

GLOBAL STATUS OF PNEUMOCOCCAL DRUG RESISTANCE

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Historically speaking, the first description of *Streptococcus pneumoniae* strains with raised penicillin MICs came from the laboratory of Dr. Maxwell Finland in the mid-1960s ¹⁾. However, results were not confirmed, and the possible significance of this phenomenon was missed. Hansman and co-workers, from Australia, first described in 1967 a case of a child colonized in the upper respiratory tract by a pneumococcus with a penicillin MIC of 0.125 µg/ml ²⁾. It was not clear at the time whether the strain was causing infection or not, but the MIC was higher than that which was seen previously. Subsequent to that in the late 1960's and early 1970's Dr. Hansman and his group described the same phenomenon in other parts of Australia but most especially a very high incidence of colonization with these strains in Papua, New Guinea ³⁻⁶⁾.

In the 1970's anecdotal reports from the US and the UK appeared of infections caused by these pneumococcus strains with partial resistance to penicillin ⁷⁻¹²⁾. In early 1977, however, our group in Durban, South Africa started to see cases of bacteremia and meningitis which, with the crude disk susceptibility testing techniques we had at our disposal at the time, we found to be caused by pneumococcus strains resistant to penicillin ¹³⁾. Subsequent MIC testing showed these strains to be indeed resistant to penicillin and, within a few weeks of this finding being communicated to other workers, these strains were isolated from Johannesburg, and other parts of South Africa. Some of these strains were multiresistant to two or more antimicrobials ¹⁴⁾.

Since these findings in the late 1970s, the incidence of pneumococci resistant to penicillin and other β-lactam and non-β-lactam antibiotics has increased ¹⁵⁾. This increase has been particularly dramatic during the past 10 years all over the world, and resistant clones have spread rapidly from country to country, and from continent to continent ¹⁶⁾. This increase must be taken in the context of the fact that over 90% of pneumococcal infections are not diagnosed bacteriologically.

Because the mechanism of β-lactam resistance in pneumococci is based upon changes in penicillin binding proteins, β-lactam MICs in this species form a continuum. It has been stated that pneumococci are susceptible to penicillin G if their MICs are <0.1 µg/ml, intermediate if MICs are 0.1-1.0 µg/ml, and resistant if MICs are >1.0 µg/ml. Most pneumococci which are fully resistant to penicillin G have a maximal MIC of 4 µg/ml, but a few strains with MICs of 8 and 16 µg/ml have been isolated ¹⁷⁾. However, this classification is not absolute and the decision as to whether strains are clinically resistant depends on other additional factors such as pharmacokinetic and pharmacodynamic parameters, site of infection, immune status of the patient, etc. For

the purpose of this presentation, pneumococci with MICs 0.1 µg/ml or greater are taken as resistant. It should also be borne in mind that the incidence of these strains in nasopharyngeal carriers (especially in the pediatric age group) is higher than that seen in isolates from clinical infections ¹⁷⁾ .

In general, the higher the penicillin MIC the higher the MIC to other β-lactams and non-β-lactam compounds, including macrolides, tetracyclines, chloramphenicol, clindamycin and cotrimoxazole¹⁷⁾. In the US, most penicillin susceptible strains (>95%) are macrolide susceptible; however, this number decreases to approximately 50% in fully penicillin resistant strains ¹⁸⁾ .

In the United States there really been no proper large state-by-state surveys of the incidence of these strains. However, available data indicate that there has been an increase in resistance to penicillin from <5% before 1989 (including <0.02% of isolates with MICs >1.0 µg/ml) to 6.6% in 1991-1992 (with 1.3% of isolates with MICs >1.0 µg/ml) and to 23.6% (360) of 1527 strains during 1994-1995 ¹⁸⁻¹⁹⁾. It is also important to note the high rates of isolation of penicillin intermediate and resistant pneumococci (approximately 30%) in middle ear fluids from patients with refractory otitis media, compared to other sites ²⁰⁾ .

In Canada, the country-wide incidence of penicillin resistant pneumococci is at least 10%, and has increased dramatically between 1990-1996. Interestingly, the highest incidence of these strains has been found in Saskatchewan, a province with a sparsely populated population ^{15, 21, 22)}. A recent multicenter study has documented an incidence of at least 20% of drug-resistant pneumococci in Mexico, and all countries in South America that have been investigated ^{15, 23-29)}.

It became very clear soon after isolates had been described in South Africa that Spain was a major focus of infection. The overall incidence of drug-resistant pneumococci in Spain seems to have peaked at approximately 50%: the incidence in Spain increased from 8.7% between 1979 and 1981 to 44.3% in 1989 and the latest figures in Spain are 51.7% ^{15, 30-34)} .

Importantly, strains with clonal epidemiological features identical to those of certain Spanish pneumococcal serotypes (e.g. 23F) have been isolated in various countries all over the world ¹⁶⁾. We are in the midst of a pandemic of drug-resistant pneumococcal clones, many of which may have originated in Spain. The incidence in Portugal is also quite high and is increasing ³⁵⁾. In other countries of Western Europe the incidence is increasing, and is now approximately 10% in Switzerland and Germany ^{15, 34, 36-38)}. What happened in France is a good sentinel indication of what is happening in the rest of the world: a logarithmic increase from 0.5% in 1984 to 36.3% in 1995 was seen ^{15, 39, 40)}. At least some strains probably originated from Spain, and then spread in the pediatric population, the most important reservoir of these organisms ^{39,40)}. In the Low Countries (Belgium and The Netherlands) the incidence is still low ^{15, 41-43)}. However, a French clone has recently appeared in a Belgian hospital ⁴⁴⁾, and it is probably only a matter of

time before these strains spread and the incidence in the Low Countries increases as well.

In Italy, published studies have documented a low incidence of penicillin resistant pneumococci (approximately 5% in Central and Northern Italy)(15,45,46). In contrast, however, the incidence of macrolide resistance in penicillin susceptible strains is high (approximately 50%)^{34, 45, 46)} : this contrasts with the situation described above in the US ¹⁸⁾. Macrolide resistance is also more prevalent in other countries in Southern Europe (Spain, France) than in the US ³⁴⁾, and a Mediterranean clone which is penicillin susceptible but resistant to other non- β -lactams has spread through Italy, Greece and Israel ⁴⁷⁾ .

In the United Kingdom, the incidence of drug-resistant pneumococci is slowly increasing ^{15, 34, 48)} . Of interest, however, is an incidence of approximately 10% in Ireland ³⁴⁾ . The incidence in Scandinavia and Austria remains low (approximately 5%) ^{15, 49-53)} .

Another European focus of drug-resistant pneumococci is Central and Eastern Europe ¹⁵⁾ . The very high reported incidence of 57.8% in Hungary ⁵⁴⁾ was probably too high, owing to the nosocomial spread of one isolate. However, a country-wide study has documented a high incidence of these strains in most regions of Hungary⁵⁵⁾ . Romania has a very high incidence of these strains ^{56, 57)}, the only other country with a reported incidence in some studies of 68 to 87% comparable to that of Korea (see further). The incidence of drug-resistant pneumococci is also high in Bulgaria ^{57, 58)} and Slovakia (especially those areas adjacent to Hungary)^{57, 59)}. Although not published in the English literature, the incidence of drug-resistant pneumococci in the former Yugoslavia is high ⁴⁴⁾ . The incidence of these strains in Russia and Central Asia is currently unknown. Recent studies have documented a high incidence of drug-resistant pneumococci in different areas of Greece ^{60, 61)} .

The position in Iceland deserves special mention. Prior to 1989, no strains of drug-resistant pneumococci were seen in that country. Suddenly, however, between 1989 and 1992 a serogroup 6B clone, probably spread from Spain, appeared and spread rapidly in the pediatric population. Although prudent use of day-care facilities and antibiotics has reduced the incidence of these strains in Iceland, they are still prevalent, illustrating once again the capability of these strains to spread intercontinentally ^{16, 62)} .

In the Middle East, the incidence of drug-resistant pneumococci is high, especially in Israel, Saudi Arabia, Lebanon, Turkey and Egypt, with reported incidences as high as 50% ^{15, 63-68)} . The incidence in Israel (in which most strains show intermediate penicillin resistance) seems highest amongst Bedouins, probably because of their communal familial patterns, with an increased chance for people to cough and sneeze on each other and thereby colonize each other ⁶⁹⁾ .

Information on the incidence of drug-resistant pneumococci in Africa is sparse ¹⁵⁾ . The occurrence of these strains in South Africa continues unabated (especially of strains with intermediate resistance)^{70, 71)}, but a high incidence of resistance has been

documented in Kenya, Ghana, Rwanda and Uganda. Recently, a high incidence of these strains has been documented in French West and North Africa, with a particularly high incidence in Senegal (>60%)⁷²⁻⁷⁷ .

Before establishment of ANSORP, very little information on the incidence of drug-resistant pneumococci was available from Asia¹⁵ . High rates were previously reported from Korea, Japan, Pakistan and Bangladesh^{15, 78-80} . Incidence rates of these strains in other Asian countries will form the subject of other presentations at the current Symposium.

The high incidence in Papua, New Guinea continues^{15, 81} . In Australia the highest incidence seems to be amongst the Aborigines in the Northern Territories⁸¹ and a low incidence of these strains is present in New Zealand¹⁵ .

What is the driving force behind the rapid development of drug-resistant pneumococci? In order to investigate this, we performed two studies on multistep resistance in subinhibitory antibiotic concentrations. In both studies, strains were subcultured a maximum of 50 consecutive times in subMIC concentrations of the antibiotic. In the first study⁸², selection of resistance to amoxicillin (with or without clavulanate), cefaclor, cefuroxime and azithromycin among six penicillin G- and azithromycin-susceptible pneumococcal strains and among four strains with intermediate penicillin sensitivities (azithromycin MICs 0.125-4 µg/ml) was performed. For only one of the six penicillin susceptible strains did subcultures in medium with amoxicillin (with or without clavulanate) lead to an increased MIC, with the MIC rising from 0.008 to 0.125 µg/ml. Five of the six penicillin-susceptible strains showed increased azithromycin MICs (0.5 to >256 µg/ml) after 17 to 45 subcultures. Subculturing in medium with cefaclor did not affect the cefaclor MICs of three strains but led to increased cefaclor MICs (from 0.5 to 2 – 4 µg/ml) for three of the six strains, with MICs of other β-lactams rising 1 to 3 twofold dilutions. Subculturing in cefuroxime led to increased cefuroxime MICs (from 0.03 - 0.06 µg/ml to 0.125 – 0.5 µg/ml) for all six strains without significantly altering the MICs of other β-lactams, except for one strain, which developed an increased cefaclor MIC. Subculturing in azithromycin did not affect β-lactam MICs. Subculturing of the four strains with decreased penicillin susceptibility in amoxicillin (with or without clavulanate) or cefuroxime did not select for cefuroxime resistance. Subculturing of one strain in cefaclor led to an increase in MIC from 0.5 to 2 µg/ml after 19 passages. In contrast to strains that were initially azithromycin susceptible, which required >10 subcultures for resistance selection, three of four strains with azithromycin MICs of 0.125 to 4 µg/ml showed increased MICs after 7 to 13 passages, with the MICs increasing to 16-32 µg/ml. All azithromycin-resistant strains were clarithromycin resistant. With the exception of strains that contained mefE at the onset, no strains that developed resistance to azithromycin contained ermB or mefE, genes that have been found in macrolide-resistant pneumococci obtained from clinic patients. Preliminary results of

resistance selection studies in our laboratory have found the same phenomenon for erythromycin, clarithromycin and roxithromycin.

In the second study ⁸³⁾, the ability of 50 sequential subcultures in subinhibitory concentrations of ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin, trovafloxacin and amoxicillin/clavulanate to select for resistance was studied in 6 penicillin susceptible and 4 penicillin intermediate pneumococci. Subculturing in ciprofloxacin, grepafloxacin, levofloxacin and sparfloxacin led to selection of mutants with raised MICs in all 10 strains with MICs rising from 0.5-4 to 4-32 µg/ml after 7-12 passages for ciprofloxacin, 0.06-0.25 to 0.5-8 µg/ml after 5-23 passages for grepafloxacin, 0.5-1 to 4-64 after 14-49 passages for levofloxacin and 0.125-0.25 to 1-16 after 8-26 passages for sparfloxacin. Subculturing in trovafloxacin led to raised MICs in 8 strains with MICs rising from 0.06-0.125 to 0.5-8 µg/ml after 6-28 passages. Subculturing in amoxicillin/clavulanate led to raised MICs in one strain with the MIC rising from 0.015-0.125 µg/ml after 24 passages. Subculturing in amoxicillin/clavulanate did not lead to raised MICs to any of the quinolones, and subculturing in quinolones did not lead to amoxicillin/clavulanate resistance. Most mutations in quinolone resistant quinolones were in *parC* and *gyrA*, but efflux pump abnormalities were found in strains resistant to ciprofloxacin and levofloxacin.

In summary, drug-resistant pneumococcal clones have spread all over the world and are now a serious therapeutic problem, particularly in meningitis and otitis media. Introduction of a conjugated vaccine which can be used in children under 2 years of age may help alleviate this serious problem. Although little can be done to prevent spread of resistant clones from country to country and from continent to continent, rational use of antimicrobials (especially of the oral class) may also help in this regard.

REFERENCES

1. Kislak JW, Razavi LMB, Daly AK, Finland M. Susceptibility of pneumococci to nine antibiotics. *Am. J. Med. Sci.* 1965; 250: 261-268.
2. Hansman D, Bullen MM. A resistant pneumococcus. *Lancet* 1967; 2: 264-265.
3. Hansman D, Glasgow H, Sturt J, Devitt HL, Douglas R. Increased resistance to penicillin of pneumococci isolated from man. *N. Engl. J. Med.* 1971; 284: 175-177.
4. Hansman D. Type distribution and antibiotic sensitivity of pneumococci from carriers in Kiriwina, Trobriand Islands (New Guinea). *Med. J. Aust.* 1972; 2: 771-773.
5. Hansman D, Devitt L, Miles H, Riley I. Pneumococci relatively insensitive to penicillin in Australia and New Guinea. *Med. J. Aust.* 1974; 2: 353-356.

6. Gratten M, Naraqi S, Hansman D. High prevalence of penicillin-insensitive pneumococci in Port Moresby, New Guinea. *Lancet* 1980; 2: 192-195.
7. Naraqi S, Kirkpatrick GP, Kabins S. Relapsing pneumococcal meningitis: isolation of an organism with decreased susceptibility to penicillin G. *J. Pediatr.* 1974; 85: 671-673.
8. Howes VJ, Mitchell RG. Meningitis due to relatively penicillin-resistant pneumococcus. *Br. Med. J.* 1976; 1: 996.
9. Paredes A, Taber LH, Yow MD, Clark D, Nathan W. Prolonged pneumococcal meningitis due to an organism with increased resistance to penicillin. *Pediatrics* 1976; 58: 378-381.
10. Mace JW, Janik DS, Sauer RL, Quilligan JJ Jr. Penicillin-resistant pneumococcal meningitis in an immunocompromised infant. *J. Pediatr.* 1977; 91: 506.
11. Cates KL, Gerrard JM, Giebink GS, et al. A penicillin-resistant pneumococcus. *J. Pediatr.* 1978; 93: 624-626.
12. Iyer PV, Kahler JH, Jacobs NM. Penicillin-resistant pneumococcal meningitis. *Pediatrics* 1978; 61: 157-158.
13. Appelbaum PC, Bhamjee A, Scragg JN, Hallett AF, Bowen AJ, Cooper RC. *Streptococcus pneumoniae* resistant to penicillin and chloramphenicol. *Lancet* 1977; 2: 995-997.
14. Jacobs MR, Koornhof HJ, Robins-Browne RM, et al. Emergence of multiply resistant pneumococci. *N. Engl. J. Med.* 1978; 299: 735-740.
15. Appelbaum PC. Antimicrobial resistance in *Streptococcus pneumoniae*: an overview. *Clin. Infect. Dis.* 1992; 15: 77-83.
16. Munoz R, Coffey TJ, Daniels M, et al. Intercontinental spread of a multiresistant clone of serotype 23F *Streptococcus pneumoniae*. *J. Infect. Dis.* 1991; 164: 302-306.
17. Jacobs MR. Treatment and diagnosis of infections caused by drug-resistant *Streptococcus pneumoniae*. *Clin. Infect. Dis.* 1992; 15: 119-127.
18. 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 30-center national surveillance study. *Antimicrob. Agents Chemother.* 1997; 40: 1208-1213.
19. Breiman RF, Butler JC, Tenover FC, Elliott JA, Facklam RR. Emergence of drug-resistant pneumococcal infections in the United States. *J. Amer. Med. Assoc.* 1994; 271: 1831-1835.
20. Jacobs MR, Dagan R, Appelbaum PC, Burch DJ. Prevalence of antimicrobial-resistant pathogens in middle ear fluid: multinational study of 917 children with acute otitis media. *Antimicrob. Agents Chemother.* 1998; 42: 589-595.
21. Jetté LP, Lamothe F, and the Pneumococcus Study Group. Surveillance of invasive *Streptococcus pneumoniae* infection in Quebec, Canada, from 1984 to

- 1986; serotype distribution, antimicrobial susceptibility, and clinical characteristics. *J. Clin. Microbiol.* 1989; 27: 1-5.
22. Simor AE, Louie M, The Canadian Bacterial Surveillance Network, Low D. Canadian National Survey of Prevalence of Antimicrobial Resistance among clinical isolates of *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 1996; 40: 2190-2193.
 23. Echaniz-Aviles G, Velazquez-Meza, Carnalla-Barajas, et al. Antimicrobial susceptibilities and capsular types of invasive *Streptococcus pneumoniae* isolated in children in Mexico City. *Microb. Drug. Res.* 1997; 3: 153-157.
 24. Di Fabio JL, Homma A, De Quadros C. Pan American Health Organization epidemiological surveillance network for *Streptococcus pneumoniae*. *Microb. Drug Res.* 1997; 3: 131-133.
 25. Brandileone MCD, Vieira VSD, Casagrande ST, et al. Prevalence of serotypes and antimicrobial resistance of *Streptococcus pneumoniae* strains isolated from Brazilian children with invasive infections. *Microb. Drug Res.* 1997; 3: 141-146.
 26. Hortal M, and the Pneumococcus Study Group. Capsular type distribution and susceptibility to antibiotics of *Streptococcus pneumoniae* clinical strains isolated from Uruguayan children with systemic infections. *Microb. Drug Res.* 1997; 3: 159-163.
 27. Sessegolo JF, Levin ASS, Levy CE, Asensi M, Facklam RR, Texeira LM. Distribution of serotypes and antimicrobial resistance of *Streptococcus pneumoniae* strains isolated in Brazil from 1988 to 1992. *Antimicrob. Agents Chemother.* 1994; 32: 906-911.
 28. Rossi A, Ruvinsky R, Ragueira M, Corso A, Pace J, Gentile A, Di Fabio JL, and the *Streptococcus pneumoniae* working group. *Microb. Drug Res.* 1997; 3: 135-140.
 29. Palavecino E, Appelbaum P, Jacobs MR. High prevalence of antibiotic-resistant pneumococci in Santiago, Chile. Abstract E-7, p. 168. Proceedings, 38th ICAAC. 1998.
 30. Baquero F, Martinez-Beltran J, Loza E. A review of antibiotic resistance patterns of *Streptococcus pneumoniae* in Europe. *J. Antimicrob. Chemother.* 1991; 28 (Suppl. C): 31-38.
 31. Baquero F. Trends in antibiotic resistance of respiratory pathogens: an analysis and commentary on a collaborative surveillance study. *J. Antimicrob. Chemother.* 1996; 38 (Suppl. A): 117-132.
 32. Liñares J, Pallares R, Alonso T, Perez JL, Ayats J, Gudiol F, Viladrich PF, Martin R. Trends in antimicrobial resistance of clinical isolates of *Streptococcus pneumoniae* in Bellvitge Hospital, Barcelona, Spain (1979-1990). *Clin. Infect. Dis.* 1992; 15: 99-105.
 33. Fenoll A, Jado I, Vicioso D, Pérez A, Casal J. Evolution of *Streptococcus pneumoniae* serotypes and antibiotic resistance in Spain: update (1990 to 1996).

- J. Clin. Microbiol. 1998; 36: 3447-3454. Felmingham D, et al. 1999. Unpublished.
34. Pato MVV, De Carvalho CB, Tomasz A, and the Multicenter Study Group. Antibiotic susceptibility of *Streptococcus pneumoniae* isolates in Portugal: a multicenter study between 1989 and 1993. *Microb. Drug Res.* 1997; 3: 59-69.
 35. Reinert RR, Lütticken R, Kaufhold A. Aktuelle Daten zur Antibiotikaempfindlichkeit von *Streptococcus pneumoniae* (Pneumokokken). Die Bedeutung von penicillinresistenten Isolat. *Mediz. Klin.* 1993; 88: 357-361.
 36. 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: 1398-1401.
 37. Wüst J, Huf E, Kayser FH. Antimicrobial susceptibilities and serotypes of invasive *Streptococcus pneumoniae* in Switzerland. *J. Clin. Microbiol.* 1995; 33: 3159-3163.
 38. Geslin P, Buu-Hoi A, Frémaux A, Acar JF. Antimicrobial resistance in *Streptococcus pneumoniae*: an epidemiological survey in France, 1970-1990. *Clin. Infect. Dis.* 1992; 15: 95-98.
 39. Geslin P, Frémaux A, Sissia G, Spicq C, Aberanne S. 1997. Centre National de Référence des pneumococques: Rapport d'activité année 1997.
 40. Enting RH, Spanjaard L, van de Beck D, Hensen EF, de Gans J, Dankert J. 1996. Antimicrobial susceptibility of *Haemophilus influenzae*, *Neisseria meningitidis* and *Streptococcus pneumoniae* isolates causing meningitis in the Netherlands, 1993-1994. *J. Antimicrob. Chemother.* 1996; 777-786.
 41. Hermans PWM, Sluijter M, Elzenaar K, et al. Penicillin-resistant *Streptococcus pneumoniae* in the Netherlands: results of a 1-year molecular epidemiologic survey. *J. Infect. Dis.* 1997; 175: 1413-1422.
 42. Verhaegen J, Glupczynski Y, Verbist L, Blogie M, Verbiest N, Vandeven J, Yourassowsky E. Capsular types and antibiotic susceptibility of pneumococci isolated from patients in Belgium with serious infections, 1980-1993. *Clin. Infect. Dis.* 1995; 20: 1339-1345.
 43. Appelbaum PC. 1999. Personal communication.
 44. Ronchetti MP, Merolla R, Bajaksouzian S, Violo G, Ronchetti R, Jacobs MR. Antimicrobial susceptibility of *Streptococcus pneumoniae* from children attending day-care centers in a central Italian city. *Clin. Microbiol. Infect.* 1998; 4: 622-626.
 45. Marchese A, Debbia E, Pesce A, Schito GC. Comparative activities of amoxicillin and 10 other oral drugs against penicillin-susceptible and -resistant *Streptococcus pneumoniae* strains recently isolated in Italy. *Clin. Microbiol. Infect.* 1998; 4: 170-173.

46. Syrogiannoopoulos G, Ronchetti F, Dagan R, Grivea I, Ronchetti MP, Porat N, Appelbaum PC, Jacobs MR. Mediterranean clone of multidrug-resistant serotype 6B *Streptococcus pneumoniae* in Greece, Italy and Israel. Abstract 566, p. 183. 36th Annual Meeting of the Infectious Disease Society of America. 1998.
47. Felmingham D, Robbins MJ, Tesfaslasie Y, Harding I, Shrimpton S, Grüneberg RN. Antimicrobial susceptibility of community-acquired lower respiratory tract pathogens isolated in the UK during the 1995-1996 cold season. *J. Antimicrob. Chemother.* 1998; 41: 411-415.
48. Hedlund J, Svenson S, Kalin M, Henrichsen J, Ollson-Liljequist B, Möllerberg G, Källenius G. Incidence, capsular types, and antibiotic susceptibility of invasive *Streptococcus pneumoniae* in Sweden. *Clin. Infect. Dis.* 1995; 21: 948-953.
49. Magnus T, Andersen BM. Serotypes and resistance patterns of *Streptococcus pneumoniae* causing systemic disease in Northern Norway. *Eur. J. Clin. Microbiol. Infect. Dis.* 1995; 14: 229-234.
50. Manninen R, Huovinen P, Nissinen A, and The Finnish Study Group For Antimicrobial Resistance. Increasing antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* in Finland. *J. Antimicrob. Chemother.* 1997; 40: 387-392.
51. Nielsen SV, Henrichsen J. 1993. Capsular serotypes and susceptibility to penicillin of pneumococci isolated from cerebrospinal fluid or blood in Denmark, 1983-1988. *Scand. J. Infect. Dis.* 1993; 25: 165-170.
52. Mittermayer H, Jebelean C, Binder L, Haditsch M, Watschinger R. 1996. Antibiotic susceptibility of pneumococci isolated in Austria over a four-year period. *Eur. J. Clin. Microbiol. Infect. Dis.* 1996; 15: 817-820.
53. Marton A, Gulyas M, Munoz R, Tomasz A. Extremely high incidence of antibiotic resistance in clinical isolates of *Streptococcus pneumoniae* in Hungary. *J. Infect. Dis.* 1991; 163: 542-548.
Marton A. Pneumococcal antimicrobial resistance: the problem in Hungary. *Clin. Infect. Dis.* 1992; 106-111.
54. Vereanu A, Mihalcu F, Ungureanu V, Buzatu S. Sensitivity to penicillin of *S.pneumoniae* strains isolated from various pathological conditions. *Roum. Arch. Microbiol. Immunol.* 1992; 51: 171-182.
55. Appelbaum PC, Gladkova C, Hryniewicz W, et al. Carriage of antibiotic-resistant *Streptococcus pneumoniae* by children in Eastern and Central Europe – a multicenter study with use of standardized methods. *Clin. Infect. Dis.* 1996; 23: 712-717.
56. Setchanova L. Clinical isolates and nasopharyngeal carriage of antibiotic-resistant *Streptococcus pneumoniae* in Hospital for Infectious Diseases, Sofia, Bulgaria, 1991-1993. *Microb. Drug Res.* 1995; 1: 79-84.

57. Trupl J, Hupkova H, Appelbaum PC, Jacobs MR. The incidence of penicillin-resistant pneumococci in the Slovak Republic. *Chemotherapy (Basel)* 1997; 43: 316-322.
58. Kanavaki S, Karabela S, Marinis E, Legakis NJ. Antibiotic resistance of clinical isolates of *Streptococcus pneumoniae* in Greece. *Antimicrob. Agents Chemother.* 1994; 32: 3056-3058.
59. Syrogiannopoulos G, Grivea IN, Beratis NG, Spiliopoulou E, Fasola EL, Bajaksouzian S, Appelbaum PC, Jacobs MR. Resistance patterns of *Streptococcus pneumoniae* from carriers attending day-care centers in Southwestern Greece. *Clin. Infect. Dis.* 1997; 25: 188-194.
60. 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: 158-163.
61. Dagan R, Yagupsky P, Goldbart A, Wasas A, Klugman K. Increasing prevalence of penicillin-resistant pneumococcal infections in children in southern Israel: implications for future immunization policies. *Pediatr. Infect. Dis. J.* 1994; 13: 782-786.
62. Raz R, Elhanan G, Shimoni Z, Kitzes R, Rudnicki C, Igra Y, Yinnon Y, and the Israeli Adult Pneumococcal Bacteremia Group. Pneumococcal bacteremia in hospitalized Israeli adults: epidemiology and resistance to penicillin. *Clin. Infect. Dis.* 1997; 24: 1164-1168.
63. Rotimi VO, Feteih J, Barbour PRH. Prevalence of penicillin-resistant *Streptococcus pneumoniae* in a Saudi Arabian hospital. *Eur. J. Clin. Microbiol. Infect. Dis.* 1995; 14: 149-151.
64. Sener B, Günalp A. Trends in antimicrobial resistance of *Streptococcus pneumoniae* in children in a Turkish hospital. *J. Antimicrob. Chemother.* 1998; 42: 381-384.
65. Uwaydah M, Jradeh M, Shibab Z. Antimicrobial resistance of clinical isolates of *Streptococcus pneumoniae* in Lebanon. *J. Antimicrob. Chemother.* 1996; 38: 283-286.
66. Ostroff SM, Harrison LH, Khallaf N, Assaad MT, Guirguis NI, Harrington S, El-Alamy M, and the Antimicrobial Resistance Surveillance Group. Resistance patterns of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolates recovered in Egypt from children with pneumonia. *Clin. Infect. Dis.* 1996; 23: 1069-1074.
67. Dagan R. 1999. Personal communication.
68. Klugman KP. Pneumococcal resistance to antibiotics. *Clin. Microbiol. Rev.* 1990; 3: 171-196.
 Koornhof HJ, Wasas A, Klugman K. Antimicrobial resistance in *Streptococcus pneumoniae*: a South African perspective. *Clin. Infect. Dis.* 1992; 15: 84-94.

69. Scott JAG, Hall AJ, Hannington A, Edwards R, Mwarumba S, Lowe B, Griffiths D, Crook D, Marsh K. 1998. Serotype distribution and prevalence of resistance to benzylpenicillin in three representative populations of *Streptococcus pneumoniae* from the coast of Kenya. *Clin. Infect. Dis.* 1998; 27: 1442-1450.
70. Paul J, Bates J, Kimari J, Gilks C. Serotypes and antibiotic susceptibilities of *Streptococcus pneumoniae* in Nairobi, Kenya. *J. Infection* 1996; 32: 139-142.
71. Joloba M, Bajaksouzian S, Fasola E, Whalen C, Jacobs MR. 1999. Unpublished.
72. Ramdani-Bouguessa N, Denine R, Rahal K. Antimicrobial resistance of *Streptococcus pneumoniae* mostly isolated from children in Algeria. Abstract E-15, p. 171. Proceedings, 38th ICAAC. 1998.
73. Ben-Rejeb S, Boukadida J, Hammami A, et al. Prospective epidemiologic study and antimicrobial susceptibility of 214 *Streptococcus pneumoniae* strains isolated in Tunisia. Abstract E-11, p. 170. Proceedings, 38th ICAAC. 1998.
74. Benbachir M, Ben Redjeb S, Boye CS-B, Dosso M. Two years surveillance of antibiotic resistance in *Streptococcus pneumoniae* in four African Countries. Abstract E-17, p. 172. Proceedings, 38th ICAAC. 1998.
75. Ubukata K, Asahi Y, Okuzumi K, Konno M, and the Working Group for penicillin-resistant *S.pneumoniae*. Incidence of penicillin-resistant *Streptococcus pneumoniae* in Japan, 1993-1995. *J. Infect. Chemother.* 1996; 1: 177-184.
76. Lee HJ, Park JY, Jang SH, Kim JH, Kim EC, Choi KW. High incidence of resistance to multiple antimicrobials in clinical isolates of *Streptococcus pneumoniae* from a university hospital in Korea. *Clin. Infect. Dis.* 1995; 20: 826-835.
77. Yoshida R, Kaku M, Kohno S, et al. 1995. Trends in antimicrobial resistance of *Streptococcus pneumoniae* in Japan. *Antimicrob. Agents Chemother.* 1995; 39: 1196-1198.
78. Gratten M, Torzillo P, Morey F, Dixon J, Erlich J, Hagger J, Henrichsen J. 1996. Distribution of capsular types and antibiotic susceptibility of invasive *Streptococcus pneumoniae* isolated from Aborigenes in Central Australia. *J. Clin. Microbiol.* 1996; 34: 338-341.
79. Pankuch GA, Jueneman SA, Davies TA, Jacobs MR, Appelbaum PC. In vitro selection of resistance to four β -lactams and azithromycin in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 1998; 42: 2914-2918.
80. Davies TA, Pankuch GA, Dewasse BE, Jacobs MR, Appelbaum. In vitro development of resistance to five quinolones and amoxicillin/clavulanate in *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 1999 (In press).