Maintaining culturability of *Streptococcus pneumoniae* (pneumococci) during transportation

J. E. MOORE*†, W. A. COULTER‡ and C. E. GOLDSMITH*

Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Belfast; †School of Biomedical Sciences, University of Ulster, Coleraine; and ‡School of Dentistry, Queen's University of Belfast, Royal Group of Hospitals, Belfast, Northern Ireland, UK

Streptococcus pneumoniae is a major pathogen commonly causing otitis media, sinusitis, meningitis, pneumonia and bacteraemia.[1] It is widely known that *S. pneumoniae* is carried asymptomatically within the healthy community, particularly in the vulnerable populations of children and the elderly, and those with underlying medical conditions.[2] Carriage rates vary depending on sampling method, sampling site and differences in storage and culture techniques, with carriage rates varying from 2% to 50% and above.[3]

This Gram-positive bacterium is relatively fragile in comparison to other Gram-positive organisms, including the staphylococci, and the pneumococcal organism undergoes autolysis when grown to stationery phase, due to the production of an autolysin enzyme. This property was first reported in 1937 by René J Dubos at the Rockefeller Institute for Medical Research in New York, (www.ncbi.nlm.nih.gov/pmc/articles/PMC2133519/pdf/873.pdf), and modern descriptions have appeared more recently.[1] This characteristic is well known among clinical microbiologists, as this trait creates practical difficulties for its successful transportation between laboratories, locally, nationally and internationally, for collaborative, epidemiological, typing and reference functions. Several previous studies have attempted to define optimal transport conditions for the successful transport of pneumococci, particularly in nasal secretions and on oropharyngeal swabs.[4,5] No study has examined its survival in pure culture during transportation mimicking its journey within the post or via courier services. Hence, this short study aims to identify a reliable transportation method to maintain culturability of clinical pneumococci successfully between clinical microbiology laboratories.

S. pneumoniae (n=5 clinical isolates [742, 031, 741, 910, 370]) were obtained from the MicroARK Strain Repository in the Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital. These isolates had previously been cultured from nasal and oropharyngeal sites in patients as part of a larger study (AMRAP/COM/2730/04) examining carriage rates of pneumococci within the community. All isolates had previously been well characterised by a combination of phenotypic and genotypic methods and subsequently had been stored in ProTec beads (Technical Service Consultants, Lancs, UK) at –80°C. *S. pneumoniae* isolates were subcultured on Columbia blood agar (CM0331 Oxoid, Basingstoke, UK) supplemented with 5% (v/v) defibrinated horse blood for 24 h at 37°C under microaerophilic conditions. Subsequently, revived cultures were passaged on a further two occasions prior to use in the current study. Cultures (24 h) were prepared as detailed above and were subcultured on three media, including (i) freshly prepared chocolate slopes (Columbia blood agar [CM0331 Oxoid]) supplemented with 5% (v/v) defibrinated horse blood, (ii) transport swabs containing Amies transport medium without charcoal (TS/5-13, Technical Service Consultants, Lancs, UK), and (iii) transport swabs containing Amies transport medium with charcoal (TS/5-14, Technical Service Consultants). All three media were stored for 16 days in the dark at ambient temperature (18–20°C) and were subcultured after seven days and at day 16 on Columbia blood agar (CM0331, Oxoid) supplemented with 5% (v/v) defibrinated horse blood for 24 h at 37°C under microaerophilic conditions. The resulting growth was scored semiquantitatively (Table 1). All isolates survived for seven days on all three media; however, at day 16, isolate 370 was non-culturable on Amies transport medium (without charcoal) and isolates 031 and 910 were lost on the chocolate slopes. A subsequent attempt to recover culturability of isolates 031 and 910 was made by the addition of Todd-Hewitt broth (5 mL; CM0189, Oxoid) to the bijou slope vials and incubation for 24 h at 37°C, followed by subsequent plating on Columbia blood agar, as described above. This enrichment procedure failed and the organisms were considered non-culturable.

Table 1. Comparison of survival of clinical isolates of *Streptococcus pneumoniae* (n=5) on i) freshly prepared chocolate slopes (Columbia blood agar (CM0331 Oxoid, Basingstoke, UK), supplemented at 45°C with 5% (v/v) defibrinated horse blood; ii) transport swabs containing Amies transport medium without charcoal (TS/5-13, Technical Service Consultants [TCS], Lancs, UK); and iii) transport swabs containing Amies transport medium with charcoal (TS/5-14, TCS).

	Transport system		
Strain reference	Chocolate slope	Amies medium (without charcoal)	Amies medium (with charcoal)
After seven days			
742	+++	+++	+++
031	++	+++	+++
741	++	+++	+++
910	+++	++	+
370	+++	++	+++
After 16 days			
742	+++	+++	+++
031	–	+++	+++
741	+	+++	+++
910	–	+++	+++
370	++	–	+++

+: growth, –: no growth.

In conclusion, this short study has confirmed the survival of culturable pneumococcal cells for at least seven days at ambient temperature on chocolate slopes, and in Amies transport media on swabs, with and without charcoal. Optimal culturability was maintained on extended storage

Correspondence to: Professor John E. Moore
Northern Ireland Public Health Laboratory, Department of Bacteriology
Belfast City Hospital, Belfast BT9 7AD, Northern Ireland, UK
Email: jemoore@niphl.dnet.co.uk

to at least 16 days in Amies transport medium containing charcoal. These data will help laboratories adopt safe and reliable procedures to ensure that pneumococcal culturability is not lost during transportation.

The authors wish to thank Alan Murphy for his help with media preparation. This work was financially supported through HSC R&D Office commissioned grant: Antimicrobial Resistance Action Plan (AMRAP) (COM/2730/04).

References

1 Todhar K. *Streptococcus pneumoniae*. In: *Todhar's online textbook of bacteriology*. 2011 (http://textbookofbacteriology.net/S.pneumoniae.html).
2 Boost MV, O'Donoghue MM, Dooley JS. Prevalence of carriage of antimicrobial-resistant strains of *Streptococcus pneumoniae* in primary school children in Hong Kong. *Epidemiol Infect* 2001; **127** (1): 49–55.
3 Uzuner A, Ilki A, Akman M *et al.* Nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* in healthy children. *Turk J Pediatr* 2007; **49** (4): 370–8.
4 Charalambous BM, Batt SL, Peek AC, Mwerinde H, Sam N, Gillespie SH. Quantitative validation of media for transportation and storage of *Streptococcus pneumoniae. J Clin Microbiol* 2003; **41** (12): 5551–6.
5 Mason CK, Goldsmith CE, Moore JE *et al*. Optimisation of storage conditions for maintaining culturability of penicillin-susceptible and penicillin-resistant isolates of *Streptococcus pneumoniae* in transport medium. *Br J Biomed Sci* 2010; **67** (1): 1–4.

Polymerase chain reaction amplification: effect of dyes and other staining agents employed in clinical microbiology laboratories

J. HIRAYAMA[*†], K. HAYASHI[*†], C. E. GOLDSMITH[*], W. A. COULTER[‡], B. C. MILLAR[*], J. S. G. DOOLEY[§], M. MATSUDA[†] and J. E. MOORE[*§]

[*]*Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital, Belfast, Northern Ireland, UK;* [†]*Laboratory for Molecular Biology, School of Environmental Health Sciences, Azabu University, Sagamihara, Japan;* [‡]*School of Dentistry, The Queen's University of Belfast, Royal Group of Hospitals, Belfast; and* [§]*School of Biomedical Sciences, University of Ulster, Cromore Road, Coleraine, Northern Ireland, UK*

Correct and reliable isolation and identification of bacterial organisms in clinical microbiology is an important function of such service laboratories. Causal organisms of infection may be presented for identification through their growth from enrichment on non-selective/selective media and directly from clinical specimens. To date, such identification has largely relied on phenotypic schema, including initial examination of colonial morphology and Gram stain, which is usually followed by some form of semi-automated identification scheme, generally based on biochemical differentials, such as the API identification schemes or the BBL Crystal scheme. Molecular methodologies, particularly employment of the 16S rDNA polymerase chain reaction (PCR) and sequencing techniques, offer an alternative laboratory mechanism for the identification of such organisms. These have a specific operational advantage when working with non-culturable organisms such as the obligate intracellular organisms *Coxiella burnetii*, *Tophereyma whipplei* or difficult-to-culture organisms such as the slow-growing *Mycobacterium* spp.

Correspondence to: Professor John E. Moore
Northern Ireland Public Health Laboratory, Department of Bacteriology
Belfast City Hospital, Belfast BT9 7AD, Northern Ireland.
Email: jemoore@niphl.dnet.co.uk

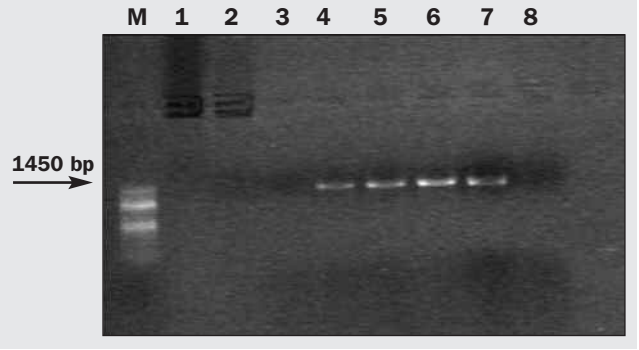

Fig. 1. Effect of the presence of crystal violet on PCR amplification of the 16S rRNA gene in *E. coli* 0157:H7 NCTC12900. Lane M: molecular weight marker (100 bp; Gibco Life Technologies, Paisley, Scotland), lane 1: crystal violet (91.9 μg), lane 2: 10^{-1} dilution (9.19 μg), lane 3: 10^{-2} dilution (0.919 μg), lane 4: 10^{-3} dilution (91.9 ng), lane 5: 10^{-4} dilution (9.19 ng), lane 6: 10^{-5} dilution (0.919 ng), lane 7: positive control (bacterial DNA present/crystal violet absent), lane 8: negative control (PCR grade water; crystal violet and bacterial genomic DNA absent).

Often, such causal organisms are seen in histopathological specimens or bacteriological glass microscope slides, but cannot be confirmed by conventional bacteriological culture techniques. Visualisation of cellular morphologies by light microscopy generally involves the employment of differential stains and dyes, which either serve to aid visualisation (eg staining spores with malachite green) or characterisation of such organisms (eg Gram stain). A wide range of dyes and stains are available and are employed for these purposes; however, there has been relatively little work performed to determine if they have any potential influence on PCR amplification. Previously, in cytopathology, cellular digests from Papanicolaou-stained cervical smears did not yield products from PCR, whereas cellular digests from unstained cervical smears always yielded PCR products.[1] Analysis of individual Papanicolaou stain reagents identified inhibition of PCR by haematoxylin and by aluminium sulphate. These inhibitors could be removed from Papanicolaou-stained cervical smears by destaining the slides with 1% HCl.[1]

The application of molecular techniques for identification and downstream molecular assays, including genotyping or other molecular characterisation assays, on archived material mounted on glass slides has the potential to provide detailed identification and epidemiological information on the causal organism seen initially by light microscopy.

Previously, the authors developed a robust method of removal of fixed oocysts of *Cryptosporidium parvum* from archived material using laser-capture microscopy (LCM).[2]