blood	Bacteraemia
M12 240157	***N. subflava***	25-44	Blood	Bacteraemia
M12 240744	***N. subflava***	0	Blood	Bacteraemia
M13 240190	***N. subflava***	1-4	Blood	Bacteraemia, Pneumonia
M13 240236	***N. subflava***	65+	Blood	Meningitis, Bacteraemia
M13 240631	***N. subflava***	5-11	Blood	Bacteraemia
M13 240728	***N. subflava***	1-4	Blood	Meningitis, Bacteraemia
M19 240062	***N. subflava***	65+	Blood	Bacteraemia
M19 240249	***N. subflava***	0	Blood	Bacteraemia
M19 240656	***N. subflava***	0	Blood	Bacteraemia, Periorbital cellulitis

- Table 2 shows a subset of isolates that possessed antigens found within licenced MenB vaccines.
- All *N. polysaccharea, N. cinerea* and *N. bergeri* isolates harboured fHbp alleles, with a variety of variant 1 (subfamily B) and variant 3 (subfamily A) variants observed. All *N. polysaccharea* and *N. bergeri* isolates possessed NHBA alleles.

Table 2 : Strains possessing alleles for meningococcal vaccine antigens.

Isolate	Species	fHbp	fHbp peptide	Pfizer subfamily	Novartis Variant Family	NHBA	NHBA peptide	NadA	NadA peptide
M12 240875	*N. bergeri*	New allele	555	A*	3*	752	207	n/a	n/a
M16 240279	*N. bergeri*	New allele	New allele	B*	1*	752	207	n/a	n/a
M16 240038	*N. cinerea*	512	437	B	1	n/a	n/a	NadA homolog with insertion	NadA homolog with insertion
M11 240331	*N. cinerea*	100	100	B	1	n/a	n/a	Partial NadA with downstream fusion	Partial NadA with downstream fusion
M12 240348	*N. cinerea*	322	275	B	1	n/a	n/a	n/a	n/a
M15 240827	*N. polysaccharea*	840	685	A	3	728	291	n/a	n/a
M17 240155	*N. polysaccharea*	169	160	A	3	1240	1136	n/a	n/a
M20 240201	*N. polysaccharea*	673	570	A	3	81	470	n/a	n/a
M21 240071	*N. polysaccharea*	673	570	A	3	81	470	n/a	n/a

*presumed variant/subfamily based on closest homology with annotated fHbp alleles.

- Table 3 contains the antimicrobial MICs and resistance-associated amino acid for the studied isolates.
- Penicillin and cefotaxime resistance were correlated with *porB* and *penA* amino acid configurations associated with resistance in gonococci. *gyrA* alterations associated with ciprofloxacin resistance in meningococci and gonococci conferred increased resistance in these strains. All isolates possessed all five *penA* mutations associated with penicillin resistance in meningococci (not shown). All species were negative for all known beta-lactamases.
- Many strains were also resistant to rifampicin and three *N. subflava* isolates had *tetM* plasmids promoting azithromycin resistance.

Table 3 : Antibiotic MIC values and relevant amino acid profiles. Breakpoints values for *N. meningitidis* and *N. gonorrhoeae* were used as indicated below.

Strain	Species	porB G120	porB A121	penA I312	penA V316	gyrA S/T91	gyrA D95	Penicillin MIC (mg/L)	Cefotaxime MIC (mg/L)	Ciprofloxacin MIC (mg/L)
M12 240875	*N. bergeri*	G	D	I	V	S	D	0.25	0.006	0.003
M16 240279	*N. bergeri*	G	D	I	V	S	D	0.25	0.012	0.004
M11 240331	*N. cinerea*	G	D	I	V	T	D	0.19	0.006	0.023
M12 240348	*N. cinerea*	G	D	I	V	T	D	0.5	0.012	0.004
M16 240038	*N. cinerea*	G	D	I	V	T	D	0.19	0.004	0.016
M13 240630A	*N. elongata*	M	D	n/a	n/a	S	D	0.023	0.004	0.047
M14 240009	*N. elongata*	M	D	n/a	n/a	S	D	0.19	0.094	0.002
M11 240095	*N. mucosa*	S	D	M	T	S	D	4	0.19	0.047
M11 240746	*N. mucosa*	S	D	M	T	S	D	6	0.38	0.016
M13 240010	*N. mucosa*	S	D	M	T	S	D	6	0.25	0.25
M13 240156	*N. mucosa*	S	D	M	T	S	Y	12	0.25	0.38
M13 240159	*N. mucosa*	T	D	M	T	S	D	2	0.19	0.023
M14 240642	*N. mucosa*	S	D	M	T	S	D	0.75	0.064	0.006
M16 240658	*N. mucosa*	S	D	M	T	S	D	1.5	0.125	0.023
M16 240664	*N. mucosa*	S	D	M	T	S	D	0.75	0.064	0.016
M19 240195	*N. mucosa*	S	D	M	T	S	D	3	0.25	0.094
M12 240690	*N. oralis*	A	G	M	T	S	D	2	0.19	0.006
M13 240387	*N. oralis*	A	G	M	T	S	D	1.5	0.125	0.016
M16 240505	*N. oralis*	A	G	M	T	S	D	0.75	0.125	0.006
M19 240699	*N. oralis*	A	G	M	T	S	D	1	0.125	0.012
M15 240827	*N. polysaccharea*	G	D	I	V	S	D	0.125	0.002	0.003
M17 240155	*N. polysaccharea*	G	D	M	T	S	D	0.5	0.032	0.008
M20 240201	*N. polysaccharea*	G	D	I	V	S	D	0.25	0.003	0.003
M21 240071	*N. polysaccharea*	G	D	I	V	S	D	0.25	0.006	0.004
M10 240439	*N. subflava*	S	D	M	T	T	D	4	0.125	0.023
M11 240194	*N. subflava*	S	D	M	T	T	D	0.75	0.032	0.023
M12 240157	*N. subflava*	S	D	M	T	T	D	1.5	0.094	0.023
M12 240744	*N. subflava*	S	D	M	T	T	D	0.75	0.064	0.008
M13 240190	*N. subflava*	S	D	M	T	T	D	4	0.19	0.023
M13 240236	*N. subflava*	S	D	M	T	T	D	1.5	0.125	0.016
M13 240631	*N. subflava*	S	D	M	T	I	D	1	0.032	0.5
M13 240728	*N. subflava*	S	D	M	T	T	D	2	0.094	0.032
M19 240062	*N. subflava*	S	D	M	T	*	*	1.5	0.064	0.094
M19 240249	*N. subflava*	S	D	M	T	T	D	3	0.125	0.023
M19 240656	*N. subflava*	S	D	M	T	I	D	1	0.125	0.38

Borderline Resistance (Meningococcal Breakpoints)

Resistant (Meningococcal and Gonococcal Breakpoints)

Resistant (Meningococcal Breakpoints Only)

Resistance-Associated Amino Acid

Resistance thresholds as per European Committee on Antimicrobial Susceptibility Testing (EUCAST)

DISCUSSION AND CONCLUSIONS

- Whilst rare, infections from typically commensal *Neisseria* species do occur and can present in serious infectious disease including bacteraemia/ septicaemia or meningitis. Clinicians should be mindful of this possibility in assessing positive blood culture results.
- *N. polysaccharea* disease has not been described in published literature. Interestingly, all cases identified were in cancer patients, suggesting an even lower risk of infection in immunocompetent individuals.
- Many of these *Neisseria* species exhibited significant resistance to antibiotics typically used to treat *N. meningitidis* and *N. gonorrhoeae,* which may lead to challenges in treating these infections. The ability of these strains to confer resistance to other species through HGT is also a concern.
- *N. bergeri, N. cinerea* and *N. polysaccharea* were shown to harbour meningococcal vaccine antigens which, if expressed, may lead to immune cross-reactivity in vaccinated individuals. Expression of fHbp in some strains of *N. cinerea* and *N. polysaccharea* has previously been confirmed; NHBA expression has not yet been demonstrated.

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