International Journal of Infectious Diseases 34 (2015) 38–40

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Short Communication

The association of serotype and pulsed-field gel electrophoresis genotype in isolates of *Streptococcus pneumoniae* isolated in Israel

M. Bar-Meir [a,b,*], G. Naaman [c], M. Assous [d], Z. Korenman [e], L. Valinsky [e], E. Picard [b,f]

[a] *Infectious Diseases, Shaare Zedek Medical Center, PO Box 3235, Jerusalem 91031, Israel*
[b] *Faculty of Medicine, Hebrew University, Jerusalem, Israel*
[c] *Pediatrics, Shaare Zedek Medical Center, Jerusalem, Israel*
[d] *Microbiology Laboratory, Shaare Zedek Medical Center, Jerusalem, Israel*
[e] *Central Laboratories, Ministry of Health, Jerusalem, Israel*
[f] *Pediatric Pulmonology, Shaare Zedek Medical Center, Jerusalem, Israel*

ARTICLE INFO

Article history:
Received 20 November 2014
Received in revised form 26 February 2015
Accepted 27 February 2015

Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
S.pneumoniae
Pulsed-field
Gel
Electrophoresis
Genotype
Serotype

SUMMARY

The relationship between *Streptococcus pneumoniae* isolates causing invasive infections in children admitted to a single center in central Israel was examined by pulsed-field gel electrophoresis (PFGE) and serotyping. Although there was a close correlation between serotype and PFGE clone, the genetic diversity varied by serotype, with some genotypes comprising multiple serotypes. Additionally, clones C and D were associated with higher penicillin minimum inhibitory concentrations. Serotyping alone may be insufficient for epidemiological mapping of pneumococcal isolates in the era of pneumococcal conjugate polysaccharide vaccines.

1. Introduction

A major concern in the era of pneumococcal conjugate polysaccharide vaccines (7-valent PCV7 and 13-valent PCV13) is the occurrence of 'serotype replacement', which is the increase in non-vaccine serotypes as causes of asymptomatic carriage and invasive disease.[1] Additionally, through the process of 'serotype switching' the bacteria can acquire a novel capsule type by the exchange of a sequence at the capsule polysaccharide synthesis (cps) locus.[2] Thus, clones associated with certain serotypes can persist in the post-vaccine era as mutants expressing non-vaccine type capsules. The phenomenon of serotype replacement has already been established for nasopharyngeal colonization after PCV7 introduction,[3] but whether replacement has occurred for invasive disease and after PCV13 introduction is unclear. The goal of the present study was to compare clonal distribution with serotype distribution of pneumococcal isolates causing invasive infections among children in the Jerusalem area in the pre-vaccine era. Characterization of the pneumococcal genetic background may provide the basis for surveillance of replacement disease in the post-vaccine era.

2. Methods

All children admitted to Shaare Zedek Medical Center with *Streptococcus pneumoniae* isolated from a normally sterile body fluid between the years 2004 and 2006 were identified. One isolate per patient was obtained for analysis. Antibiotic susceptibility testing was performed by agar dilution method on Mueller–Hinton (Oxoid, Basingstoke, UK) blood agar plates. Serotyping was performed by capsular reaction testing (Quellung test), using commercially available antisera (Statens Serum Institute, Denmark). Chromosomal DNA fragments for pulsed-field gel electrophoresis (PFGE) were generated by *Apa*I for serotypes 6 and 8 and by *Sma*I for all other serotypes, and were analyzed as described previously.[4]

PFGE genotype was defined as isolates showing >90% identity in the dendrogram created using Bionumerics ver. 7.1 (Applied Maths, Belgium) and the UPGMA/Dice coefficients, with a band position tolerance of 2%, in accordance with published interpretive criteria.[5]

* Corresponding author. Tel.: +972 2 6666340; fax: +972 2 6666840.
E-mail address: mbarmeir@gmail.com (M. Bar-Meir).

http://dx.doi.org/10.1016/j.ijid.2015.02.023

The statistical analysis was performed using SPSS software v. 17 (SPSS Inc., Chicago, IL, USA). Analysis of variance was used for comparisons between clones. Simpson's index of diversity was used to estimate the bacterial population diversity. This index is a measure of the probability that two isolates chosen randomly will belong to the same clone.[6]

3. Results

During the study period, 96 children had *S. pneumoniae* isolated from a normally sterile body fluid. One isolate per patient was obtained for analysis; seven did not grow from the frozen stocks. Of the remaining 89 isolates, all were blood isolates, except for two pleural fluid isolates. The mean age of the patients was 29 months (range 2 days to 13 years). Overall, 44 (49%) patients had pneumonia, 34 (38%) had bacteremia without a focus, seven (8%) had meningitis, and four patients had bacteremia with another focus of infection (three cellulitis, one peritonitis). One patient died of pneumococcal sepsis.

Eighty-two (92%) isolates belonged to PCV13. The non-PCV13 serotypes were 12F ($n = 4$) and one each of serotypes 8, 6C, and 15B. The 89 isolates were represented by 38 different PFGE genotypes, with 67% belonging to nine major genotypes (Table 1). All major genotypes comprised one serogroup each. Serotypes 18C, 19A, and 19F were represented by three to seven different clones. Twenty-nine genotypes contained only one isolate each of serotypes 1, 3, 4, 5, 6B, 6C, 8, 9 V, 14, 15B, 18B, 18C, 19A, 19F, and 23F. The genetic diversity of the isolates was 88% when serotype distribution was considered and 92% when the distribution of major PFGE genotypes was considered (p = not significant).

The penicillin minimum inhibitory concentration (MIC) for all isolates ranged from 0.002 mg/l to 1 mg/l. Isolates of PFGE groups C and D (corresponding with serotypes 14 and 23F) exhibited higher median penicillin MICs with a wider distribution compared to the other clones ($p = 0.0001$). Erythromycin resistance (MIC $\geq$1 mg/l) was frequent among isolates of PFGE types F and G, corresponding with serotypes 6A and 6B. PFGE groups A and C were associated with pneumonia (odds ratio (OR) 3, 95% confidence interval (CI) 0.8–12 and OR 1.5, 95% CI 0.3–7.8, respectively) and PFGE group B was associated with bacteremia (OR 3.1, 95% CI 0.9–11), but none of these associations were statistically significant.

Table 1
Correlation between major pneumococcal genotypes and serotypes

Major PFGE clone	Serotype											Total
	1	5	14	23F	12F	6A	6B	19F	19A	18C	Other	
A	17											17
B		16										16
C			9									9
D				3								3
E					4							4
F						4						4
G							2					2
H								2				2
I	3											3
Other[a]	1	1	3	3	0	0	2	2	7	3	7	29
Total	21	17	12	6	4	4	4	4	7	3	7	89

PFGE, pulsed-field gel electrophoresis.
[a] 'Other' represents 29 separate PFGE clones. For example, serotype 19A is represented by seven different PFGE genotypes and 18C by three different PFGE genotypes. Serotype 14 is represented by PFGE genotype C along with three isolates with different genotypes. For serotype 23F, three isolates belong to PFGE genotype D and three belong to three different genotypes.

4. Discussion

This study demonstrated a close correlation between serotype and PFGE genotype, although genetic diversity varied by serotype. For example, serotypes 6A and 12F were found to be mostly clonal, while serotypes 18C and 19A had high genetic diversity and were represented by multiple clones.

A similar phenomenon was found in a recent study from southern Israel,[7] where serotype 5 was represented by only one clone and serotype 15B/C by 12 minor clones. Since clonal distribution in that study differed between acute otitis media and invasive infections, it was suggested that, in addition to the capsule, the genetic background of the bacteria may also play a role in dictating virulence. Another study that investigated the persistence of fluoroquinolone-resistant *S. pneumoniae* in a long-term care facility, demonstrated how a capsular switch along with multiple antimicrobial resistance mutations occurred within a single clone.[8] Taken together, these data highlight the need to continue long-term genetic cloning for surveillance of replacement disease in the era of pneumococcal conjugate vaccines.

Additionally, we detected a correlation between PFGE clone and penicillin MIC, with clones C and D presenting higher penicillin MICs. This is consistent with studies from around the world, which have demonstrated differences in resistance profile among strains of the same genetic clone.[9,10] Horizontal transfer of penicillin resistance genes between pneumococcal clones or from viridans streptococci has been described, with some clones being more 'promiscuous', e.g., more likely to transfer multiple genes to other clones.[11]

Clones C and D corresponded with serotypes 14 and 23F, and both belong to the so-called 'pediatric serotypes', which tend to be more antibiotic-resistant.[12] We did not find isolates with dual non-susceptibility to penicillin and macrolides. This is in agreement with the most recent European Antimicrobial Resistance Surveillance System report, which noted dual non-susceptibility in Europe to be below 5%.[13]

In summary, the data presented here suggest that serotyping alone would be insufficient for surveillance in the era of the conjugated vaccines, and clonal distribution should be monitored to detect replacement disease.

Acknowledgement

We thank Shepard Schwartz for reviewing the manuscript.
Funding: None.
Conflict of interest: All authors have no conflict of interest.

References

1. Weinberger DM, Malley R, Lipsitch M. Serotype replacement in disease after pneumococcal vaccination. *Lancet* 2011;**378**:1962–73.
2. Croucher NJ, Harris SR, Fraser C, Quail MA, Burton J, van der Linden M, et al. Rapid pneumococcal evolution in response to clinical interventions. *Science* 2011;**331**:430–4.
3. Dagan R. Serotype replacement in perspective. *Vaccine* 2009;**27**(Suppl 3):C22–4.
4. Sandgren A, Sjostrom K, Olsson-Liljequist B, Christensson B, Samuelsson A, Kronvall G, Henriques Normark B, et al. Effect of clonal and serotype-specific properties on the invasive capacity of *Streptococcus pneumoniae*. *J Infect Dis* 2004;**189**:785–96.
5. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;**33**:2233–9.
6. Brueggemann AB, Griffiths DT, Meats E, Peto T, Crook DW, Spratt BG. Clonal relationships between invasive and carriage *Streptococcus pneumoniae* and serotype- and clone-specific differences in invasive disease potential. *J Infect Dis* 2003;**187**:1424–32.
7. Porat N, Benisty R, Trefler R, Givon-Lavi N, Dagan R. Clonal distribution of common pneumococcal serotypes not included in the 7-valent conjugate vaccine (PCV7): marked differences between two ethnic populations in southern Israel. *J Clin Microbiol* 2012;**50**:3472–7.

8. Ben-David D, Schwaber MJ, Adler A, Masarwa S, Edgar R, Navon-Venezia S, et al. Persistence and complex evolution of fluoroquinolone-resistant *Streptococcus pneumoniae* clone. *Emerg Infect Dis* 2014;**20**:799–805.
9. Pinar A, Köseoğlu O, Yenişehirli G, Sener B. Molecular epidemiology of penicillin-resistant *Streptococcus pneumoniae* in a university hospital, Ankara, Turkey. *Clin Microbiol Infect* 2004;**10**:718–23.
10. Dobay O, Rozgonyi F, Hajdú E, Nagy E, Knausz M, Amyes SG. The relationship between serotypes and PFGE genotypes in isolates of *Streptococcus pneumoniae* from Hungary. *Clin Microbiol Infect* 2005;**11**:673–6.
11. Wyres KL, Lambertsen LM, Croucher NJ, McGee L, von Gottberg A, Liñares J, et al. The multidrug-resistant PMEN1 pneumococcus is a paradigm for genetic success. *Genome Biol* 2012;**13**:R103.
12. Liñares J, Ardanuy C, Pallares R, Fenoll A. Changes in antimicrobial resistance, serotypes and genotypes in *Streptococcus pneumoniae* over a 30-year period. *Clin Microbiol Infect* 2010;**16**:402–10.
13. Antimicrobial resistance surveillance in Europe. Annual report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). *Surveillance Report* 2012.