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Improved methods of detection of meningococcal DNA from oropharyngeal swabs from cases and contacts of meningococcal disease

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SUMMARY

In the UK the increasing use of pre-admission parenteral antibiotic therapy in meningococcal disease has lessened the value of routine cultures as a tool to confirm diagnosis, and laboratory confirmation of invasive meningococcal infection is achieved increasingly by non-culture, nucleic acid amplification methods. The purpose of this study was to evaluate a DNA extraction and meningococcal-specific DNA amplification methodology for detection of meningococci from oropharyngeal swabs.

One hundred and six swabs from suspected or confirmed cases of meningococcal disease, and 94 swabs from contacts of meningococcal disease cases were examined. Of laboratory-confirmed cases, 38/65 (58·5%) yielded a positive oropharyngeal swab PCR result and 5/24 (20·8%) swabs from suspected but laboratory-unconfirmed cases were PCR positive. No significant differences in PCR positivity rates were found between the types of swab transport systems utilized, but transport time to the testing laboratory was found to affect PCR positivity ($P < 0{\cdot}05$).

Application of meningococcus-specific PCR to oropharyngeal swabs, in addition to routine culture of swabs, can provide valuable epidemiological information as well as case confirmation for contact management. PCR amplification of meningococcal PCR from oropharyngeal swabs will also increase the ascertainment in swabbing surveys carried out as part of meningococcal disease outbreak investigation and management.

INTRODUCTION

Neisseria meningitidis is the major cause of bacterial meningitis and an important cause of septicaemia within the UK [1]. Licensed polysaccharide vaccines are available for serogroups A, C, W-135 and Y, but for serogroup C are not immunogenic in infants less than 2 years of age [2]. Oligosaccharide-protein conjugate vaccines for serogroup C meningococci have recently undergone phase II trials in young infants, proving to be safe and immunogenic [3, 4], and widespread vaccination is now being undertaken in the UK [5]. Since the new conjugated vaccines will be introduced without randomized controlled trials of efficacy, it will be of importance to evaluate the effect of vaccination pressure on the ecology of populations of carried meningococci and on their serogroup composition.

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Studies of meningococcal carriage in individual cases and in populations have been hampered by the relative insensitivity of isolation from oropharyngeal swabs, with even those obtained promptly from meningococcal disease patients yielding an organism in only about 50% of cases [6]. Also, many meningococcal carrier isolates are non-serogroupable [7] as it is the non-capsular state which facilitates invasion [8, 9]. Recently, it has been demonstrated that phenotypically non-serogroupable isolates can be serogrouped genotypically by using a polymerase chain reaction (PCR) assay based upon a meningococcal sialyltransferase (*siaD*) [10, 11]. This assay has already proved to be valuable in determining more accurately the incidence of carriage of the outbreak strain in both serogroup B and C outbreaks [10].

The insensitivity of traditional methods of culture has highlighted the need for a more sensitive technique for detection of meningococci from oropharyngeal swabs. The use of non-culture DNA-based assays such as the Taqman™ meningococcal-specific PCR-based assay has been shown to improve rates of laboratory confirmation of meningococcal disease [12] especially in cases where pre-admission use of antibiotics has resulted in the inability to culture organisms. Combining an effective DNA extraction method for oropharyngeal swabs with established Taqman™ assays offers potential advantages for assessing carriage rates of meningococci as well as providing an additional method to establish the serogroup of non-culture confirmed cases of meningococcal disease.

In this study a DNA extraction method from oropharyngeal swabs, obtained from clinically confirmed cases of meningococcal disease and contacts, was evaluated using Taqman™ assays previously developed [12]. These Taqman™ assays involve a screening assay, the *ctrA* assay, to detect the presence of meningococcal DNA, followed by a serogrouping (*siaD*) assay on any screen-positive samples to determine whether the samples are serogroupable for B or C.

METHODS

Samples

Oropharyngeal swabs were obtained from either clinically confirmed cases or contacts of confirmed cases. Three types of swab were received for analysis: (i) plain swabs and swabs preserved in Amies Transport Medium (ii) with or (iii) without charcoal [13].

Oropharyngeal swab DNA extraction

Swabs were first emulsified in 200 μl of sterile water in 1·5 ml screwcap microtubes, then inverted, cut to fit in the microtube and centrifuged in an Eppendorf microfuge for 5 min at 12000 ***g***. Following centrifugation, swabs were discarded and the supernatant retained and boiled for 10 min. Supernatants were placed at −20 °C for 1 min, centrifuged for 5 min at 12000 ***g***, divided into aliquots and stored at −80 °C until analysis.

Culture and phenotyping

Cultures received at the MRU were confirmed as meningococci by Gram stain, oxidase test and latex agglutination. Confirmed meningococci were then serogrouped by co-agglutination using rabbit polyclonal serogroup-specific antisera as described previously [14].

PCR of CSF or blood

Specimens received from suspected cases of meningococcal disease were heated to 100 °C for 10 min and then centrifuged at 12000 ***g*** for 10 min. Meningococcal DNA was detected using the Taqman™ *ctrA* assay and serogrouping of specimens was performed using the *siaD* assay as described previously [12].

Taqman™ PCR assays

Two Taqman™ PCR assays were used: a screening assay based on the detection of the capsule transfer gene (*ctrA*) and a serogrouping assay utilizing the sialyltransferase gene (*siaD*) for detection of serogroups B and C. The assays were performed as described previously [12] using 2 μl of supernatant in each reaction.

Statistics

To determine if there was any significant difference between the types of swabs used in this study, a heterogenous χ^2 test was performed. An analysis of proportions test was used to determine if transport time to the MRU was a significant factor.

RESULTS

Oropharyngeal swabs were received from 106 patients with suspected or confirmed meningococcal disease; 82 of these were laboratory-confirmed cases of which 65 were culture and/or PCR (from blood or cerebrospinal fluid (CSF)) confirmed cases, while 17 yielded an isolate from a throat swab only. From the 106 patients, a single clinical specimen (blood or CSF) was submitted to the MRU for testing from 51 patients, 2 specimens were submitted from 39 patients, and 3 specimens from the remaining 16 patients. Additionally, 94 oropharyngeal swabs were received from contacts of clinically confirmed cases. Serogrouping results from the oropharyngeal swabs were compared with the serogrouping results of the culture and/or PCR specimens.

Laboratory-confirmed cases

Culture and PCR results of specimens from clinically confirmed cases of meningococcal disease and results of oropharyngeal swab PCR tests are shown in Table 1. From the 65 cases confirmed by culture and/or PCR from a CSF or blood specimen, 38 oropharyngeal swabs (59%) yielded a supernatant that tested positive by the *ctrA* assay, of which 27 (13 serogroup B and 14 serogroup C) yielded a serogroup-specific result in the *siaD* PCR assay.

Of these 65 cases, 26 had an oropharyngeal swab cultured; 9 of these did not yield a meningococcus on culture. The remaining 17 were culture positive for *N. meningitidis*; 8 stains were phenotypically serogroup B and 9 were serogroup C. Of the 17 oropharyngeal culture positive swabs, 14 (82%) yielded a positive *ctrA* result of which 1 serogrouped as B and 7 as serogroup C. The 11 swabs from cases that were culture negative yielded 5 (45%) positives when tested by *ctrA* PCR, of which 3 serogrouped as B and 1 as C.

Case confirmation from throat swab only

Seventeen clinical cases yielded isolates from oropharyngeal swabs only. Of these, 2 were non-serogroupable (NG), 1 was serogroup A, 5 were serogroup C and 9 serogroup B. Nine of the 17 oropharyngeal swabs were positive by the *ctrA* assay of which 3 serogrouped as B and 2 as C. The *siaD* serogroup results for the oropharyngeal swabs were the same as those of the corresponding culture.

Table 1. *Culture and PCR results of specimens from laboratory confirmed and laboratory-unconfirmed cases of meningococcal disease compared to PCR results from swab*

Culture result from swab		Culture from blood/CSF					*ctrA* from blood/CSF			*siaD* from blood/CSF			*ctrA* from swab		*siaD* from swab		
		Pos					Pos			Pos					Pos		
Serogroup	Number of swabs	B	C	W135	Neg	NR*	Pos	Neg	NR	B	C	Neg	ND†	Pos	Neg	B	C	Neg	ND
B	17	1	0	0	0	16	8	6	3	8	0	0	9	13	4	8	0	5	4
C	14	0	5	0	0	9	5	6	3	0	5	0	9	10	4	0	10	0	4
Other	3‡	0	0	0	0	3	0	2	1	0	0	0	3	0	3	0	0	0	3
Negative	11	3	1	2	0	5	5	5	1	3	1	1	6	6	5	3	1	2	5
Swab not cultured	61	19	11	0	1	30	23	29	9	11	9	3	38	23	38	6	7	10	38

* NR, not received.
† ND, *siaD* assay not performed as screen *ctrA* negative.
‡ 1 serogroup A and 2 non-serogroupable.

Table 2. *Culture results of oropharyngeal swabs from contacts of meningococcal disease compared to PCR swab results*

				siaD result				
		ctrA result (%)		Pos				
Isolate	Number	Pos	Neg	B	C	B and C†	Neg	ND*
B	5	4 (80)	1 (20)	2	0	1	1	1
C	3	3 (100)	0 (0)	0	3	0	0	0
NG	5	1 (20)	4 (80)	0	0	0	1	4
W135	1	0 (0)	1 (100)	0	0	0	0	1
29E	1	0 (0)	1 (100)	0	0	0	0	1
Y	1	0 (0)	1 (100)	0	0	0	0	1
Negative	78	8 (10·3)	70 (89·7)	1	0	0	7	70

* ND, *siaD* assay not performed as screen negative.

† One sample was proved to be positive for both serogroup B and C. Previous studies have demonstrated that individuals can occasionally carry more than one meningococcal strain simultaneously [15].

Suspected, laboratory-unconfirmed cases

Twenty-four oropharyngeal swabs were received from clinically suspected, laboratory-unconfirmed cases. Nineteen of these swabs were negative when extracted and tested by PCR; the remaining 5 (20·8%) were *ctrA* screen positive, of which 1 serogrouped as B and 2 as C.

PCR of swabs from contacts of meningococcal disease

A comparison of culture and PCR results from contacts of meningococcal disease is shown in Table 2 [15]. In total, 94 swabs were received. PCR screening (*ctrA*) gave 8 positive results in contrast to a positive culture in 16 cases. All culture serogrouping results for serogroups B and C were concordant with those obtained by swab *siaD* PCR with the exception of a single swab which yielded a serogroup B isolate but was found to be positive for both serogroups B and C by *siaD* PCR. In addition to these, a further eight culture negative swabs were found to be positive.

Comparison of *ctrA* PCR results from blood/CSF with *ctrA* PCR results from swabs

Oropharyngeal swabs from meningococcal disease cases ($n = 106$) were investigated using the *ctrA* PCR screening assay and 52 (49·1%) were positive. Of these 106 oropharyngeal swabs there was a total of 89 blood or CSF samples that had a corresponding oropharyngeal swab. On testing these blood or CSF specimens with the *ctrA* PCR screening assay, 41 (46·1%) were positive (Table 1).

Oropharyngeal swab type

The effect of different oropharyngeal swab type was investigated. Comparisons could only be performed using swabs from clinically confirmed cases, as the swabs from the contacts were all submitted in Amies Transport Medium with charcoal. The swabs received from the clinically confirmed cases comprised of 60 swabs in Amies Transport Medium with charcoal, 39 swabs in Amies Transport Medium without charcoal and 7 dry swabs. Similar percentages of positive PCR results from swabs in Amies with charcoal (51·7%), Amies without charcoal (33·3%) and dry swabs (42·9%) were noted for culture and/or PCR confirmed cases. In testing the swabs with the *ctrA* PCR, there were no significant differences ($P = 0{\cdot}0654$), between swabs in Amies Transport Medium with charcoal and swabs in Amies without charcoal. The dry swabs were excluded from the analysis owing to the small sample size.

Time from disease onset to collection of oropharyngeal swab

The mean time between onset of disease to the oropharyngeal swab being taken was 1·6 days. Nine swabs were taken more than 5 days after onset of disease of which 5 (56%) were positive on the *ctrA* Taqman™ assay, with 2 serogrouping as B and 1 as C.

Transport time to MRU

Of the 65 swabs from laboratory-confirmed cases, 18 were received at the MRU more than 5 days after collection. Eight of these were screen positive, 2 of which were serogroup C positive, with the remaining 6 failing to serogroup. Of the 17 swabs that were culture positive, only 1 was received more than 5 days after being taken, and was shown to be *ctrA* and serogroup C positive.

Of the 24 swabs from suspected but laboratory-unconfirmed cases, 4 were received more than 5 days after collection. Three were negative and 1 was *ctrA* and serogroup B positive. Of the 94 swabs received from contacts, only 5 were received more than 5 days after collection; of these, 3 were *ctrA* positive.

There was a significant difference in screen PCR positivity ($P < 0{\cdot}05$) between the swabs from laboratory-confirmed cases that took more than 5 days to arrive at the laboratory and those that arrived within 5 days of being taken.

DISCUSSION

In the majority of cases of meningococcal disease, laboratory confirmation of the diagnosis is still achieved by culture of a meningococcus by conventional techniques from blood, CSF or oropharyngeal swab or by visualization of Gram negative diplococci in CSF. However, the increasing use of pre-admission parenteral antibiotic treatment has resulted in a decrease in the proportion of cases being confirmed by these conventional methods and has highlighted the need for alternative methods of laboratory diagnosis.

In 1995, PCR assays for the confimation of meningococcal disease were introduced as a national reference service at the PHLS MRU. Between July 1996 and June 1997 an additional 543 cases of meningococcal disease were confirmed using PCR alone, representing an increase of 35% in the number of laboratory confirmed cases during that time period [16].

Numerous studies have focused on the extraction, amplification and detection of meningococcus-specific DNA from clinical specimens such as blood and CSF [10–12] but so far, none has utilized oropharyngeal swabs.

The application of DNA extraction and amplification techniques to oropharyngeal swabs has been undertaken for the detection of *Bordetella pertussis* by PCR amplification of a region of the adenylate cyclase gene [17] and also by a multiplex PCR-based assay [18]. The method reported by Wadowsky and colleagues [18] involved swabs placed into vials containing 0·5 ml of saline, being vortexed and the swab wrung against the wall of the vial. The method performed by Douglas and colleagues [17] was similar to the one employed here, with the swab being immersed in distilled water, followed by three centrifugation steps with the eluate being boiled as the final step. Both methods improved the detection of *B. pertussis* with Wadowsky and colleagues (1996) [17] reporting sensitivity and specificity of 98·9 and 99·7%, respectively for PCR compared with values of 73·4 and 100% for culture.

The extraction method used here proved successful in improving the rate of detection of meningococci from throat swabs, with 58·5% of samples from laboratory-confirmed cases of meningococcal disease yielding a PCR positive oropharyngeal swab. Although a positive PCR oropharyngeal swab result was not obtained from all laboratory-confirmed cases, positive culture and/or PCR results are not obtained in all cases of meningococcal disease. The use of PCR on clinical specimens such as blood and CSF is recognized as a reliable tool for confirming meningococcal infection. Our data show that the extraction method and subsequent assays yield results that compare favourably with that of PCR on deep (blood or CSF) specimens, with 49·1% of swabs positive on the *ctrA* assay compared with 46·1% of clinical specimens.

Due to this study being an uncontrolled observational study, factors such as swab type and delays between sampling could not easily be assessed. However, statistical analysis of data on the length of time the swabs took to reach the laboratory revealed that swabs taking more than 5 days to reach the laboratory were less likely to yield a positive result than those that arrived promptly. One limitation of this study is that PCR positivity rates are likely to be improved if fresh swabs, rather than those that have already been used for culture, and arriving at the laboratory for testing within 24 h of collection, are used. PCR amplification is potentially more rapid than culture, with a result being available within 4 h of the swab arriving at the laboratory, compared with culture, which requires an overnight incubation, followed by conventional strain characterization. The current Taqman™ *ctrA* PCR screening assay only detects DNA from sialic-acid containing meningo-

cocci due to sequence variation in the 5′ end of the *ctrA* gene [19]. This assay needs refining to include all meningococci regardless of serogroup. Also the current Taqman™ serogrouping assay only amplifies and detects the sialyltransferase genes of serogroups B and C meningococci. A PCR ELISA format has been described for serogroups Y and W135 [11] which when redeveloped for the Taqman™ platform will provide additional serogroup information.

The results obtained in this study illustrate the possibility of using alternative samples such as saliva or gargles for the confirmation of both meningococcal carriage and disease. Holdsworth and colleagues described a case in which a security guard contracted meningococcal conjunctivitis after being spat in the face by an individual who was subsequently confirmed as a carrier, indicating that meningococci may sometimes be present in saliva [20]. Obtaining a saliva sample for the detection of meningococcal carriage is less invasive than obtaining an oropharyngeal swab. Further studies need to be performed to determine the proportion of individuals who have meningococci in their oropharynx who also have the bacteria or bacterial DNA present in their saliva. This would best be performed on an age group exhibiting high carriage rates, such as university students or military recruits [21] and comparing PCR and culture detection.

Our findings demonstrate that application of PCR techniques to oropharyngeal swabs from cases and contacts of meningococcal disease can increase the yield of meningococci substantially over that which can be achieved by culture alone. Though the significance of the detection of a meningococcus or meningococcal DNA from the oropharynx of a case is less certain than that from deep isolates, positive oropharyngeal swabs may provide additional evidence in support of the diagnosis, and may be the only way in which serogroup information is obtained. The improved meningococcal yield as a result of applying PCR techniques to oropharyngeal swabs is also relevant to population studies of meningococcal carriage.

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REFERENCES

1. Kaczmarski EB. Meningococcal disease in England and Wales: 1995. CDR Rev 1997; **7**: R55–9.
2. Lepow ML, Goldschneider I, Gold R, Randolph M, Gotschlich E. Persistence of antibody following immunisation of children with groups A and C meningococcal polysaccharide vaccine. Pediatrics 1977; **60**: 673–80.
3. Fairley CK, Begg N, Borrow R, Fox AJ, Jones DM, Cartwright KAV. Conjugate meningococcal serogroup A and C vaccine: reactogenicity and immunogenicity in United Kingdom infants. J Infect Dis 1996; **174**: 1360–3.
4. Richmond P, Borrow R, Miller E, et al. Meningococcal serogroup C conjugate vaccine is immunogenic in infancy and primes for memory. J Infect Dis 1999; **179**: 1569–72.
5. Donaldson L, Moores Y, Howe J. Introduction of immunisation against group C meningococcal infection. CMO Letter PL/CMO/99/02, 1999.
6. Cartwright KAV, Stuart JM, Robinson PM. Meningococcal carriage in close contacts of cases. Epidemiol Infect 1991; **106**: 133–41.
7. Cartwright KAV, Stuart JM, Jones DM, Noah ND. The Stonehouse survey: oropharyngeal carriage of meningococci and *Neisseria lactamica*. Epidemiol Infect 1987; **99**: 591–601.
8. Hammerschmidt S, Hilse R, van Putten JPM, Gerardy-Schahn R, Unkmeir A, Frosch M. Modulation of cell surface sialic acid expression in *Neisseria meningitidis* via a transposable genetic element. EMBO J 1996; **15**: 192–8.
9. Swartley JS, Stephens DS. Identification of a genetic locus involved in the biosynthesis of *N*-acetyl-D-mannosamine, a precursor of the ($\alpha 2 \rightarrow 8$)-linked polysialic acid capsule of serogroup B *Neisseria meningitidis*. J Bacteriol 1994; **176**: 1530–4.
10. Borrow R, Claus H, Guiver M, et al. Non-culture diagnosis and serogroup determination of meningococcal B and C infection by a sialyltransferase (*siaD*) PCR ELISA. Epidemiol Infect 1997; **118**: 111–7.
11. Borrow R, Claus H, Chaudry U, et al. *SiaD* PCR ELISA for confirmation and identification of serogroup Y and W135 meningococcal infections. FEMS Microbiol Lett 1998; **159**: 209–14.
12. Guiver M, Borrow R, Marsh J, et al. Evaluation of the Applied Biosystems Automated Taqman PCR system for the detection of meningococcal DNA. FEMS Immunol Med Microbiol 2000; **28**: 173–9.
13. Amies CR. A modified formula for the preparation of Stuarts Transport Medium. Can J Publ Hlth 1967; **58**: 296–300.
14. Eldridge J, Sutcliffe EM, Abbott JD. Serological grouping of meningococci and detection of antigen in

cerebrospinal fluid by co-agglutination. Med Lab Sci 1978; **35**: 63–6.
15. Schoenbach EB, Phair JJ. Appraisal of the techniques employed for the detection of subclinical (inapparent) meningococcal infections Am J Hyg 1948; **47**: 271–81.
16. Kaczmarski EB, Ragunathan PL, Marsh J, Gray SJ, Guiver M. Creating a national service for the diagnosis of meningococcal disease by polymerase chain reaction. Commun Dis Publ Hlth 1998; **1**: 54–6.
17. Douglas E, Coote JG, Parton R, McPheat W. Identification of *Bordetella pertussis* in nasopharyngeal swabs by PCR amplification of a region of the adenylate cyclase gene. J Med Microbiol 1993; **38**: 140–4.
18. Wadowsky RM, Michaels RH, Libert T, Kingsley LA, Ehrlich GD. Multiplex PCR-based assay for the detection of *Bordetella pertussis* in nasopharyngeal swab specimens. J Clin Microbiol 1996; **34**: 2645–9.
19. Frosch M, Müller D, Bousset K, Müller A. Conserved outer membrane protein of *Neisseria meningitidis* involved in capsule expression. Infect Immun 1992; **60**: 798–803.
20. Holdsworth G, Jackson H, Kaczmarski E. Meningococcal infection from saliva. Lancet 1996; **348**: 1443.
21. Cartwright K. Meningococcal carriage and disease. In: Meningococcal disease. Cartwright K, ed. Chichester, England: John Wiley & Sons Ltd., 1995: 115–46.