These data suggest that the former three plasmid DNAs had identical nucleotide sequences and that the latter two had very similar sequences. At present, however, the functions of all these plasmids are unknown. To determine these functions, nucleotide sequence information will be required.

Recently, On and colleagues analysed 29 strains of *C. lari* – a species that has proved both genotypically and phenotypically diverse – by extensive phenotypic characterisation, whole-cell protein profiling, amplified fragment length polymorphism analysis, 16S rDNA sequencing and DNA – DNA hybridisation.[17] Results led them to propose the existence of three *C. lari* subspecies, namely *C. lari* subsp. *lari* ('classical' strains), *C. lari* subsp. *ureasum* (UPTC strains) and *C. lari* subsp. *subantarcticus* (subantarctic animal isolates).[17]

It is anticipated that the present study will stimulate greater interest in UPTC as a *C. lari* subspecies, and that molecular characterisation, including nucleotide sequencing of the plasmid DNAs of the UPTC strains detected in the present study, may result in an understanding of the function of the plasmids. □

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Multilocus sequence typing and *porA* gene sequencing differentiates strains of *Neisseria meningitidis* during case clusters

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Notified cases of meningococcal disease (MD) in Scotland and many other countries within Europe have increased in recent years[1] and are often associated with epidemic strains such as C:2a:P1.5 from the ET-37 complex.[2] A number of case clusters due to these strains have occurred in schools, universities and other close-contact situations.[3-5] Characterisation of *Neisseria meningitidis* strains isolated during case clusters is important for public health management, and indicates the necessity for molecular techniques to differentiate strains.[6]

In Scotland, serogroup C disease became prevalent from the mid-1990s and was the predominate serogroup in 1998, accounting for 46% of all serogroupable invasive isolates.[1] Strains isolated during case clusters could not be differentiated if they were C:2a:P1.5 because there was no provision for genotyping. Therefore, multilocus sequence typing (MLST)[7] and *porA* gene sequencing[8] were introduced as a national service in 2000, in order to fully characterise all meningococcal isolates in Scotland.

Although MLST is a recently-described method, it has proved to be highly discriminatory for the differentiation of a number of bacterial pathogens.[9] Here, we describe the use of MLST and *porA* gene sequencing to differentiate *N. meningitidis* strains in two clusters.

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Table 1. Summary of phenotypic and genotypic results from five patients in two case clusters

Patient no.	Isolate type	Phenotypic characterisation			Allelic profile [a]	MLST type	Variable regions		
		Serogroup	Serotype	Sero-subtype		(ST)	VR1	VR2	VR3
1	Blood	C	2A	P1.5	2 3 4 3 8 4 6	11	5-1	10-4	36b
2	Eye	C	2A	P1.5	2 3 4 3 8 4 6	11	5-1	10-4	36b
3	Throat	B	1	P1.14	4 10 15 9 8 11 17	283	7	14	35
4	Throat	B	NT[b]	P1.16	7 5 1 13 13 18 15	1199	7	16	35
5	CSF[c]	B	-	-	2 6 4 - 9 - 6	-	-	-	-

[a] *Allelic profile corresponds to the nucleotide sequences of the seven housekeeping genes, namely abcZ, adk, aroE, fumC, gdh, pdhC and pgm*
[b] *NT: non-typable with monoclonal antibodies*
[c] *Direct from clinical sample*

N. meningitidis strains isolated from patients with MD were sent by area diagnostic microbiology laboratories to the Scottish Meningococcus and Pneumococcus Reference Laboratory (SMPRL) in Glasgow. Culture confirmation of MD was performed on presumed isolates of *N. meningitidis* using standard procedures for the biochemical identification, serogrouping and serotyping/subtyping of meningococci.[10-12] MLST and *porA* gene sequencing were performed using a semi-automated system, as described previously.[13] DNA sequence data were edited accordingly and entered onto the MLST and *porA* databases (http://neisseria.org/nm/typing/pora/ and http://www.mlst.net/new/index.htm, respectively). Currently, the *porA* website does not permit VR3 queries, so the data relating to VR3 sequences were aligned with published data.[4,13]

The first case cluster occurred within a family, and MLST and *porA* gene sequencing were used to fully characterise strains of *N. meningitidis* isolated from two siblings, a girl and a boy. The girl (patient 1), aged three years, presented with a temperature and bilateral purulent conjunctivitis, and *N. meningitidis* C:2a:P1.5 was isolated from both throat and conjunctival swabs. Two non-blanching petechiae were also noted on the nape of her neck, although otherwise she was well.

The boy (patient 2), aged four years, presented with abdominal pain and otitis media, and his chest was covered with molluscum contagiosum. He was discharged from hospital. Two weeks later he presented with a temperature, painful hip and six non-blanching petechiae on his back. He had also complained of a painful knee the previous day. There were no signs of meningism. Four days later he developed molluscum contagiosum on his left cheek and herpes simplex on his chin. *N. meningitidis* C:2a:P1.5 was isolated from blood cultures.

Both children recovered uneventfully and without any recorded sequelae. As the isolates were phenotypically identical, further genotypic characterisation was performed. MLST and *porA* gene sequencing indicated that the strains were also genotypically identical at all seven MLST loci and three *porA* variable regions (Table 1). It is possible that infection passed from the girl to the boy or that infection was acquired from another close contact, although this was not ascertained. Unfortunately, it was not possible to take throat swabs from the remaining family and other close contacts to determine throat carriage in these individuals. Although the family cluster was not a major public health threat, the use of MLST and *porA* gene sequencing indicated that it is very useful in such cases.

The second case cluster was potentially high profile as two cases occurred in one health board area, one of which attended university in another health board area. In addition, these cases were putatively linked to another case in the health board area of the university.

The first patient was an 18-year-old male university student (patient 3) who presented with symptoms of meningitis and was hospitalised with suspected MD. *N. meningitidis* was isolated from a throat swab and the patient subsequently died. He had been away from the university for approximately 10 days due to holidays.

An 18-year-old female (patient 4) presented to her general practitioner in the same health board area with recurrent sore throat, and *N. meningitidis* was isolated from a swab. She had attended a party the previous week at which patient 3 had been present, although they were not close friends and did not knowingly have any close contact. Both isolates were sent to the reference laboratory for confirmation and typing.

A further complication arose when a man in the health board area of the university presented with septicaemia. This patient, a 20-year-old (patient 5), worked in commercial premises frequented by students near the university. Meningococcal PCR testing on a plasma sample showed that he was infected with *N. meningitidis* serogroup B.

Phenotypic analysis of the isolates from patients 3 and 4 showed that they were *N. meningitidis* B:nt:P1.16 and *N. meningitidis* B:1:P1.14, respectively. Although phenotypically distinct, it was thought that the cases in this cluster were not linked; however, type and subtype of the infecting organism in patient 5 was not determined and may have been identical to that of patient 3 or 4. Even so, MLST and *porA* gene sequencing were used as an additional tool,

before phenotypic results were available, and showed that the isolates were quite distinct genotypically (Table 1).

All MLST data from this study are available on the MLST website (http://www.mlst.net/new/index.htm).

Although MLST has been used previously to differentiate meningococcal strains in a university outbreak,[3] we believe this to be the first time that a national MLST and *porA* gene sequencing service has been used to discriminate strains in a family cluster and potential university cluster.

MLST and *porA* gene sequencing can be performed quickly – often more quickly than phenotypic methods, if appropriate facilities exist – so that strains can be differentiated within the timescale required for public health management. The service provided in Scotland, for example, can provide results within 24 hours.

In conclusion, MLST and *porA* gene sequencing provides a rapid and portable typing method for differentiating strains that appear identical phenotypically, and can be introduced as a national service for typing cluster strains. □

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Prevalence of *Campylobacter* species among HIV/AIDS patients in Nigeria

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Numerous opportunistic pathogens of viral, bacterial, fungal and parasitic origin are associated with human immunodeficiency virus (HIV) infection and acquired immune deficiency syndrome (AIDS). Such infections are incriminated as the most important causes of morbidity and mortality in HIV/AIDS patients,[1] and some are associated with HIV/AIDS infections in Lagos, Nigeria.[2]

Diarrhoea is the most common gastrointestinal symptom of AIDS and affects 50-90% of patients.[3] *Campylobacter* spp. are known to be one of the more prevalent organisms in HIV diarrhoea,[4] and have been mentioned among paediatric patients.[5]

This study aims to establish the prevalence of *Campylobacter* spp. associated with diarrhoea among HIV/AIDS patients in Lagos, their prevalent biotypes, antibiotic susceptibility patterns and plasmid profiles.

Of 160 stool and rectal swab samples collected from confirmed cases of HIV/AIDS, 40 isolates were obtained. *Campylobacter* spp. were isolated from six (7%) out of 84 patients with diarrhoea and two (2.5%) out of 76 patients without diarrhoea. Other bacterial agents included *Enterobacter* spp. (12 [7.5%]), *Salmonella paratyphi* A (8 [5%]) and *Escherichia coli* (4 [2.5%]), followed by *Shigella sonnei, Klebsiella pneumoniae, Proteus vulgaris* and *Candida* spp. (two each [1.25%]).

All HIV-infected patients were heterosexual, with an age range between 15 and 55 years, and diarrhoea was more prevalent in males (120 [75%]) than females (40 [25%]).

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