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Molecular testing of respiratory swabs aids early recognition of meningococcal disease in children☆,☆☆

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Abstract

Early meningococcal disease (MD) diagnosis is difficult. We assessed rapid molecular testing of respiratory specimens. We performed genotyping of respiratory swabs, blood, and cerebrospinal fluid from children with suspected disease and nasal swabs (NSs) from matched controls. Thirty-nine of 104 suspected cases had confirmed disease. Four controls were carriers. Throat swab ctrA and porA testing for detection of disease gave a sensitivity of 81% (17/21), specificity of 100% (44/44), positive predictive value (PPV) of 100% (17/17), negative predictive value (NPV) of 92% (44/48), and relative risk of 12. NS ctrA and porA testing gave a sensitivity of 51% (20/39), specificity of 95% (62/65), PPV of 87% (20/23), NPV of 77% (62/81), and relative risk of 4. Including only the 86 NSs taken within 48 h of presentation, the results were sensitivity of 60% (18/30), specificity of 96% (54/56), PPV of 90% (18/20), NPV of 82% (54/66), and relative risk of 5. Swab type agreement was excellent (kappa 0.80, $P < 0.001$). There was exact phylogenetic agreement from different specimen sites for individuals. Carried genosubtypes were P1.7 and P1.21-7. Prehospital rapid molecular testing of easily obtained respiratory specimens could accelerate diagnosis of MD.

Keywords: Early; Molecular; Meningococcal; Diagnosis; Children

1. Introduction

Neisseria meningitidis is a major cause of morbidity and mortality in children inducing epidemic as well as sporadic disease (Brooks et al., 2006). The risk of meningococcal disease (MD) varies with environmental and host factors and with the characteristics of the strain acquired (Bygraves et al., 1999). Incidence shows a bimodal distribution with peaks in early childhood and late adolescence and an increase in the winter months.

The incubation period is usually 3 to 5 days. Nasopharyngitis is a common prodrome causing diagnostic difficulty (Young et al., 1972). In a previous study, we reported that this prodrome is likely to be caused by MD itself rather than by respiratory coinfection (Dunlop et al., 2007). This led us to investigate molecular testing of respiratory specimens for early MD diagnosis in children with suspected MD and the additional benefits of sequence-based genotyping.

Diagnosing MD by molecular testing of respiratory specimens is complicated by asymptomatic carriage of *N. meningitidis* in the nasopharynx of some individuals. Carriage rates assessed by culture vary from 2% in children under 5 years of age to 25% in late adolescence

Abbreviations: CSF, cerebrospinal fluid; MD, meningococcal disease; NPV, negative predictive value; NS, nasal swab; PCR, polymerase chain reaction; PPV, positive predictive value; TS, throat swab.

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(Cartwright et al., 1987). In approximately a quarter of individuals, meningococcal acquisition results in prolonged carriage. In 33%, carriage is for a few days or weeks and in the remainder it is transient (Andersen et al., 1998; Tzeng and Stephens, 2000). Different strains may persist for variable times, and carriage of multiple strains simultaneously has been observed (Jordens et al., 2002). High carriage rates of a hypervirulent strain tend to lead to increased disease incidence (Cartwright et al., 1987). A disease isolate has colonized a laboratory worker over a prolonged period (Woods and Cannon, 1990). Although seasonal variation, temperature, and humidity have an impact on disease, they have little, if any, effect on prevalence of carriage.

MD usually presents as septicaemia or meningitis. Early diagnosis is difficult for both with initial non-specific signs such as lethargy, fever, irritability, leg pain, abnormal skin colour, and cold peripheries (Thompson et al., 2006).

Bacterial cultures of blood, cerebrospinal fluid (CSF), throat swabs (TSs), and skin scrapings are too slow to aid initial clinical management, generally taking at least 48 h, and with studies reporting blood culture positivity rates of just 4.6–54% , the lower rates following prehospital antibiotic administration, they do not always identify the infective agent (Cartwright et al., 1992; Hackett et al., 2002; Ragunathan et al., 2000; Wylie et al., 1997). By contrast, molecular polymerase chain reaction (PCR) testing identifies bacterial DNA and can detect both viable and nonviable organisms within a few hours.

This study aimed to assess whether molecular detection of *N. meningitidis* in paediatric respiratory specimens might aid MD diagnosis. Other aims were to compare findings for throat and nasal swabs (NSs) and to investigate local meningococcal molecular epidemiology and carriage rates using genosubtyping.

2. Materials and methods

2.1. Patient recruitment and sample collection

Children under 15 years of age presenting to the Royal Belfast Hospital for Sick Children and other paediatric units in Northern Ireland with suspected MD were prospectively identified and invited to take part. Identification involved medical assessment with detection of fever, a petechial rash, or signs of meningitis, and allocation to a specific suspected MD clinical care pathway. The care pathway included forwarding blood to the Meningococcal Reference Unit (MRU), Manchester, UK, for meningococcal PCR for diagnostic and epidemiologic purposes (Corless et al., 2001).

Suspected MD cases had blood and TS specimens taken on admission as part of their routine care. Rayon tipped swabs were applied to the mucosal surface and gently rotated for a few seconds. Those for molecular testing were transferred to 1.8-mL polypropylene tubes containing 800 μL of lysis buffer, briefly vortexed, and stored at −70 °C. CSF was obtained in selected cases where meningitis was suspected and a lumbar puncture was not contraindicated.

Clinical circumstances sometimes dictated a variance in sampling, for example, endotracheal intubation having been performed before obtaining 2 TSs.

Following informed consent, a NS was taken from the anterior nares. Age- and sex-matched controls were prospectively recruited after each suspected MD case, and NSs were taken. Controls were selected from children attending orthopaedic, surgical, or dental outpatients, or from the wider community. Exclusion criteria were having been hospital inpatients within the last month, being treated with antibiotics, or having a chronic illness predisposing to infective disease.

There was statutory notification to public health of suspected MD cases with subsequent classification, by the Communicable Disease Surveillance Centre Northern Ireland, as either confirmed MD or other disease based on bacterial cultures, PCR results from the MRU, and clinical course.

2.2. DNA Extraction

Total nucleic acids were extracted from swab specimens (200-μL aliquots of lysis buffer), plasma (200 μL), and CSF (200 μL) using the protocol of the QIAamp DNA Blood BioRobot 9604 Kit (Qiagen, Qiagen House, Fleming Way, Crawley, West Sussex). The elution volume was 50 μL. For those CSF specimens of less than 200 μL, a manual extraction was carried out using the QIAamp DNA Blood Mini Kit (Qiagen). Yeast transfer RNA (Sigma Aldrich, Poole, UK) was added during the extraction procedures for CSF specimens with a working concentration of 100 ng/μL.

Extracts (50 μL, in Qiagen Buffer AE) were stored at −20 °C prior to testing.

2.3. PCR Assays

Meningococcal PCRs were performed on respiratory, CSF, and blood specimens using nested PCR (nPCR) and seminested PCR (snPCR) assays locally and also on blood by single-round Taqman® PCR at the MRU (Corless et al., 2001).

Primer sets (Table 1) were selected from published data or designed for this study using the Lasergene v. 5.08 software package (DNASTAR, Wisconsin). Primer set A (nPCR): Primers NM 1A and NM 1B (this study) and NM 1C and NM 1D are specific for the capsule transfer *ctrA* gene and detect serogroups A, B, C, X, Y, Z, W135, and 29E, and some nongroupable strains of *N. meningitidis* (Corless et al., 2001). The first-round PCR reaction (NM 1A and NM 1B) generates an amplicon of 324 base pairs (bp). The second-round PCR reaction (NM 1C and NM 1D) generates an amplicon of 111 bp.

Primer set B (snPCR): Primers NM 3C2 and NM 3B/NM 3D were modified from previously published primers and amplify the hypervariable VR1 region of the *porA* gene of *N. meningitidis* (Diggle and Clarke, 2003). The first-round