Penicillin-Resistant *Streptococcus pneumoniae* in the Netherlands: Results of a 1-Year Molecular Epidemiologic Survey

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The molecular epidemiologic characteristics of penicillin-resistant pneumococci in the Netherlands were investigated in 1995. Dutch electronic surveillance data showed that 0.7% of all pneumococci were intermediately resistant and 0.4% were highly resistant to penicillin. From March 1995 to March 1996, 89 penicillin-resistant isolates were collected by 39 medical microbiology laboratories. Thirty different genotypes were observed by restriction fragment end labeling. Twenty-one DNA types were unique, whereas 9 distinct genotypes were shared by ≥2 isolates. Different serogroups were found within 6 of the 9 genetically identical clusters of penicillin-resistant isolates, suggesting that horizontal transfer of capsular genes is common. Finally, nosocomial transmission of penicillin-resistant pneumococci was observed among 21 elderly adults with chronic obstructive pulmonary disease. This study demonstrates that multiple clones of penicillin-resistant pneumococci have been introduced in the Netherlands, a country with a low prevalence of pneumococcal infection. Some clones spread among the population in and outside hospitals.

Streptococcus pneumoniae is a common cause of serious and life-threatening infections, such as pneumonia, bacteremia, and meningitis, and of noninvasive infections, such as otitis media and sinusitis [1]. The emergence of pneumococcal resistance to penicillin, particularly in combination with other resistance determinants, poses serious problems for the institution of adequate antimicrobial therapy and subsequent reduction of transmission.

Soares et al. [2] reported the spread of a multiresistant clone of serotype 6B from Spain to Iceland in the late 1980s. This resulted in an epidemic of the clone, which was isolated with a frequency of up to 12% in 1992 [3]. In 1991, Munoz et al. [4] reported evidence for the intercontinental spread of a multiresistant clone of *S. pneumoniae* serotype 23F from Spain to the United States. This clone subsequently disseminated throughout the United States. In contrast to the clonal spread of pneumococcal resistance, an extensive genetic diversity has been observed among penicillin-resistant pneumococci in South Africa [6] and Kenya [7]. The increasing emergence of penicillin-resistant strains in the latter countries is thought to be due to horizontal transfer of genes of altered penicillin-binding proteins (PBPs) with lowered affinity to penicillin and other β-lactam antibiotics.

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Because of the worldwide increase in drug resistance and the clinical implications of penicillin resistance among pneumococci, we investigated the epidemiologic characteristics of penicillin-resistant pneumococci in the Netherlands over 1 year.

Materials and Methods

Bacterial isolates. Thirty-nine Dutch medical microbiology laboratories participated in this study and sent all penicillin-resistant pneumococci isolated between March 1995 and March 1996 to our laboratory. These 39 laboratories offer microbiologic services to the majority of the Dutch hospitals and cover 81% of the Dutch population. The participating hospitals, which provide medium, high, and intensive care, are distributed throughout the Netherlands. The laboratories performed susceptibility testing on all pneumococcal isolates; all penicillin-resistant pneumococci were included in this study. Duplicate patient isolates were excluded. Eighteen of the 39 laboratories collected surveillance data on the prevalence of penicillin-resistant pneumococci in 1995. From these data, we calculated the average prevalence of penicillin-resistant pneumococci.

Eighty-nine resistant isolates (MIC ≥0.1 mg/L) were received and characterized in this study. Sources were blood (n = 4), cerebrospinal fluid (n = 3), sputum (n = 60), nasopharynx (n = 19), pus (n = 2), and vaginal tissue (n = 1). We also analyzed 153 penicillin-susceptible pneumococcal isolates from Dutch patients with meningitis (provided by J. Dankert, National Reference Center for Bacterial Meningitis, Academic Medical Center, Amsterdam). This collection included all pneumococcal meningitis strains isolated in the Netherlands in 1994.

Reference strains belonging to pandemic clone 23F [4] were isolated in Spain, South Africa, Germany, and the United States (provided by R. Hakenbeck, Max-Planck Institute für moleculare

Table 1. Susceptibility of penicillin-resistant pneumococci to other classes of antibiotics.

Antibiotic	Range of concentrations tested	MIC									
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	>8
Penicillin	0.015–32	—	—	—	3	9	11	44	22	—	—	—
Cotrimoxazole	0.06–16	—	—	—	3	4	—	3	2	32	39	6
Doxycycline	0.06–16	—	—	—	34	9	1	—	4	15	15	11
Erythromycin	0.06–8	—	—	4	37	5	—	—	2	—	1	40
Rifampicin	0.015–4	18	57	14	—	—	—	—	—	—	—	—
Vancomycin	0.06–8	—	—	—	1	18	70	—	—	—	—	—
Sparfloxacin	0.03–2	—	—	—	19	56	14	—	—	—	—	—

NOTE. In total, 89 pneumococci were tested for each antibiotic.

Genetik, Berlin, and F. Tenover, CDC, Atlanta). Reference strains belonging to the pandemic clone 9V [8] were isolated in Spain (R. Hakenbeck).

Biochemical characterization, serotyping, and susceptibility testing. Isolates were confirmed as *S. pneumoniae* by investigation of their susceptibility to optochin and to bile solubility [9]. They were serotyped on the basis of capsular swelling (Quellung reaction) observed microscopically after suspension in antisera prepared at Statens Seruminstitut (Copenhagen) [10].

The susceptibility of pneumococcal strains was determined by agar dilution method. The MIC was defined as the lowest concentration of the antimicrobial agent that prevented visible growth. For this purpose, serial $\log_2$ concentrations of antibiotics were prepared in agar (IsoSensitest; Oxoid, Unipath, Basingstoke, UK) supplemented with 5% horse blood. Table 1 shows the concentration ranges of the various antibiotics. Pneumococcal isolates were removed from storage at −70°C, and subcultured at 37°C on Columbia agar (Oxoid) supplemented with 5% sheep blood, using 5% CO_2. Bacterial suspensions were prepared in 0.9% NaCl from 24-h agar cultures and adjusted to a McFarland turbidity of 0.5. Suspensions were further diluted (1:10) in saline. The inocula were applied to the test plates, using a multipoint inoculator, resulting in $\sim 10^4$ cfu per spot. MICs were read after 24 h of incubation at 37°C with 5% CO_2. Quality control strains included in each run were *S. pneumoniae* ATCC 49619, *S. pneumoniae* 19857 (clinical isolate, laboratory control), and *Staphylococcus aureus* ATCC 29213.

The antimicrobial agents tested were penicillin G (Sigma, St. Louis), erythromycin (Abbott Laboratories, Queensborough, UK), doxycycline (Pfizer, Brussels), vancomycin (Eli Lilly, Indianapolis), rifampicin (Sigma), cotrimoxazole (1:19 trimethoprim and sulfamethoxazole [Sigma]) and sparfloxacin (Rhone-Poulenc Rorer, Vitry sur Seine, France). We used antibiotic breakpoints to discriminate between susceptible and nonsusceptible strains in accordance with National Committee for Clinical Laboratory Standards guidelines for susceptibility testing [11].

Restriction fragment end labeling (RFEL) and BOX polymerase chain reaction (PCR) typing. Pneumococcal strain typing by RFEL was done as described by Van Steenbergen et al. [12] and adapted by Hermans et al. [13]. Briefly, purified pneumococcal DNA was digested by the restriction enzyme *Eco*RI. The DNA restriction fragments were end labeled at 72°C with [α-^{32}P]dATP using DNA polymerase (Goldstar; Eurogentec, Seraing, Belgium). After the radiolabeled fragments were denatured and separated electrophoretically on a 6% polyacrylamide sequencing gel containing 8 *M* urea, the gel was transferred onto filter paper, vacuum-dried (HBI, Saddlebrook, NY), and exposed for variable times at room temperature to ECL hyperfilm (Amersham Laboratories, Amersham, UK). BOX PCR typing was done as described [14].

PBP genotyping. Genetic polymorphism of PBP genes 1A, 2B, and 2X was investigated by restriction fragment length polymorphism analysis. For this purpose, we amplified the genes by PCR and analyzed the digested DNA products by agarose gel electrophoresis. PCR amplification of the PBP genes was done in a 50-μL PCR buffer system containing 75 m*M* TRIS-HCl, pH 9.0, 20 m*M* $(NH_4)_2SO_4$, 0.01% (wt/vol) of Tween 20, 1.5 m*M* $MgCl_2$, 0.2 m*M* dNTP, 10 pmol of individual primers, 0.5 U of DNA polymerase (Eurogentec), and 10 ng/μL purified chromosomal DNA. Cycling in a programmable thermal controller (PTC-100; MJ Research, Watertown, MA, USA) consisted of the following steps: predenaturation at 94°C for 1 min, 30 1-min cycles at 94 and 52°C, 2-min cycles at 72°C, and final extension at 72°C for 3 min. The primers used to amplify PBP 1A, 2B, and 2X genes have been described [4, 15, 16].

The amplification products (5 μL) were digested by restriction endonuclease *Hin*fI and separated by electrophoresis in 2.5% agarose gels containing 0.5× TBE (44.5 m*M* TRIS, 44.5 m*M* boric acid, 1.25 m*M* EDTA) and 0.1 μg/mL ethidium bromide (5 mm thick, Agarose MP; Boehringer Mannheim, Almere, Netherlands). Gels were run in 0.5× TBE containing 0.1 μg/mL ethidium bromide at a constant current of 20 mA for 4 h and photographed (Polaroid MP4 Land camera; Polaroid 667 films). Before electrophoresis, samples were mixed with a 5× concentrated layer mix consisting of 50% glycerol in water and 0.8 mg/mL bromo phenol blue.

Computer-assisted analysis of DNA band patterns. RFEL autoradiographs were converted to images (Image Master DTS; Pharmacia Biotech, Uppsala, Sweden) and analyzed by computer (Windows version Gelcompar software version 4; Applied Math, Kortrijk, Belgium). We analyzed DNA fragments with molecular weights of 160–400 bp after normalizing the DNA banding patterns by using the pneumococcus-specific bands present in the RFEL banding patterns of all strains. The banding patterns were analyzed by the unweighted pair group method, using arithmetic averages [17] and by applying the Jaccard similarity coefficient to

Table 2. Resistance patterns of penicillin-resistant pneumococci.

Resistance pattern	Strains with penicillin MIC		
	≤0.5 (n = 23)	1 (n = 44)	2 (n = 22)
P	4	0	0
PC	10	14	11
PD	1	0	0
PE	0	1	0
PCD	3	3	0
PCE	1	4	2
PDE	1	0	0
PCDE	3	22	9

NOTE. P, penicillin G; C, cotrimoxazole; D, doxycycline; E, erythromycin.

peaks [18]. Computer-assisted analysis and methods and algorithms followed the manufacturer's instructions. A tolerance of 1.5% in band positions was allowed during comparison of DNA patterns. Identical DNA types were arbitrarily defined as RFEL homologies >95%.

Results

Molecular epidemiology of Dutch penicillin-resistant pneumococci. The average prevalence of penicillin-resistant pneumococci in 1995 was calculated using electronic surveillance data from 18 of 39 Dutch medical microbiology laboratories participating in the study. By combined data analysis, 2653 pneumococci were isolated by the laboratories. Of these, 0.7% (n = 19) were intermediately resistant (MIC = 0.1–1 mg/L) and 0.4% (n = 11) were highly resistant (MIC > 1 mg/L). From March 1995 to March 1996, the 39 laboratories sent us 89 penicillin-resistant strains (MIC ≥0.1 mg/L): 67 (75%) were intermediately resistant to penicillin, and 22 (25%) were highly resistant. Most of these strains had MICs near the resistance breakpoint, 1 mg/L penicillin (table 1).

Forty-eight percent of the penicillin-resistant strains were resistant to erythromycin, 46% to doxycycline, and 93% to cotrimoxazole. The MIC distributions of erythromycin, doxycycline, and cotrimoxazole were clearly bimodal. The MIC distribution of rifampicin, vancomycin, and sparfloxacin did not show a distinct resistant subpopulation. The latter antibiotics were active against all isolates. Table 2 summarizes the resistance patterns of the penicillin-resistant pneumococci and shows that those with MICs ≥1 mg/L tended to be more frequently resistant to ≥4 antibiotics (resistance pattern PCDE).

The penicillin-resistant pneumococci were analyzed for genotype using RFEL. Twenty-one DNA types were uniquely present, and 9 distinct genotypes were shared by ≥2 isolates. The latter group comprised 68 strains, 76% of the collection (figure 1A, table 3). Identical DNA types were arbitrarily defined as RFEL homologies >95%. However, RFEL type 19 was exceptional, consisting of a group of DNA types with homologies ≥91%. Due to the presence of three very faint bands that variably appeared in this type, even when a single strain was analyzed repeatedly, we considered these 9 strains as a group sharing RFEL type 19. Genetic clustering shown by RFEL analysis was confirmed by BOX PCR typing. Except for RFEL type 15, the RFEL clusters also displayed identical genotypes by BOX PCR. The dichotomy observed in RFEL cluster type 15 using BOX PCR typing was restricted to a single DNA fragment band difference (data not shown).

The degree of genetic clustering was much higher among the penicillin-resistant strains compared with a group of 153 penicillin-susceptible strains, since the DNA banding patterns of only 51 penicillin-susceptible strains (33%) were observed more than once (figure 1B). In addition, no overlap was observed among RFEL types between penicillin-resistant and -susceptible pneumococci (data not shown).

Within clusters of identical genotypes, different serotypes were observed. Six of the 9 RFEL clusters displayed ≥2 serotypes. The predominant RFEL type 15 harbored 11 strains of serogroup 19 and 11 strains of serogroup 23. These 2 distinct serogroups in RFEL type 15 were congruent with the 2 different BOX PCR types observed within this RFEL type. The predominant RFEL type 23 comprised 11 strains of serogroup 9 and 7 of serogroup 14 (figures 1A, 2, table 3). Furthermore, different resistance patterns were observed within clusters of identical RFEL types. The predominant RFEL type 15 harbored the resistance patterns PCD, PCDE, and PCE. RFEL type 23 displayed PC, PCDE, PCE, PDE, and PE patterns (figure 1A, table 3). RFEL type 15 was identical to the pandemic clone 23F (initially isolated in Spain [4]) when the Dutch RFEL types were compared with a computerized data library of >50 distinct RFEL types, which represent drug-resistant pneumococci found worldwide. RFEL type 23 was identical to clone 9V, which spread from Spain to France [8] (figure 2).

The 89 penicillin-resistant strains and the collection of susceptible strains were further analyzed by PBP typing. Twenty-four distinct PBP types were observed among the resistant strains (figure 1A, table 3). PBP type 1 was observed in 48 of 89 strains. This PBP cluster included a significant proportion of the strains displaying the predominant RFEL types 15, 23, and 26 (figure 1A, table 3). Nine types were present among the susceptible group of strains. No overlap of PBP types was observed between the penicillin-resistant and -susceptible strains (data not shown).

Nosocomial transmission of multiresistant pneumococci in the Netherlands. We investigated the epidemiologic relatedness of the patients infected with resistant pneumococci with identical RFEL types (homology ≥95%). The isolates belonging to RFEL cluster 15 were isolated by nine laboratories. Two laboratories isolated RFEL type 15 pneumococci from 4 and 11 patients, respectively. The patients' clinical histories revealed that nosocomial transmission of multiresistant pneumo-

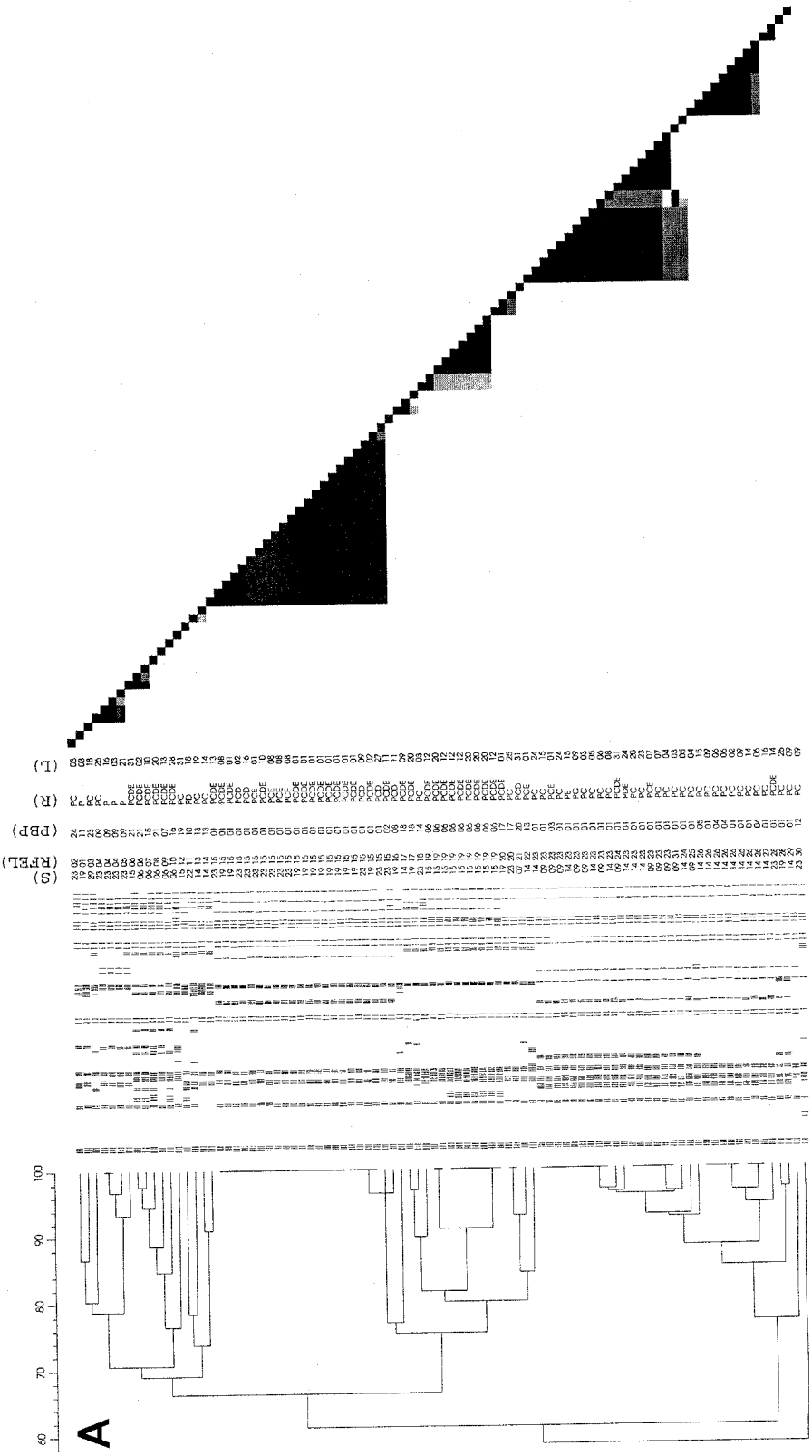

Figure 1. Dendrogram of restriction fragment end labeling (RFEL) band patterns of 89 penicillin-resistant (**A**) and 153 susceptible *S. pneumoniae* strains (**B**). Similarity matrices are also depicted: Similarities >95% are in black; similarities of 90%–95% are in gray. Characters in brackets indicate serogroup (S), RFEL type, penicillin-binding protein (PBP) type, resistance profile (R), and laboratory/hospital code (L). Arbitrary cutoff value for identical RFEL type is 95%, except for RFEL type 19 (see text for details). Resistant antibiotics are P, penicillin G; C, cotrimoxazole; D, doxycycline; and E, erythromycin.

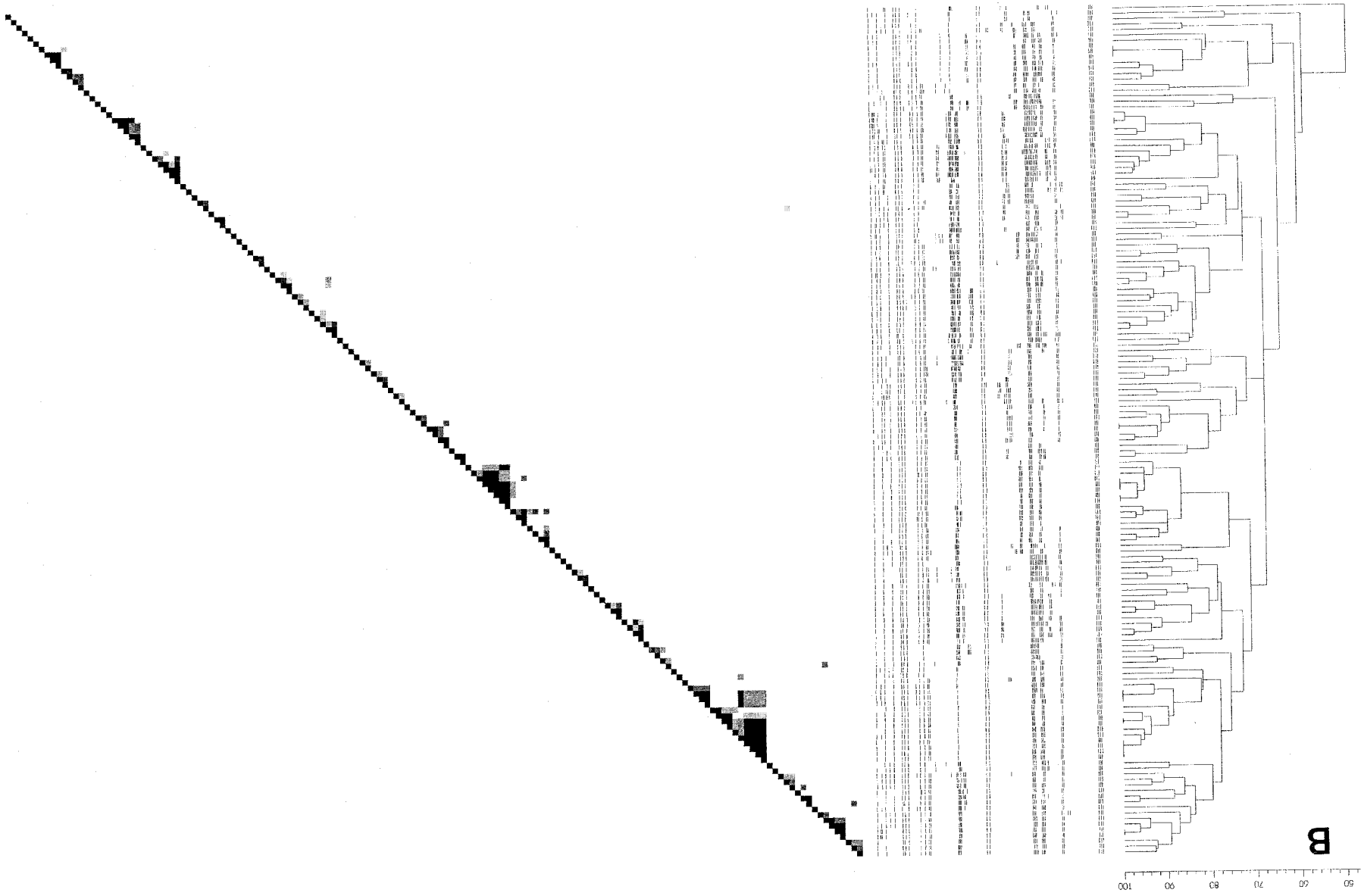

Table 3. Genotypic and phenotypic properties of penicillin-resistant pneumococci within 9 restriction fragment end labeling (RFEL) clusters.

RFEL cluster type	PBP type	Serogroup	Resistance pattern	No.* of strains
15	1	19	PCDE	9,† 1, 1
15	1	23	PCE	3,† 1
15	1	23	PCD	1, 1, 1, 1
15	1	23	PCDE	1, 1
15	2	23	PCDE	1
23	1	9	PC	2, 1, 1, 1, 1, 1, 1
23	1	9	PCDE	1
23	1	9	PCE	1
23	3	9	PCE	1
23	1	14	PC	1, 1, 1, 1, 1
23	1	14	PE	1
23	1	14	PDE	1
26	1	14	PC	2, 1, 1, 1
26	4	14	PC	3
26	5	14	PC	1
19	6	15	PCDE	5,† 4†
4	9	23	P	1, 1
4	9	23	PC	1
28	1	19	PC	1
28	1	23	PCDE	1
20	17	19	PCDE	1
20	17	23	PC	1
6	21	6	PCDE	1
6	21	15	PCDE	1
17	18	19	PCDE	1
17	22	14	PCDE	1

NOTE. Twenty-one strains with unique RFEL types are not included in this table. PBP, penicillin-binding protein; P, penicillin G; C, cotrimoxazole; D, doxycycline; E, erythromycin.

* No. sent by individual laboratories.

† Confirmed hospital outbreaks.

cocci had occurred in two hospitals, involving 3 and 9 patients. In both hospitals, the patients had chronic obstructive pulmonary disease, and penicillin-resistant pneumococci were isolated from sputum. The time period during which patients shared the same rooms in hospitals 1 and 8 is shown in figure 3A, B, respectively.

An epidemiologic link between hospitals 1 and 8 could not be identified. Of interest was that patient 3, who was involved in the pneumococcal outbreak in hospital 1, had a multiresistant pneumococcus isolated in 1993. RFEL analysis clearly demonstrated that the strain isolated in 1993 had an RFEL type identical to that observed in 1995 (data not shown). These data suggest the patient carried the multiresistant pneumococcus several years and was likely the index case in the nosocomial outbreak.

Within each of the two hospital outbreaks, the resistance pattern and the isolate serogroups were identical. However, the two hospitals had different resistance patterns and strain serogroups, indicating significant genetic diversity among these groups.

The isolates belonging to RFEL cluster 19 were isolated in two separate hospitals from 4 and 5 patients, respectively. Investigation of the patients' clinical histories pointed to a clonal spread of multiresistant pneumococci within and between the hospitals. Similar to the outbreaks in hospitals 1 and 8, the patients also had chronic obstructive pulmonary disease and penicillin-resistant pneumococci isolated from sputum. Figure 3C demonstrates the time period during which patients occupied the same rooms in hospitals 12 and 20. Since 1 of the patients was transferred from hospital 12 to hospital 20 (figure 3C), we assume this patient was the vehicle for the interhospital transmission of the multidrug-resistant strain.

Discussion

We investigated the molecular epidemiology of 89 penicillin-resistant pneumococcal strains collected from March 1995 to March 1996 throughout the Netherlands, a country where pneumococcal resistance remains rare. Of the isolates, 0.7% had intermediate-level resistance and 0.4% had high-level resistance. Most strains had MICs near the resistance breakpoint (1–2 mg/L). Therefore, infections by these organisms (other than in cases of meningitis) are expected to respond to intravenous treatment with penicillin.

Similar low rates of resistance have been reported in neighboring countries (e.g., Germany [19] and the UK [20]), although in the United Kingdom, resistance has risen from 1.5% in 1990 to 3.9% in 1995. In other European countries (e.g., France [21] and Spain [22]), penicillin-resistant pneumococci are much more common. Many of our penicillin-resistant strains were also resistant to cotrimoxazole, doxycycline, and erythromycin, similar to findings in the United States [23]. Rifampicin, vancomycin, and sparfloxacin were still active against all isolates. In addition, the penicillin-resistant pneumococci with MICs $\geqslant$1 mg/L tended to be more frequently multiply resistant against 4 antibiotics (resistance pattern PCDE). We hypothesize that horizontal cotransfer of antibiotic resistance genes other than PBP genes occurs frequently among pneumococci with a high level of resistance to penicillin.

Thirty distinct RFEL types were observed among the penicillin-resistant isolates. Pneumococcal strains with identical RFEL types were invariably received from two or more laboratories. The degree of genetic homogeneity was significantly higher than with a group of 153 penicillin-susceptible strains isolated from patients with meningitis. In addition, no overlap was observed among RFEL types between penicillin-resistant and -susceptible pneumococci. These observations suggest that despite the extremely low prevalence of penicillin resistance in the Netherlands, multiple clones of penicillin-resistant pneu-

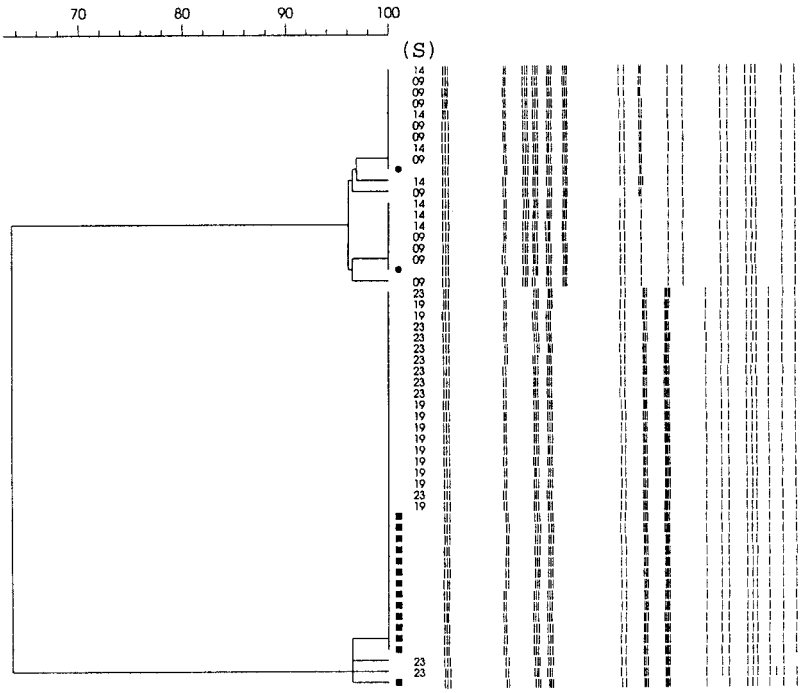

Figure 2. Genetic homology between Dutch penicillin-resistant pneumococci of restriction fragment end labeling types 15 and 23 and strains belonging to pandemic clones 23F (■) and 9V (●). Serogroups (S) are also depicted.

mococci have been introduced. The capacity of some penicillin-resistant pneumococci to spread in the Netherlands is evident, but a widespread epidemic of resistant pneumococcal strains, observed in other countries [2, 4, 5, 8, 24–29], has not yet materialized. Clearly, the very young and the elderly are at the highest risk for such an epidemic. Factors such as frequent use of β-lactam antibiotics may contribute to this risk.

Serogroups 14 (n = 22), 23 (n = 20), 19 (n = 16), 9 (n = 12), and 15 (n = 11) were frequently encountered. Different serogroups were found in 6 of 9 genetic clusters of penicillin-resistant isolates. This suggests frequent horizontal transfer of capsular genes in vivo. The high frequency of capsular transfer may have consequences with regard to the outcome of the current vaccine strategy, which focuses on the use of capsular polysaccharides representing a restricted number of capsular types. The use of multivalent conjugate vaccines may shift the capsular distribution toward capsular types not represented in the vaccines.

When the RFEL types were compared with those in a computerized data library of >80 distinct drug-resistant pneumococci collected worldwide (Hermans PWM, unpublished data), RFEL type 15 was identical to the pandemic clone 23F initially isolated in Spain [4]. Similar to our observations, Coffey and coworkers [15] reported that the pandemic clone 23F harbors both serogroups 23 and 19. In addition, Barnes et al. [30] reported capsular transformation of the latter clone from serotype 23F to serotype 14. In addition, RFEL type 23 resembled the Spanish/French clone 9V [8]. So far, capsular diversity within clone 9V has not been observed. Our data demonstrate that clone 9V (RFEL type 23) harbors at least 2 capsular serogroups: 9 and 14.

PBP genotype analysis revealed that PBP type 1 was predominant in 48 of 89 resistant strains. Of interest, PBP type 1 strains represented 8 distinct RFEL types. Since the genetic relatedness of the RFEL types is low, we hypothesize that identical PBP types result from horizontal transfer of PBP genes coding for low-affinity PBPs within these genetically distinct families. Detailed characterization of the PBP genes to investigate their clonal relatedness is underway.

Within four hospitals, the penicillin-resistant *S. pneumoniae* isolates invariably displayed identical RFEL type, resistance pattern, and serogroup. Two hospital outbreaks involving 3 and 9 patients were caused by isolates of RFEL type 15. Case-history studies of the patients did not suggest hospital-to-hospital transmission. Since the resistance patterns and the serotypes differed among the isolates from the two hospitals, we conclude that these nosocomial outbreaks were caused by two distinct lineages derived from the alarming clone 23F.

In two other hospitals, nosocomial transmission of strains displaying RFEL type 19 was observed. Interhospital transmission was highly suggestive, since 1 patient was transferred from one hospital to the other, and the resistance pattern and the serogroup of the strains from the two hospitals were identical. RFEL type 19 did not show any genetic relatedness with the DNA banding patterns shown in the international data library of >80 distinct RFEL types representing drug-resistant pneumococci collected worldwide (Hermans P. W. M., unpublished data). The patients infected nosocomially with penicillin-resistant pneumococci in-

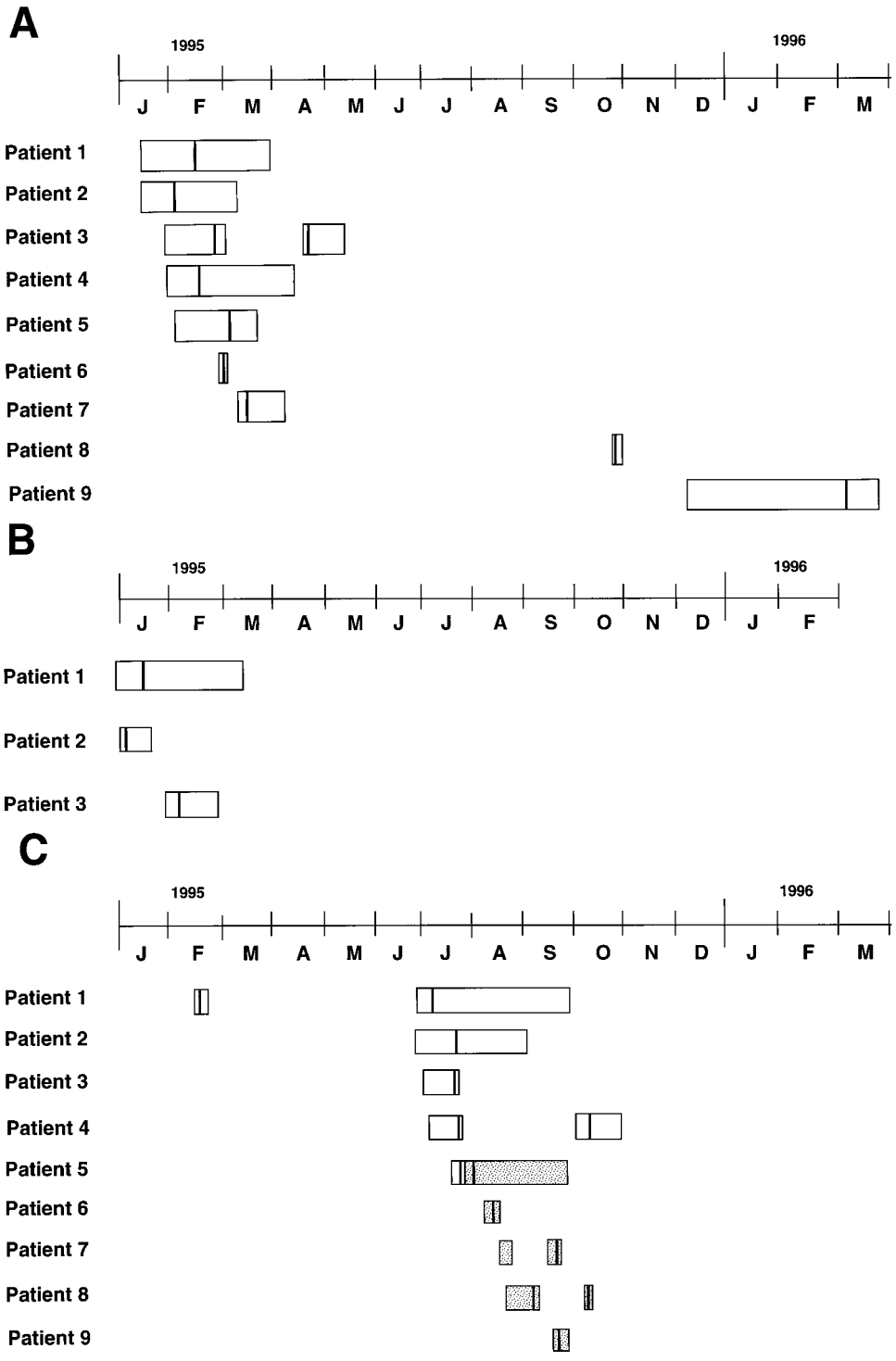

Figure 3. Schematic drawing indicating nosocomial transmission of penicillin-resistant pneumococci in hospitals 1 (**A**), 8 (**B**), and 12 (open bars) and 20 (shaded bars) (**C**). Letters on time axis indicate months of year; vertical lines in bars indicate time points when pneumococci were isolated from patients.

variably had chronic obstructive pulmonary disease. We therefore conclude that this disease is an important risk factor for the acquisition and spread of penicillin-resistant pneumococci.

Several pneumococcal outbreaks in hospitals have been reported [31–38], and outbreaks have been investigated in other institutional settings, such as prisons [39], shelters for the homeless [40], nursing homes [41], and day care facilities [30, 42, 43].

RFEL types 15, 19, 23, and 26 were most frequently observed among Dutch penicillin-resistant isolates. Only two laboratories collected isolates with RFEL type 19, whereas RFEL

types 15, 23, and 26 were collected by 8, 13, and 6 laboratories, respectively. These data suggest that strains with RFEL types 15, 23, and 26 either have been present longer in the Dutch community or have spread more quickly within the population compared with strains with RFEL type 19. In addition, different serogroups and resistance patterns were present within RFEL types 15 and 23. Since both types have also been observed in other countries, we conclude that multiple entry into the Netherlands might explain the diversity of serogroups and resistance patterns within these genotypes.

Our data demonstrate the epidemic potential of certain penicillin-resistant pneumococcal clones that, due to their relative resistance to penicillin and other commonly used antimicrobial agents, may spread in subpopulations regularly exposed both to the pneumococcus and to the drugs. Restrictive antibiotic policies remain the major defense against the epidemic dissemination of such strains. In addition, the emergence of multidrug-resistant pneumococci raises the issue of the need for isolation facilities in hospitals and for vaccination of patients at risk for pneumococcal disease.

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References

1. Caputo GM, Appelbaum PC, Liu HH. Infections due to penicillin-resistant pneumococci. Arch Intern Med **1993**;153:1301–10.
2. Soares S, Kristinsson KG, Musser JM, Tomasz A. Evidence for the introduction of a multiresistant clone of serotype 6B *Streptococcus pneumoniae* from Spain to Iceland in the late 1980s. J Infect Dis **1993**;168:158–63.
3. Kristinsson KG, Hjalmarsdottir MA, Steingrimsson O. Increasing penicillin resistance in pneumococci in Iceland. Lancet **1992**;339:1606–7.
4. Munoz R, Coffey TR, Daniels M, et al. Intercontinental spread of a multiresistant clone of serotype 23F *Streptococcus pneumoniae.* J Infect Dis **1991**;164:302–6.
5. McDougal LK, Facklam R, Reeves M, et al. Analysis of multiply antimicrobial-resistant isolates of *Streptococcus pneumoniae* from the United States. Antimicrob Agents Chemother **1992**;36:2176–84.
6. Smith AM, Klugman KP, Coffey TJ, Spratt BG. Genetic diversity of penicillin-binding protein 2B and 2X genes from *Streptococcus pneumoniae* in South Africa. Antimicrob Agents Chemother **1993**;37:1938–44.
7. Kell CM, Jordens JZ, Daniels M, et al. Molecular epidemiology of penicillin-resistant pneumococci isolated in Nairobi, Kenya. Infect Immun **1993**;61:4382–91.
8. Gasc AM, Geslin P, Sicard AM. Relatedness of penicillin-resistant *Streptococcus pneumoniae* serogroup 9 strains from France and Spain. Microbiology **1995**;141:623–7.
9. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. Washington, DC: American Society for Microbiology, **1995**.
10. Facklam RR, Washington JA II. Streptococcus and related catalase-negative gram-positive cocci. In: Balows A, Hausler WJ, Hermann RL, Isenberg HD, Shadomy HJ, eds. Manual of clinical microbiology, 5th ed. Washington, DC: American Society for Microbiology, **1991**:238–57.
11. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing. 6th informational supplement; document M100-S6. Wayne, PA: NCCLS, **1995**.
12. Van Steenbergen TJM, Colloms SD, Hermans PWM, de Graaff J, Plasterk RHA. Genomic DNA fingerprinting by restriction fragment end labeling (RFEL). Proc Natl Acad Sci USA **1995**;92:5572–6.
13. Hermans PWM, Sluijter M, Hoogenboezem T, Heersma H, van Belkum A, de Groot R. Comparative study of five different DNA fingerprint techniques for molecular typing of *Streptococcus pneumoniae* strains. J Clin Microbiol **1995**;33:1606–12.
14. Van Belkum A, Sluijter M, De Groot R, Verbrugh H, Hermans PWM. A novel BOX repeat PCR assay for high-resolution typing of *Streptococcus pneumoniae* strains. J Clin Microbiol **1996**;34:1176–9.
15. Coffey TJ, Dowson CG, Daniels M, et al. Horizontal transfer of multiple penicillin-binding protein genes, and capsular biosynthetic genes, in natural populations of *Streptococcus pneumoniae.* Mol Microbiol **1991**;5:2255–60.
16. Dowson CG, Hutchison A, Spratt BG. Extensive re-modelling of the transpeptidase domain of penicillin-binding protein 2B of a penicillin-resistant South African isolate of *Streptococcus pneumoniae.* Mol Microbiol **1989**;3:95–102.
17. Romesburg HC. Cluster analysis for researchers. Malabar, FL: Krieger, **1990**:9–28.
18. Sneath PHA, Sokal RR. Numerical taxonomy. San Francisco: Freeman, **1973**:131–2.
19. Reinert RR, Queck A, Kaufhold A, Kresken M, Lütticken R. Antimicrobial resistance and type distribution of *Streptococcus pneumoniae* isolates causing systemic infections in Germany, 1992–1994. Clin Infect Dis **1995**;21:1398–401.
20. Johnson AP, Speller DCE, George RC, Warner M, Domingue G, Efstratiou A. Prevalence of antibiotic resistance and serotypes in pneumococci in England and Wales: results of observational surveys in 1990 and 1995. BMJ **1996**;312:1454–6.
21. Bédos JP, Chevret S, Chastang C, Geslin P, Régnier R, and French Cooperative Pneumococcus Study Group. Epidemiological features of and risk factors for infection by *Streptococcus pneumoniae* strains with diminished susceptibility to penicillin: findings of a French survey. Clin Infect Dis **1996**;22:63–72.
22. Gómez J, Ruiz-Gómez J, Hernández-Cardona JL, Núñez ML, Canteras M, Valdés M. Antibiotic resistance patterns of *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis:* a prospective study in Murcia, Spain, 1983–1992. Chemotherapy **1994**;40:299–303.
23. Doern GV, Brueggemann A, Holley HP Jr, Rauch AM. Antimicrobial resistance of *Streptococcus pneumoniae* recovered from outpatients in the United States during the winter months of 1994 to 1995: results of a 3-center national surveillance study. Antimicrobl Agents Chemother **1996**;40:1208–13.
24. Lefèvre JC, Bertrand MA, Faucon G. Molecular analysis by pulsed-field gel electrophoresis of penicillin-resistant *Streptococcus pneumoniae* from Toulouse, France. Eur J Clin Microbiol Infect Dis **1995**;14:491–7.

25. Lefèvre JC, Gasc AM, Lemozy J, Sicard AM, Faucon G. Pulsed field gel electrophoresis for molecular epidemiology of penicillin resistant *Streptococcus pneumoniae* strains. Pathol Biol **1994**;42:547–52.
26. McDougal LK, Rasheed JK, Biddle JW, Tenover FC. Identification of multiple clones of extended-spectrum cephalosporin-resistant *Streptococcus pneumoniae* isolates in the United States. Antimicrob Agents Chemother **1995**;39:2282–8.
27. Munoz R, Musser JM, Crain M, et al. Geographic distribution of penicillin-resistant clones of *Streptococcus pneumoniae:* characterization by penicillin-binding protein profile, surface protein A typing, and multilocus enzyme analysis. Clin Infect Dis **1992**;15:112–8.
28. Reichmann P, Varon E, Günther E, et al. Penicillin-resistant *Streptococcus pneumoniae* in Germany: genetic relationship to clones from other European countries. J Med Microbiol **1995**;43:377–85.
29. Sibold C, Wang J, Henrichsen J, Hakenbeck R. Genetic relationships of penicillin-susceptible and -resistant *Streptococcus pneumoniae* strains isolated on different continents. Infect Immun **1992**;60:4119–26.
30. Barnes DM, Whittier S, Gilligan PH, Soares S, Tomasz A, Henderson FW. Transmission of multi-drug resistant serotype 23F *Streptococcus pneumoniae* in group day care: evidence suggesting capsular transformation of the resistant strain in vivo. J Infect Dis **1995**;171:890–6.
31. Berk SL, Gage KA, Holtsclaw-Berk SA, Smith JK. Type 8 pneumococcal pneumonia: an outbreak on an oncology ward. South Med J **1985**;78:159–61.
32. Cartmill TDI, Panigrahi H. Hospital outbreak of multiresistant *Streptococcus pneumoniae* [letter]. J Hosp Infect **1992**;20:130–1.
33. Denton M, Hawkey PM, Hoy CM, Porter C. Co-existent cross-infection with *Streptococcus pneumoniae* and group B streptococci on an adult oncology unit. J Hosp Infect **1993**;23:271–8.
34. Gould FK, Magee JG, Ingham HR. A hospital outbreak of antibiotic-resistant *Streptococcus pneumoniae.* J Infect **1987**;15:77–9.
35. Mandigers CMPW, Diepersloot RJA, Dessens M, Mol SJM, Van Klingeren B. A hospital outbreak of penicillin-resistant pneumococci in the Netherlands. Eur Respir J **1994**;7:1635–9.
36. Millar MR, Brown NM, Tobin GW, Murphy PJ, Windsor ACM, Speller DCE. Outbreak of infection with penicillin-resistant *Streptococcus pneumoniae* in a hospital for the elderly. J Hosp Infect **1994**;27:99–104.
37. Moore EP, Williams EW. Hospital transmission of multiply antibiotic-resistant *Streptococcus pneumoniae.* J Infect **1988**;16:199–200.
38. Reichler MR, Rakovsky J, Sláčiková M, et al. Spread of multidrug-resistant *Streptococcus pneumoniae* among hospitalized children in Slovakia. J Infect Dis **1996**;173:374–9.
39. Hoge CW, Reichler MR, Dominguez EA, et al. An epidemic of pneumococcal disease in an overcrowded, inadequately ventilated jail. N Engl J Med **1994**;331:643–8.
40. Mercat A, Nguyen J, Dautzenberg B. An outbreak of pneumococcal pneumonia in two men's shelters. Chest **1991**;99:147–51.
41. Quick RE, Hoge CW, Hamilton DJ, Whitney CJ, Borges M, Kobayashi JM. Underutilization of pneumococcal vaccine in nursing homes in Washington State: report of a serotype-specific outbreak and a survey. Am J Med **1993**;94:149–52.
42. Cherian T, Steinhoff MC, Harrison LH, Rohn D, McDougal LK, Dick J. A cluster of invasive pneumococcal disease in young children in child care. JAMA **1994**;271:695–7.
43. Reichler MR, Allphin AA, Breiman RF, et al. The spread of multiply resistant *Streptococcus pneumoniae* at a day care center in Ohio. J Infect Dis **1992**;166:1346–53.