

INTRODUCTION

Pneumococcal infections are a leading cause of death in the World, irrespective of country development. While children account for a significant proportion of pneumococcal associated deaths globally, in the developed countries most deaths occur among the elderly.

There are >90 different pneumococcal serotypes, but only a few cause the majority of invasive pneumococcal disease (IPD). The most common serotypes varies in time, place and age group, among other factors.

To prevent IPD there are currently three vaccines available – two polysaccharide conjugate vaccines, covering 10 and 13 serotypes (PCV10 and PCV13, respectively) and one polysaccharide vaccine, targeting 23 serotypes (PPV23). In Portugal, PCV13 has been the most widely used pneumococcal vaccine in children since its introduction, in early 2010 (>60% coverage in 2012). In 2015, PCV13 vaccine was included in the national immunization program (NIP) for children. For adults, PCV13 and PPV23 are available since 2012 and 1996, respectively, but with low usage.

OBJECTIVES

The aim of this study was to document changes in serotype distribution and antimicrobial susceptibility of isolates causing IPD in adults, after PCV13 received an adult indication and before the introduction of PCV13 in the NIP for children.

METHODS

A total of 1163 isolates were collected from adults with IPD – 404 in 2012, 383 in 2013 and 376 in 2014. Isolates were collected in a laboratory-based surveillance system that includes 31 microbiology laboratories throughout Portugal. Most isolates were recovered from blood (n=1066). The remaining isolates were collected from cerebral spinal fluid (n=59), pleural fluid (n=26), peritoneal fluid (n=9) and other normally sterile sites (n=3). Age distribution was as follows: 231 isolates were from patients 18-49 yrs, 267 from patients 50-64 yrs and 665 from patients ≥65 yrs.

All isolates were serotyped by Quellung reaction and tested for antimicrobial susceptibility using Etest strips or Kirby-Bauer disk diffusion technique, according to the CLSI recommendations and interpretative criteria [1].

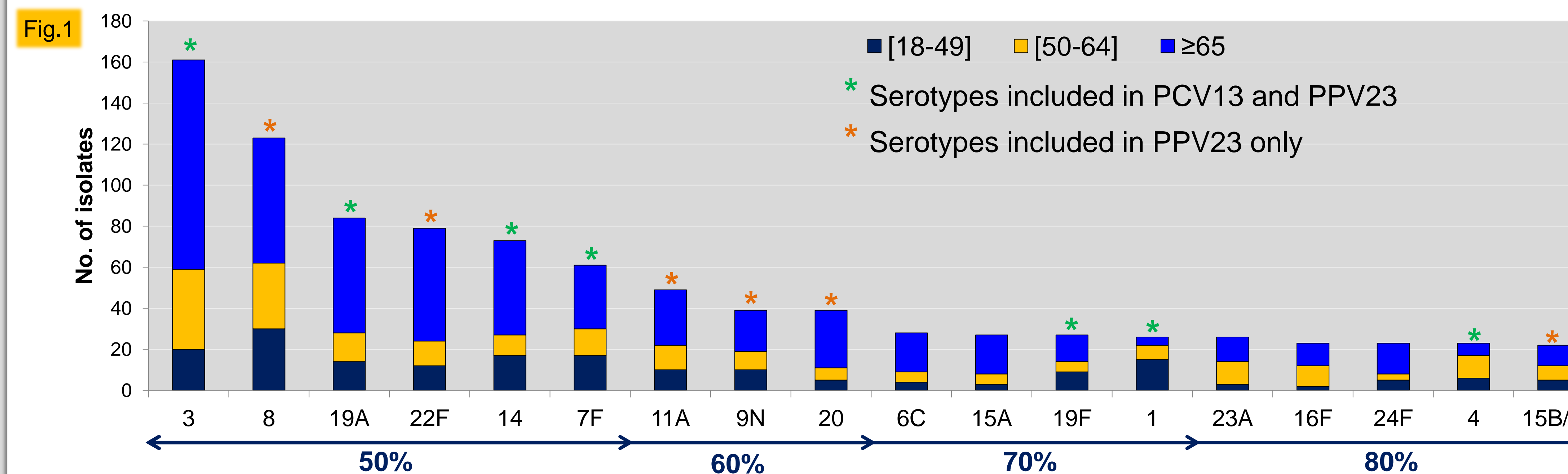
Simpson's index of diversity (SID) was used to measure population diversity (<http://www.comparingpartitions.info>). Differences were evaluated by Fisher exact test and the Cochran-Armitage test (CA) was used for trends. A p<0.05 was considered significant for all tests. Previously published data from 2008 to 2011 [2,3] were included for comparison.

REFERENCES

- [1] Clinical and Laboratory Standards Institute (2014). *Performance standards for 482 antimicrobial susceptibility testing - twenty-fourth informational supplement*. 483 Wayne, PA: Clinical and Laboratory Standards Institute.
- [2] Horácio, A. N., Diamantino-Miranda, J., Aguiar, S. I., Ramirez, M., Melo-Cristino, J., and the Portuguese Group for the Study of Streptococcal Infections (2012). Serotype changes in adult invasive pneumococcal infections in Portugal did not reduce the high fraction of potentially vaccine preventable infections. *Vaccine* 30, 218–224. doi:10.1016/j.vaccine.2011.11.022.
- [3] Horácio, A. N., Diamantino-Miranda, J., Aguiar, S. I., Ramirez, M., Melo-Cristino, J., and the Portuguese Group for the Study of Streptococcal Infections (2013). The majority of adult pneumococcal invasive infections in Portugal are still potentially vaccine preventable in spite of significant declines of serotypes 1 and 5. *PLoS ONE* 8, e73704. doi:10.1371/journal.pone.0073704.

RESULTS

Serotype 3 was the most frequent cause of adult IPD in Portugal during 2012-2014



- Serotype diversity was high in 2012-2014, with 54 different serotypes detected (SID=0.944, CI95%: 0.939-0.949);
- 50% of adult IPD was caused by only six serotypes and was potentially vaccine preventable;
- However, non-vaccine serotypes 6C, 15A, 23A, 16F and 24F were also common in adult IPD in Portugal;
- Serotype 1 was associated with younger patients (CA p<0.001), as found in previous studies [2, 3].

Fig. 1- Pneumococcal serotypes causing 80% of adult IPD in Portugal in 2012-2014. Serotypes are ordered by frequency and organized according to three age groups – 18-49 yrs, 50-64 yrs and ≥65 yrs. Serotypes targeted by PCV13 and PPV23 are marked with asterisks.

The proportion of adult IPD caused by PCV13 serotypes decreased in 2012-2014, due to declines in PCV13 serotypes 7F and 19A

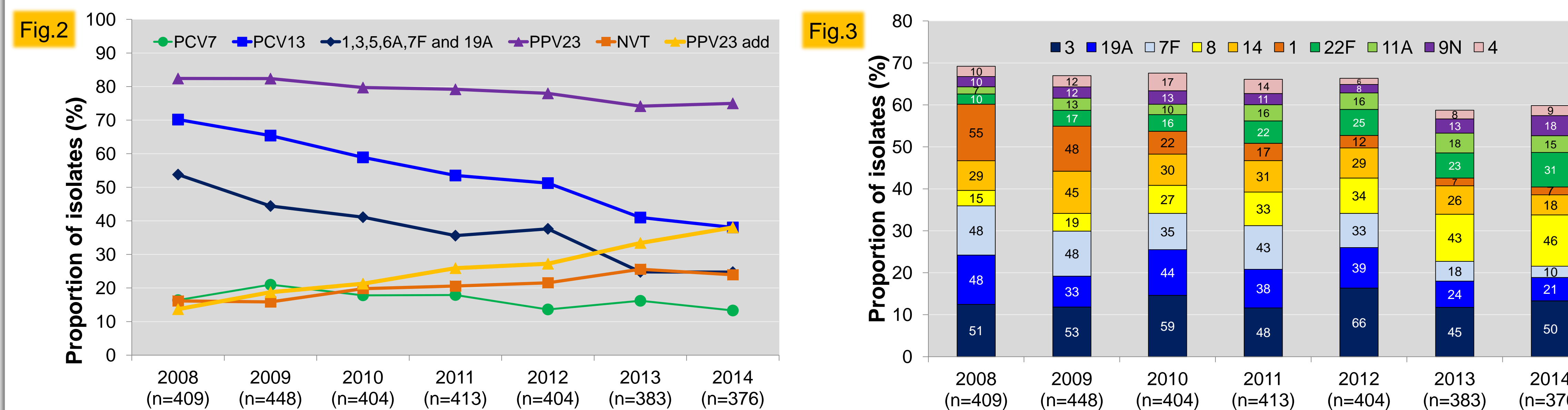


Fig. 2 – Proportion of isolates expressing serotypes included in pneumococcal vaccines causing adult IPD in Portugal, 2008-2014. PCV7 (serotypes 4, 6B, 9V, 14, 18C, 19F and 23F) was available for children in Portugal outside the NIP since 2001 and was replaced by PCV13 in 2010. “1, 3, 5, 6A, 7F and 19A” – additional serotypes of PCV13 relative to PCV7; “PPV23 add” – additional serotypes of PPV23 relative to PCV13. Fig. 3 – The 10 most frequent serotypes found causing adult IPD in Portugal from 2008 to 2014. Inside bars are absolute numbers.

- The proportion of IPD caused by PCV13 serotypes declined from 2012 to 2014 (p<0.001), continuing the trend initiated in 2009 (Fig. 2);
- In contrast, adult IPD caused by serotypes found only in PPV23 continued to increase (Fig. 2);
- Serotypes 7F and 19A presented the major decreases from 2012 to 2014 (significant after FDR for serotype 7F), while serotypes 8 and 22F are the most important emerging serotypes (Fig. 3);
- Serotype 3 has been the most frequent cause of adult IPD in Portugal, presenting only minor fluctuations (Fig. 3).

Antimicrobial resistance declined from 2012 to 2014

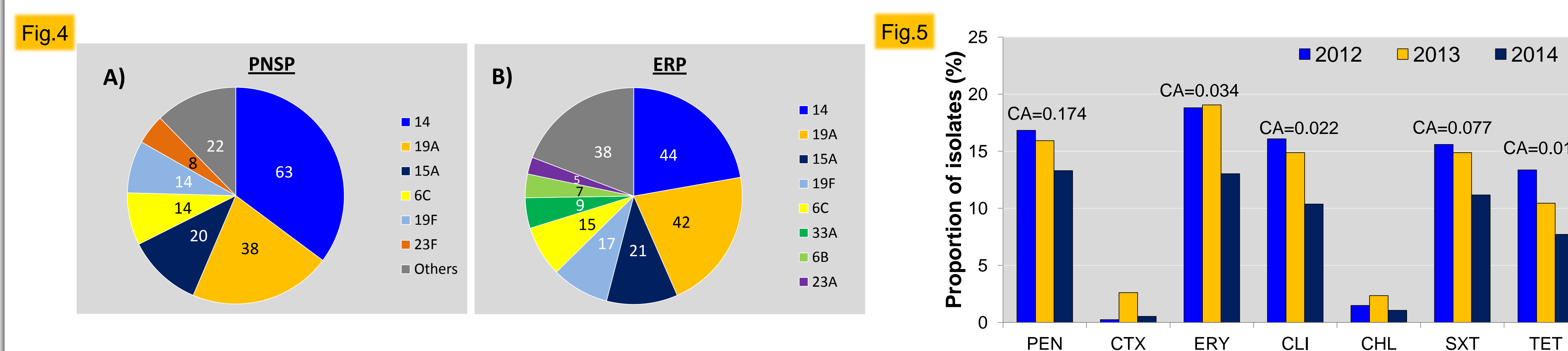


Fig. 4 – Serotypes of PNSP (A) and ERP (B) causing adult IPD, 2012-2014. Absolute numbers of non-susceptible isolates are shown. Fig. 5 – Evolution of antimicrobial resistance of the isolates causing adult IPD in Portugal, 2012-2014. “PEN”- penicillin, “CTX” – cefotaxime, “ERY” – erythromycin, “CLI” – clindamycin, “CHL” – chloramphenicol, “SXT” – trimethoprim sulfamethoxazole, “TET” – tetracycline.

- Serotypes 14 and 19A were the most frequent cause of penicillin and erythromycin resistance in 2012-2014 (Fig. 4);
- However, in 2014, serotype 15A became the most frequent serotype among erythromycin resistant pneumococci (ERP) and the second most frequent serotype among penicillin non-susceptible pneumococci (PNSP), behind serotype 14;
- Despite the increase in serotype 15A, the net result was a global decline in resistance for several antibiotics (Fig. 5);
- Of the serotypes expressed by PNSP and ERP in 2012-2014, 72% and 61%, respectively, were of serotypes included in PCV13.

CONCLUSIONS

- The proportion of adult IPD caused by PCV13 serotypes and by resistant isolates decreased in the post-PCV13 period, probably due to a herd effect from children vaccination;
- Further decreases are expected with the availability of PCV13 in the

- NIP for children, since 38% of adult IPD was still caused by PCV13 serotypes in 2014;
- In 2014, 75% of adult IPD was potentially vaccine preventable with PPV23, but serotypes 8 and 22F continue to increase in adult IPD.