Erythromycin resistance among invasive pneumococci in Scotland, 1994–2003

S. C. CLARKE, J. A. REID, L. THOM, M. A. DIGGLE, S. McCHLERY, K. SCOTT and G. F. S. EDWARDS
Scottish Meningococcus and Pneumococcus Reference Laboratory, Glasgow, UK

Streptococcus pneumoniae is a major cause of meningitis, septicaemia, pneumonia, sinusitis and otitis media, and remains a leading cause of morbidity and mortality worldwide, especially in the young and old.[1] In Scotland, between 1999 and 2001, the overall incidence of invasive pneumococcal disease (IPD) was 11 cases per 10^5 persons.[2]

Although polysaccharide vaccines have been used successfully for some years, conjugate vaccines are now providing a significant step forward in the fight against pneumococcal disease.[3] However, conjugate vaccines do not represent the long-term solution to further vaccine development. Therefore, epidemiological data relating to serotype distribution, as well as sequence type (ST) and antibiotic susceptibility, are required.

Increasing amounts of information are becoming available about the molecular relationships between different pneumococcal clones.[4-8] In the UK, serotype 14 pneumococci are the most prevalent in IPD, accounting for 18.5% of cases in England and Wales during 1995–1997.[9]

Resistance of pneumococci to erythromycin is increasing worldwide and it is thought that the degree of resistance affects the clinical impact of erythromycin useage.[10] Thus, the aim of this study is to determine the incidence of erythromycin resistance among invasive pneumococci in Scotland between 1994 and 2003, and of the serotypes associated with such resistance.

Invasive pneumococci received between 1994 and 2003 from diagnostic microbiology laboratories around Scotland were included. Serotyping was performed by latex agglutination and co-agglutination[11] and minimum inhibition concentrations (MICs) for erythromycin were determined using E-Test (Bio-Stat, Stockport, UK).

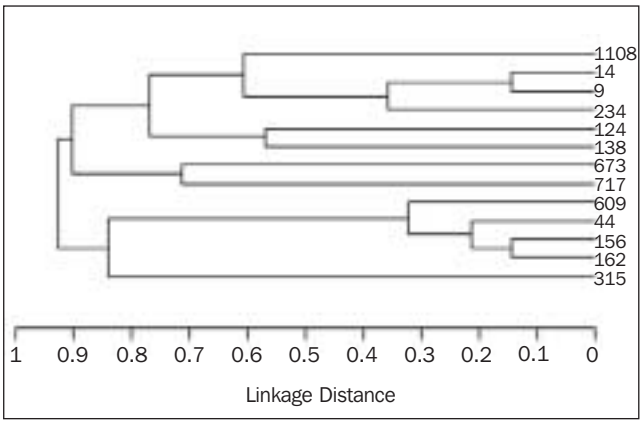

Fig. 1. Genetic relationship between STs among erythromycin-resistant invasive pneumococci in Scotland, 2003.

Correspondence to: Dr S.C. Clarke
Department of Improving Health and Quality, Portsmouth City PCT, Finchdean House, Milton Road, Portsmouth PO3 6DP, UK
Email: stuartcclarke@hotmail.com

Antibiotic susceptibility levels were determined using current British Society for Antimicrobial Chemotherapy (BSAC) guidelines (www.bsac.org.uk). Pneumococci were considered resistant to erythromycin when the MIC was ≥ 1 mg/L.

For multilocus sequence typing (MLST), chromosomal DNA was prepared as described previously[12] and MLST was performed using the method of Enright and Spratt.[5] Briefly, internal fragments of seven housekeeping genes – *aroE, gdh, gki, recP, spi, xpt* and *ddl* – were amplified by the polymerase chain reaction (PCR) and nucleotide sequences were determined for both strands. Sequence types were assigned by reference to the pneumococcal MLST database (http://spneumoniae.mlst.net) and further analysis of alleles and STs was performed using the Sequence Type Analysis and Recombination Tests (START) software package (http://pubmlst.org/software/analysis/start/).[13]

A total of 3961 invasive pneumococci were received between 1994 and 2003. Of these, 431 (10.9%) were resistant to erythromycin (Table 1). The incidence of erythromycin resistance was particularly low in 1994, but it increased in 1995 and 1996 to a level that has not changed substantially since (12–14%; Table 2). Most erythromycin resistance was low-level (<32 mg/L), none had an MIC of 32–256 mg/L, and only 21 had an MIC > 256 mg/L.

Erythromycin resistance in pneumococci is commonly mediated by methylation of the ribosomal 23S RNA (drug target) or drug efflux, resulting in two major phenotypes, MLSB (high-level) and M (low-level).[10] Pneumococci of the MLS_B phenotype carry the *ermB* gene and elicit erythromycin resistance by ribosomal modification, while M phenotype pneumococci are resistant to macrolides via an active efflux mechanism requiring the *mefA* or *mefE* genes.[10] The data presented here suggest that erythromycin-resistant pneumococci causing invasive infection in Scotland are of the M phenotype.

A total of 373 (87%) pneumococci were serotype 14, the most common serotype associated with erythromycin resistance (Table 2). Only 10 other serotypes showed erythromycin resistance, although the resistance was not common among these serotypes. These are interesting data as enhanced pneumococcal surveillance began in 1999 and the quality of the dataset improved between 1999 and 2003 (data not shown). However, the percentage of pneumococci resistant to erythromycin out of the total number of invasive pneumococci received each year has not increased. As expected, the most common serotype associated with erythromycin resistance was serotype 14. This is not only the most common serotype causing IPD in Scotland but also frequently shown to be erythromycin-resistant. Encouragingly, six of the serotypes that showed erythromycin resistance, including serotype 14, are included in the 7-valent pneumococcal conjugate vaccine.

In order to determine the genetic background of the erythromycin-resistant pneumococci, MLST was performed only on those isolated during 2003. Among the 78 pneumococci isolated in 2003, only 13 different STs were found (Fig. 1). However, 63 (80.8%) of these were ST9. The isolates grouped into two lineages, although most STs were not closely related, even though they were

Table 1. Summary of erythromycin MICs among 431 invasive pneumococci in Scotland, 1994–2003.

Year	Number (%)	MIC mg/L (*n*)
1994	8 (3.8)	3 (5), 6 (1), 8 (1), >256 (1)
1995	16 (7.8)	1 (1), 1.5 (4), 2 (3), 4 (1), 8 (3), 16 (2), 24 (1), >256 (1)
1996	21 (10.7)	1.5 (1), 2 (2), 3 (2), 4 (1), 6 (3), 8 (6), 12 (2), 16 (1), >256 (3)
1997	11 (6.0)	1 (1), 1.5 (4), 2 (3), 3 (2), 5 (1)
1998	19 (12.0)	1 (1), 1.5 (4), 2 (6), 3 (2), 4 (2), 6 (1), 8 (2), >256 (1)
1999	39 (13.3)	1 (1), 1.5 (15), 2 (11), 3 (3), 4 (6), 6 (2), >256 (1)
2000	74 (10.9)	1 (8), 1.5 (6), 2 (13), 3 (16), 4 (16), 6 (8), 8 (2), 12 (3), >256 (2)
2001	77 (13.8)	1 (2), 1.5 (6), 2 (9), 3 (6), 4 (14), 6 (16), 8 (11), 12 (4), 16 (3), 32 (1), >256 (5)
2002	88 (14.2)	1.5 (3), 2 (9), 3 (16), 4 (27), 6 (14), 8 (14), 9 (1), 16 (1), >256 (3)
2003	78 (11.7)	1 (2), 1.5 (2), 2 (5), 3 (5), 4 (23), 6 (16), 8 (10), 12 (8), 16 (2), 24 (1), >256 (4)

Table 2. Serogroups/types associated with erythromycin resistance among 431 invasive pneumococci in Scotland, 1994–2003.

Serogroup/type	Number (% of total)
4	2 (0.5)
6	19 (4.4)
8	1 (0.2)
9	15 (3.5)
11	1 (0.2)
12	1 (0.2)
14	373 (86.5)
15	1 (0.2)
19	8 (1.9)
23	7 (1.6)
33	3 (0.7)

erythromycin-resistant. This would suggest that they may have different ancestors and the above data would suggest they have the same erythromycin resistance mechanism, namely the M phenotype.

Clones belonging to the ST9 group are recognised by the Pneumococcal Molecular Epidemiology Network (PMEN) as members of a number of disease-associated antibiotic-resistant pneumococci that have spread successfully worldwide (www.sph.emory.edu/PMEN). The ST9 clone is known as England-9 and is erythromycin-resistant.[14]

Invasive pneumococcal disease remains an important cause of morbidity and mortality in Scotland and elsewhere. The present study has demonstrated that erythromycin resistance is probably associated with the M phenotype and that the common circulating serotype is serotype 14, the main clone of which is ST9. Other serotypes are not commonly associated with erythromycin resistance. Furthermore, earlier data from one geographical area within Scotland and performed over a three-year period[15] are confirmed by data from a 10-year period and for the whole of Scotland. However, further studies are required to ascertain the genetic relationship between erythromycin-resistant and erythromycin- susceptible pneumococci causing invasive infection.

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Comparison of *in vitro* susceptibilities to levofloxacin and ciprofloxacin with *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* isolated from cystic fibrosis patients in Northern Ireland

A-J. McKNIGHT* A. SHAW* C. E. GOLDSMITH* L. CLARKE*
B. C. MILLAR* J. McCAUGHAN†, J. S. ELBORN‡,§,
A. REID# and J. E. MOORE*

**Northern Ireland Public Health Laboratory, Department of Bacteriology, Belfast City Hospital; †Department of Medical Microbiology, The Royal Group of Hospitals; ‡Northern Ireland Regional Adult Cystic Fibrosis Centre and §Department of Respiratory Medicine, Belfast City Hospital; and #Northern Ireland Regional Paediatric Cystic Fibrosis Centre, The Royal Belfast Hospital for Sick Children, Belfast, Northern Ireland*

In vitro antibiotic susceptibility (E-test) studies were performed on *Pseudomonas aeruginosa* (n=50) and *Stenotrophomonas maltophilia* (n=10) obtained from the sputa of adult and paediatric patients with cystic fibrosis (CF). In general, *S. maltophilia* showed greater susceptibility to levofloxacin than to ciprofloxacin, which was in contrast to the susceptibility of *P. aeruginosa*, which showed that generally isolates were more susceptibility to ciprofloxacin than to levofloxacin. As the optimal treatment of CF-related infections due to these organisms is not fully known, these data may help in deciding upon appropriate empirical oral fluoroquinolone treatment of *P. aeruginosa* and *S. maltophilia* in CF patients.

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

Cystic fibrosis is the most common fatal genetic disease of Caucasians and has an approximate incidence of 1 in 2500 live births and a carriage rate of 1 in 20 individuals. Patients with CF suffer recurrent and chronic respiratory tract infections and most of the associated morbidity and mortality is due to such infections throughout their life.[1] These chronic infections are usually dominated by multidrug-resistant Gram-negative organisms, especially the pseudomonads, particularly *P. aeruginosa*; however. other multidrug-resistant organisms, including *S. maltophilia*, have recently been reported to be more prevalent.[2] A comprehensive review of these organisms may be found elsewhere.[3,4]

Growing antibiotic resistance in *P. aeruginosa* and *S. maltophilia* isolated from the sputa of adult and paediatric patients with CF is a major problem in CF centres in Northern Ireland, across Britain and worldwide. Furthermore, as there is relatively little information in the literature on the comparison of the *in vitro* susceptibility of Gram-negative organisms to the fluoroquinolones, any study examining potential improvements in efficacy should be encouraged.

P. aeruginosa isolates (n=50) from 45 patients (15 adults [8 female, 7 male]; 30 paediatric patients [16 male, 14 female]) with well-characterised CF were obtained from freshly expectorated sputum specimens. In addition, *S. maltophilia* (n=10) isolates from an equal number of adult patients with CF (5 females, 5 males]) were obtained.

The identity of these isolates was confirmed phenotypically by the API20NE scheme (bioMerieux, Les Halles, France) and genotypically in the case of *P. aeruginosa* isolates by polymerase chain reaction (PCR) amplification of the *groES* gene locus, as previously described.[5] An additional phenotypic confirmation assay was performed on all *S. maltophilia* isolates by examining for synergy between augmentin and azotreonam discs.

The minimum inhibitory concentrations (MICs) of levofloxacin and ciprofloxacin were determined by the E-test method (AbBiodisk), following the manufacturer's instructions, and all assays were performed on Mueller-Hinton agar (Oxoid, Dorset, UK). Susceptibility breakpoints were taken as ≤ 2 μg/mL for levofloxacin and ≤ 1 μg/mL for ciprofloxacin, according to NCCLS guidelines.[6] Antibiotic susceptibility of the 50 *P. aeruginosa* isolates and 10 *S. maltophilia* isolates, to the two fluoroquinolones tested, are shown in Table 1.

The E-test method was selected because of its reliability in testing susceptibility to the fluoroquinolones,[7] its ease of use and its definition of the MIC.[8] In general, *S. maltophilia* showed greater susceptibility to levofloxacin than to ciprofloxacin, as demonstrated by: a) fewer *in vitro* resistant isolates recorded for levofloxacin versus ciprofloxacin; b) a diminished range of MICs recorded for levofloxacin compared to ciprofloxacin; and c) reduced MIC_{50} and MIC_{90} values recorded for levofloxacin compared to ciprofloxacin.

This contrasts to the susceptibility of *P. aeruginosa*, where isolates generally showed a greater susceptibility to ciprofloxacin than to levofloxacin. Furthermore, there was no marked difference in susceptibility of *P. aeruginosa* isolated from either patient group studied.

Two isolates of *P. aeruginosa* and one isolate of *S. maltophilia*