Conjugate vaccines have reduced pneumococcal disease in vaccinated children and unvaccinated adults, but non-vaccine serotypes are of concern, particularly if antibiotic resistant. We reviewed *Streptococcus pneumoniae* collected via: (i) the British Society for Antimicrobial Chemotherapy (BSAC) surveillances from 2001–2014; (ii) Public Health England’s (PHE) invasive isolate surveillance from 2005–2014 and (iii) referral to PHE for resistance investigation from 2005–2014. Serotype 15A increased in all series, with many representatives showing triple resistance to macrolides, tetracyclines and penicillin. 15A was consistently among the 10 most prevalent serotypes from 2011 in PHE and BSAC invasive isolate/bacteraemia surveillance but never previously; 26–33% of these invasive 15A isolates had triple resistance. BSAC respiratory isolates were only serotyped in 2013/14 and 2014/15 (October to September); 15A was most prevalent serotype in both periods, comprising 9–11% of isolates, 38–48% of them with triple resistance. Serotype 15A represented 0–4% of *S. pneumoniae* referred to PHE for reference investigation annually until 2008 but rose to 29% (2013) and 32% (2014). Almost all multidrug-resistant 15A isolates were sequence type (ST) 63 variants, whereas susceptible 15A isolates were clonally diverse. The rise of serotype 15A suggests that pneumococcal conjugate vaccines will need ongoing adaptation.

**Introduction**

Seven-valent pneumococcal conjugate vaccine (Prevenar 7, PCV7) first became available internationally in 2000, and protects against invasive *Streptococcus pneumoniae* infection by serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. Numerous countries have reported that deployment reduced the incidence of invasive (i.e. blood and cerebrospinal fluid (CSF)) *S. pneumoniae* disease both in children, who are vaccinated, and in elderly adults, who benefit through reduced carriage and transmission of virulent serotypes by children [1-4]. Antibiotic resistance was historically concentrated in five PCV7-targeted serotypes (6B, 9V, 14, 19F and 23F) [5] and several countries have reported reductions in the prevalence of resistance as these were displaced [6]. United Kingdom (UK) experience conforms to these general patterns [7], with the caveat that penicillin-non-susceptible *S. pneumoniae* were uncommon before the vaccine’s introduction to the childhood schedule in 2006/07, meaning that little further fall occurred; macrolide resistance was reduced, reflecting displacement of a resistant serotype 14 lineage [8,9].

The success of PCV7 was partly offset by rises in other serotypes; notably 19A, where multidrug resistance to antibiotics became frequent [10,11]. This was countered by replacing PCV7 with a 13-valent conjugate vaccine (PCV13), additionally covering serotypes 1, 3, 5, 6A, 7F and 19A. PCV13 replaced PCV7 in the UK in April 2010 and this switch was followed by (i) reduced infant carriage of these additional serotypes [12], and (ii) a further 56% reduction in invasive disease incidence from a post-PCV7 baseline [13]. Again, however, rises are being seen in other, non-vaccine, serotypes, principally 8, 10A, 12F, 15A and 24F [13]. Serotype 15A is of particular interest since multidrug-resistant isolates belonging to this serotype have been reported as far apart as east Asia [14-16], North America [17,18], Norway [19], Italy [20] and Australia [21]. Here, we explore the rise...
| Rank | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | % for top 10\(^a\) |
|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-------------------|
| 2001 (n = 227) |     |     |     |     |     |     |     |     |     |     | 71.4%            |
|  14 |  8  |  9V | 23F |  3  |  4  |  6B | 12F | NA  | NA  |  1  |      | 69.5%            |
| 2002 (n = 220) |     |     |     |     |     |     |     |     |     |     | 72.0%            |
|  14 |  9V |  6B | 19F | 23F | NA  | NA  |  1  | 22F |  8  |  4  |  7F | 6A              |
| 2003 (n = 239) |     |     |     |     |     |     |     |     |     |     | 67.2%            |
|  14 |  9V |     |     |  1  |  4  |  8  | 23F |  3  | 19F |  6B | 18C             |
| 2004 (n = 241) |     |     |     |     |     |     |     |     |     |     | 67.5%            |
|  14 |  9V |  6A | 23F |  6A |  4  |  6B |  7F | NA  | NA  |  8  | 18C             |
| 2005 (n = 230) |     |     |     |     |     |     |     |     |     |     | 73.6%            |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2006 (n = 231) |     |     |     |     |     |     |     |     |     |     | 73.6%            |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2007 (n = 216) |     |     |     |     |     |     |     |     |     |     | 71.6%            |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2008 (n = 201) |     |     |     |     |     |     |     |     |     |     | 68.7%            |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2009 (n = 213) |     |     |     |     |     |     |     |     |     |     | 70.4%            |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2010 (n = 249) |     |     |     |     |     |     |     |     |     |     | 69.4%            |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2011 (n = 230) |     |     |     |     |     |     |     |     |     |     | 66.8%            |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2012 (n = 229) |     |     |     |     |     |     |     |     |     |     | 3.2%             |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2013 (n = 235) |     |     |     |     |     |     |     |     |     |     | 3.6%             |
|  14 |  9V |  8  |  7F | 23F |  3  |  4  |  6A | NA  | NA  |  8  | 18C             |
| 2014 (n = 247) |     |     |     |     |     |     |     |     |     |     | 3.2%             |

NA: not applicable.

Green: covered by PCV7; yellow: additional types covered by PCV13; pink: not covered by any conjugate vaccine.

\(^a\) When there is a tie for tenth rank, only one of the tied serotypes is counted into the percentage total for the top 10.
of serotype 15A *S. pneumoniae* in the UK and Ireland at epidemiological and molecular levels, using data from both the British Society for Antimicrobial Chemotherapy (BSAC) and Public Health England (PHE) surveillances.

**Methods**

**British Society for Antimicrobial Chemotherapy surveillance**

The BSAC Bacteraemia and Respiratory Surveillance Programmes have been described previously [22-24]. Both collect isolates from across the UK and Republic of Ireland. The Bacteraemia programme runs on the calendar year. Until 2009 we asked participating laboratories to send up to 10 consecutive bloodstream *S. pneumoniae* isolates per annum from each of 25 hospital laboratories; from 2010 we have similarly sought seven consecutive bloodstream isolates per annum from each of 40 hospital laboratories. Isolates have been serotyped throughout, and results were reviewed across the years 2001 to 2014, inclusive. The Respiratory Programme runs on an October–September year, designated e.g. 2013/14, so that isolates from each winter peak of respiratory disease are not split between calendar years. It examines consecutive isolates from lower respiratory tract infections (LRTIs) in non-hospitalised patients or those hospitalised for less than 48 hours. Until 2013/14 the BSAC Respiratory Surveillance Programme did not routinely serotype isolates, therefore only 2013/14 and 2014/15 data were reviewed. In both these years the surveillance sought 14 consecutive LRTI *S. pneumoniae* isolates from each of the same 40 laboratories contributing to the Bacteraemia surveillance. Actual numbers of isolates collected in both surveillances were somewhat below these targets (see Results) and, in most years, one or two recruited laboratories failed to collect, and were subsequently dropped and replaced by alternative sites. Hospital laboratory mergers, mostly in the past 5 years, have also meant that participating microbiology laboratories increasingly source isolates from multiple hospitals, augmenting representativeness.

**Public Health England reference laboratory submissions**

Besides surveillance isolates from invasive infections, PHE receives variable numbers of *S. pneumoniae* as reference submissions from respiratory and other non-sterile sites, principally eye and ear infections. Most are sent for investigation because the sender perceives them to have unusual resistance patterns, although senders’ definitions of unusual vary and may be contingent on the site of the infection. Over 95% of isolates are from laboratories in England, Wales and Northern Ireland, with the remaining ca 5% largely from Scotland and the Republic of Ireland. Data were reviewed across the period 2005 to 2014.

**Identification, serotyping and susceptibility testing**

All surveillance and referred isolates were confirmed as forming alpha-haemolytic colonies on horse blood agar and being inhibited by a 5 µg optochin (ethylhydrocortreine hydrochloride) disc (Oxoid-Thermofisher, Basingstoke, UK). Isolates with atypical colonial morphology, or which could not be serotyped (below), were confirmed as being lysed within 30 min by 2% sodium deoxycholate, and being catalase-negative when tested with 3% hydrogen peroxide. For serotyping, isolates were grown overnight in Todd Hewitt broth at 35°C with 5% CO2, harvested by centrifugation at 453 g for 30 min, then re-suspended in a small residual volume of broth and subjected to slide agglutination tests with standard antisera (Statens Serum Institut, Copenhagen, Denmark) [26]. Agar dilution susceptibility tests were performed in accordance with BSAC guidelines [27], using IsoSensitest agar (Oxoid-Thermofisher) supplemented with 5% defibrinated horse blood and incubated at 35–37°C in a 5% CO2 atmosphere. ‘Triple resistance’ was defined as resistant to erythromycin (minimum inhibitory concentration (MIC) > 0.5 mg/L) and tetracycline (MIC > 2 mg/L), and non-susceptible to penicillin (MIC > 0.06 mg/L), based on EUCAST breakpoints [28].

**DNA extraction, sequencing and bioinformatic analysis**

Isolates were grown on horse blood agar (PHE Media Services) and treated by the Qiagen-recommended method for lysis of Gram-negative bacteria (Qiagen, Manchester, UK), which is effective for *S. pneumoniae* and simpler than the Gram-positive protocol. DNA was extracted from the lysates using a QIAasympophy SP automated instrument (Qiagen) and a QIAasympophy DSP DNA Mini Kit, using a tissue extraction protocol. DNA concentrations were measured using the Quant-IT Broad Range DNA Kit (Life Technologies, Paisley, UK) and GloMax 96 Microplate Luminometer (Promega, Southampton, UK). After adjusting to a concentration of 10–30 ng/µL, DNA was sent for whole genome sequencing (WGS) by Illuma methodology. The resulting data were automatically analysed using a bespoke bioinformatic pipeline for *S. pneumoniae*, developed by PHE. Among other things, this (i) checks species
identification by a kmer method and (ii) automatically assigns MLST sequence types (STs), identified by mapping the reads against all *S. pneumoniae* allele variants held in the MLST database [29], using a modification of the short-read sequence typing (SRST) software [30]. Resistance genes affecting susceptibility for macrolides and tetracyclines were identified, and their sequences reviewed.

### Results

#### Serotype trends, British Society for Antimicrobial Chemotherapy bacteraemia surveillance

Prior to widespread UK deployment of PCV7 in the 2006/07 season, *S. pneumoniae* belonging to its target serotypes accounted for around half (44.4–53.6% in each of the years 2001 to 2006 inclusive) of all the *S. pneumoniae* collected in the BSAC bacteraemia surveillance but these declined to 4.7% of isolates by 2013 and 2.0% in 2014. Serotype 14 was the most common type in 6 of the 7 years from 2001 to 2007, comprising 13–20% of all isolates (Table 1) and accounting for 61% of all erythromycin-resistant isolates. By 2013, however, serotype 14 had only a single representative (0.4%), and none in 2014. Other serotypes became relatively more frequent as the PCV7 types declined, notably 7F and 19A, whereas serotype 1 had been expanding since 2001. These three types are within the spectrum of PCV13 and have declined, with variable rapidity, following its replacement of PCV7 in 2010. A further PCV13 type, serotypes 3, shows much less evidence of decline, as also noted elsewhere [13].

Serotype 15A isolates were encountered in each year from 2010 and the serotype was in the top 10 from 2011 onwards, whereas previously the type was sporadic. Other types that had long been encountered at moderate to low prevalence also became more prominent.

#### Table 2

Major serotypes and associations with resistance among *Streptococcus pneumoniae* from the British Society for Antimicrobial Chemotherapy Respiratory Surveillance, United Kingdom and Republic of Ireland, 2013/14 and 2014/15 (n=805)

| Serotype | October 2013 to September 2014 | October 2014 to September 2015 |
|----------|--------------------------------|--------------------------------|
|          | Count | % of total isolates | No (%) with triple resistance | Count | % of total isolates | No (%) with triple resistance |
| 15A      | 34    | 9.1 | 13 (38.2%) | 46 | 10.7 | 22 (47.8%) |
| 23B      | 26    | 6.9 | 1 (3.8%) | 21 | 4.9 | 0 |
| 3        | 22    | 5.9 | 0 | 26 | 6.0 | 0 |
| 11A      | 21    | 5.6 | 1 (4.8%) | 34 | 7.9 | 1 (2.9%) |
| 23A      | 21    | 5.6 | 0 | 30 | 7.0 | 4 (13.3%) |
| 22F      | 19    | 5.1 | 0 | 17 | 4.0 | 0 |
| 6C       | 18    | 4.8 | 0 | 12 | 2.8 | 0 |
| 19A      | 17    | 4.5 | 5 (29.4%) | 14 | 3.3 | 4 (28.6%) |
| 24F      | 16    | 4.3 | 0 | 12 | 2.8 | 1 (8.3%) |
| 35F      | 14    | 3.7 | 0 | 14 | 3.3 | 0 |
| 10A      | 14    | 3.7 | 0 | 12 | 2.8 | 0 |
| 31       | 14    | 3.7 | 0 | 16 | 3.7 | 0 |
| 16F      | 12    | 3.2 | 1 (8.3%) | 19 | 4.4 | 0 |
| 35B      | 11    | 2.9 | 0 | 3 | 0.7 | 0 |
| 17F      | 11    | 2.9 | 0 | 16 | 3.7 | 0 |
| 19F      | 11    | 2.9 | 3 (27.3%) | 14 | 3.3 | 5 (35.7%) |
| 33F      | 11    | 2.9 | 0 | 18 | 4.2 | 0 |
| 8        | 10    | 2.7 | 0 | 12 | 2.8 | 1 (8.3%) |
| Other serotypes, with ≥10 isolates in one or both years | 73 | 19.4 | 3 (4.9%) | 85 | (21.7) | 10 (2.3%) |
| PCV7 serotypes | 17 | 4.5 | NA | 20 | 4.6 | NA |
| PCV13 serotypes | 63 | 16.8 | NA | 67 | 15.6 | NA |
| Total | 375 | 100 | 27 (7.2%) | 430 | 100 | 49 (11.4%) |

NA: not applicable; PCV: pneumococcal conjugate vaccine.

*In 2013/14, three 6B isolates had triple resistance; the 10 ‘Other serotype’ isolates with triple resistance in 2014/15 comprised three nontypeable, two 12F and single representatives of 6B, 7F, 9N, 9V and 23.*
including serotypes 8, and (albeit with considerable year-on-year variation) 22F.

Triple resistance was seen in just 60/3,206 isolates (1.97%) throughout the period reviewed and its prevalence exceeded 10% only among isolates of serotypes 37 (2/3 isolates), 6B (13/90 isolates, 14.4%) and, most strikingly, 15A (13/50, 26.0%). Triple-resistant serotype 15A *S. pneumoniae* were received in every year from 2011, although never previously. This observation, along with increasing numbers of 15A isolates among PHE reference submissions (below), prompted the present analysis.

### Table 3
Predominant serotypes among *S. pneumoniae* serotyped by the Respiratory and Vaccine Preventable Bacteria Reference Unit, Public Health England from invasive infections, 2005–2014 (n = 45,645)

| Rank | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    |
|------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 2005 | 14    | 1     | 8     | 9V    | 4     | 23F   | 3     | 6B    | 7F    | 19F   |
| n = 4,662 | 701   | 528   | 357   | 333   | 327   | 271   | 250   | 248   | 208   | 195   |
| 2006 | 14    | 1     | 9V    | 8     | 23F   | 4     | 3     | 6B    | 7F    | 19F   |
| n = 4,857 | 660   | 611   | 337   | 321   | 300   | 288   | 268   | 256   | 249   | 210   |
| 2007 | 1     | 14    | 9V    | 8     | 23F   | 4     | 3     | 6A    | 23F   | 6B    |
| n = 4,673 | 583   | 449   | 351   | 348   | 316   | 278   | 238   | 237   | 231   | 197   |
| 2008 | 1     | 14    | 9V    | 8     | 23F   | 4     | 3     | 6A    | 23F   | 6B    |
| n = 4,978 | 592   | 474   | 372   | 359   | 328   | 307   | 239   | 238   | 206   | 189   |
| 2009 | 1     | 7F    | 8     | 3     | 22F   | 19A   | 6A    | 14    | 9V    | 23F   |
| n = 5,000 | 553   | 501   | 490   | 438   | 423   | 393   | 189   | 148   | 131   | 118   |
| 2010 | 7F    | 19A   | 8     | 1     | 3     | 22F   | 12F   | 33F   | 6C    | 11A   |
| n = 4,881 | 675   | 640   | 445   | 362   | 362   | 361   | 361   | 361   | 361   | 102   |
| 2011 | 7F    | 19A   | 8     | 1     | 3     | 22F   | 12F   | 33F   | 6C    | 11A   |
| n = 4,549 | 665   | 538   | 424   | 391   | 382   | 348   | 139   | 131   | 126   | 124   |
| 2012 | 8     | 19A   | 22F   | 19A   | 19A   | 19A   | 19A   | 22F   | 19A   | 19A   |
| n = 4,092 | 485   | 456   | 369   | 357   | 276   | 243   | 176   | 155   | 148   | 125   |
| 2013 | 8     | 7F    | 22F   | 19A   | 3     | 15A   | 12F   | 1     | 24F   | 33F   |
| n = 3,995 | 545   | 415   | 362   | 320   | 293   | 274   | 203   | 174   | 153   | 141   |
| 2014 | 8     | 12F   | 22F   | 19A   | 15A   | 15A   | 7F    | 9N    | 33F   | 24F   |
| n = 3,959 | 599   | 336   | 334   | 243   | 229   | 224   | 219   | 219   | 170   | 168   |

PCV: pneumococcal conjugate vaccine.
Green: covered by PCV7.
Yellow: additional types covered by PCV13.
Pink: not covered by any conjugate vaccine.
99% of isolates are from England, Wales and Northern Ireland, with the remaining few from Scotland, Crown Dependencies, Republic of Ireland and elsewhere.

Serotypes among British Society for Antimicrobial Chemotherapy respiratory isolates
Unlike those collected in the BSAC Bacteraemia Surveillance, *S. pneumoniae* from the BSAC Respiratory Surveillance were not routinely serotyped until 2013/14, when 15A proved to be the most frequent serotype (Table 2), comprising 34.9% of all 375 isolates collected, with a similar pattern in 2014/15, when 15A comprised 46/430 (10.7%) of isolates. What is more, 15A was one of only four serotypes (the others being 6B, 19A and 19F) where triple resistance was seen in over 10% of representatives. Overall, triple resistance was seen in 13/34 (38.2%) serotype 15A isolates vs
Serotypes that reached a top-10 ranking in any surveillance year in Republic of Ireland and elsewhere.

99% of isolates are from England, Wales and Northern Ireland, with the remaining few from Scotland, Crown Dependencies, Republic of Ireland and elsewhere. Serotypes that reached a top-10 ranking in any surveillance year in Table 3 are line-listed.

| Serotype | Total | Triple resistance | % Triple resistance |
|----------|-------|-------------------|---------------------|
| 15A      | 330   | 104               | 31.5                |
| 6B       | 420   | 51                | 12.1                |
| 19F      | 401   | 45                | 11.2                |
| 19A      | 987   | 83                | 8.4                 |
| 23F      | 360   | 15                | 4.2                 |
| 24F      | 124   | 5                 | 4.0                 |
| 9V       | 562   | 19                | 3.4                 |
| 14       | 1,145 | 27                | 2.4                 |
| 6A       | 366   | 3                 | 0.8                 |
| 8        | 1,197 | 3                 | 0.3                 |
| 6C       | 205   | 2                 | 1.0                 |
| 9N       | 261   | 1                 | 0.4                 |
| 3        | 777   | 2                 | 0.3                 |
| 33F      | 239   | 2                 | 0.8                 |
| 1        | 1,195 | 1                 | 0.1                 |
| 22F      | 761   | 1                 | 0.1                 |
| 12F      | 474   | 1                 | 0.2                 |
| 4        | 334   | 0                 | 0                   |
| 7F       | 1,155 | 0                 | 0                   |
| All others | 2,258 | 0                 | 0                   |
| All isolates and serotypes | 13,551 | 469 | 3.5 |

99% of isolates are from England, Wales and Northern Ireland, with the remaining few from Scotland, Crown Dependencies, Republic of Ireland and elsewhere. Serotypes that reached a top-10 ranking in any surveillance year in Table 3 are line-listed.

14/341 (4.1%) of all other isolates in 2013/14 (p<0.001, logistic regression adjusted for clustering by centre); there was an even sharper difference, 24/46 (52.2%) vs 25/384 (6.5%) (p<0.001, in 2014/15.

Also notable was the fact that PCV7 serotypes accounted for only 17/375 (4.5%) of all the respiratory S. pneumoniae in 2013/14 and PCV13 types for just 63/375 (16.8%); corresponding figures in 2014/15 were 18/430 (4.3%) for PCV7 types and 68/430 (15.8%) for PCV13 types. The sole previous season when S. pneumoniae from the Respiratory Programme were typed was 2005/06, immediately before UK introduction of PCV7 [24]. Then, among 749 isolates, 312 (41.7%) belonged to PCV7 types and 450 (60.1%) to PCV13 types (assuming all serogroup 7 isolates belonged to serotype 7F) whereas 36 (4.8%) belonged to serogroup 15, which was not split to its component (15A/B/C/F) serotypes. The declines in PCV7 types, PCV13 types, and the rise in serotype 15A (compared with all serotype 15 in 2005/06) were all highly significant (p<0.001, logistic regression adjusted for clustering by centre).

As in the BSAC series, serotype 15A first appeared in the top 10 in 2011. It then advanced to seventh rank by 2012 and sixth rank in both 2013 and 2014, accounting for 5.7% of isolates (224/3,959) in the latter year. Again, the proportion of resistance was striking: among the 330 tested, fully 104 (31.5%) of bloodstream 15A S. pneumoniae for all years pooled had triple resistance, whereas triple resistance rates for all other isolates that ever featured in the top 10 were under 12.5% (Table 4). Proportions of serotype 15A isolates, taking 2005–2014 pooled, rose with the patient’s age, from 1.3% in the 0–5 year age group to 1.4% in the 6–35 year age group, 0.6% in the 36–45 year age group, 1.7% in the 46–55, 56–65 and 66–75 year age groups, reaching 2.4% in the 76–85 year age group and 3.1% among the over-85 year-olds (p<0.001). Triple resistance was represented among serotype 15A S. pneumoniae throughout the surveillance period reviewed, with proportions as follows: 2005, 0/3 isolates with triple resistance; 2006, 1/4; 2007, 2/10; 2008, 4/13; 2009 7/13; 2010, 18/34; 2011, 10/33; 2012, 15/50; 2013, 19/63 and 2014, 33/114.

The isolates tested for antibiotic susceptibility and resistance (n=13,551, annual range 1,159–2,966 p.a.) are a subset of those in Table 3 and comprise all isolates from hospitals that participate in the EARS-net surveillance along with those bloodstream isolates where the referring laboratory specifically sought susceptibility testing. Inclusion of the latter group may over-represent resistant organisms, although there is no reason why it should do so disproportionately within particular serotypes.

Serotype trends, isolates referred to Public Health England for investigation of resistance

Between 2005 and 2014, 1,536 S. pneumoniae from respiratory, ear and eye infections were referred to PHE (Table 5) for investigation of unusual resistance. These submissions constitute a heavily biased sample and lack a denominator, but do provide a rolling snapshot of S. pneumoniae isolates that sending laboratories
Table 5
Predominant serotypes among respiratory, ear and eye isolates of Streptococcus pneumoniae received by the Public Health England Colindale reference service, 2005–2014 (n=1,536)

|                     | Number of Isolates of indicated serotype in year: | Grand total | No with triple resistance | % with triple resistance |
|---------------------|--------------------------------------------------|-------------|----------------------------|--------------------------|
|                     | 2005  | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 | 2005  | 2006 | 2007 | 2008 | 2009 | 2010 | 2011 | 2012 | 2013 | 2014 |            |            |            |
| Serotype 19F        | 15    | 31   | 41   | 50   | 55   | 35   | 23   | 14   | 12   | 14   | 290   | 232  | 80.0 |
| Serotype 19A        | 2     | 10   | 15   | 27   | 45   | 44   | 28   | 17   | 8    | 17   | 213   | 169  | 79.3 |
| Serotype 15A        | 0     | 4    | 3    | 8    | 23   | 22   | 17   | 26   | 31   | 31   | 165   | 137  | 83.0 |
| Serotype 6B         | 5     | 18   | 39   | 35   | 25   | 13   | 4    | 7    | 3    | 2    | 151   | 104  | 68.9 |
| Non-typeable rough  | 3     | 8    | 16   | 23   | 33   | 18   | 10   | 15   | 5    | 2    | 133   | 81   | 60.9 |
| Serotype 9V         | 7     | 36   | 25   | 15   | 18   | 10   | 0    | 0    | 1    | 2    | 114   | 16   | 14.0 |
| Serotype 14         | 9     | 18   | 21   | 6    | 11   | 11   | 4    | 2    | 3    | 1    | 86    | 30   | 34.9 |
| Serotype 23F        | 7     | 9    | 6    | 16   | 16   | 9    | 3    | 2    | 1    | 3    | 72    | 35   | 48.6 |
| Serotype 35B        | 1     | 3    | 4    | 9    | 7    | 6    | 9    | 4    | 7    | 4    | 54    | 9    | 16.7 |
| No serotype data    | 1     | 2    | 0    | 0    | 0    | 6    | 1    | 1    | 5    | 26   | 42    | 22   | 52.4 |
| Serotype 6A         | 0     | 3    | 5    | 5    | 3    | 6    | 1    | 2    | 0    | 1    | 26    | 7    | 26.9 |
| Serotype 11A        | 0     | 0    | 1    | 4    | 3    | 3    | 5    | 2    | 4    | 23   | 23    | 12   | 52.2 |
| Serotype 3          | 0     | 1    | 3    | 0    | 3    | 4    | 1    | 2    | 4    | 21   | 21    | 3    | 14.3 |
| Serotype 1          | 0     | 0    | 1    | 2    | 3    | 5    | 1    | 1    | 1    | 0    | 14    | 1    | 7.1  |
| Serotype 13         | 0     | 2    | 0    | 1    | 6    | 1    | 0    | 0    | 1    | 11   | 11    | 0    | 0.0  |
| All other types a   | 8     | 5    | 5    | 9    | 16   | 17   | 15   | 10   | 13   | 23   | 121   | 38   | 31.4 |
| Total               | 58    | 150  | 185  | 207  | 268  | 210  | 120  | 108  | 97   | 133  | 1,536 | 896  | 58.3 |
| 15A as % typed      | 0.0   | 2.7  | 1.6  | 3.9  | 8.6  | 10.8 | 14.3 | 24.3 | 33.7 | 29.0 | 11.0  |     |     |

95% of isolates were from England, Wales and Northern Ireland with the remainder from Scotland, Crown Dependencies, Republic of Ireland or elsewhere.

a Not accounting for > 10 isolates in total over the surveillance period.
b Excludes ‘no data row’ above from denominator.

(mostly in England and Wales) consider to be concerning. Overall, 896/1,536 (58.3%) had triple resistance. In the earlier years members of serotypes 19F, 9V, 6B and 14 dominated, collectively accounting for 82.8–92.4% of referrals from 2005 to 2007, before declining from the start of the ‘PCV7 era’. Serotype 19A accounted for a growing proportion of referrals from 2005, peaking at 23.3% in 2011, while serotype 15A represented just 0–4% of submissions throughout the period 2005 to 2008 but thereafter increased progressively, becoming the most commonly referred serotype in 2012. In 2013, it accounted for 31/92 of all submissions where typing was undertaken, and for 31/107 in 2014. These proportions were greater than ever previously achieved by any other serotype. Fully 83.0% of serotype 15A isolates (137/165) had the triple resistance vs 68.9–80.0% among serotype 9V, 19A and 19F referrals, with lower proportions for other serotypes (Table 4).

Genomic sequencing and phenotypes of serotype 15A isolates
Genomic sequencing was performed on 156 serotype 15A S. pneumoniae. These represented a diversity of resistance patterns, and including 50 with triple resistance; a limitation was that all 156 sequenced isolates dated from 2013 and 2014. MLST types were deduced from the sequence data, and 78 (50%) of the isolates were identified as belonging to ST63 (n=61) or its single or double locus variants (n=17). All of these 78 ST63-related isolates were resistant to erythromycin (also clindamycin, not shown) and 49 (62.8%) had the triple resistance profile (Table 6). The macrolide and clindamycin resistance correlated with the consistent presence of erm(B) genes, as detected by WGS. All 78 ST63-related isolates were found also to carry the tetracycline-resistance determinant, tet(M); those (n=65, 83.3%) that expressed tetracycline resistance had the intact gene, whereas those (n=13, 16.7%) that were tetracycline-susceptible (all of them classical ST63 isolates) had a deletion of two nucleotides at codon 339, generating a premature stop codon and thereby inactivating the gene. Most of the 49 isolates with triple resistance were susceptible to alternative agents: 37 remained susceptible to ampicillin, 47 to moxifloxacin, 48 to ceftaxime and all 49 to vancomycin, all based on EUCAST breakpoints. Sequence types (STs) 3811 (n=19), 58 and its single locus variants (SLVs) (n=21), and 73 and its SLVs (n=11) were all heavily represented among all these, just one isolate had triple resistance and three or fewer were non-susceptible to any one of erythromycin, tetracycline or penicillin.

WGS data were available for a further 141 non-15A S. pneumoniae, predominantly investigated owing to multidrug resistance. Six had ST63-related profiles and
these all had triple resistance; three expressed serotype 19F, one serotype 21 and one 23F; the final isolate was typed using antisera as serotype 20 but was predicted to be serotype 11A based on WGS; review suggests that the original serotype determination was in error. The association with 19F (a PCV7 serotype) is notable (see Discussion), but members of this serotype were highly variable in terms of ST; among a total of 25 serotype 19F isolates sequenced, 22 with triple resistance, we recorded 12 different known STs, along with two new variants. No single ST had more than four representatives.

**Discussion**

Deployment of PCVs has had clear public health benefits. The incidence of invasive pneumococcal disease has been reduced not only in vaccinated children, but also in elderly adults, who benefit from herd immunity [31]. There is also evidence of impact on non-invasive disease: thus, PCV7 deployment in the UK in 2006 also was followed by a 19% reduction in hospital admissions for community-acquired pneumonia (CAP) among children aged <2 years, reversing a rising trend that had persisted during the preceding decade [32]. A similar reduction was reported in Italy [33]. Moreover, a Cochrane review concluded that PCV7 reduced the incidence of acute otitis media in healthy vaccinated children, although with less impact for those with a history of the illness or deemed to be ‘high risk’ [34]. Lastly, active PCV13 vaccination was recently shown to achieve a 50% reduction in the incidence of bacteraemia and non-invasive pneumonia in elderly adults, again reflecting displacement of vaccine serotypes [35].

A limitation to this pattern of successes is, however, that the PCV vaccines cover only the most prevalent pneumococcal serotypes, leaving scope for expansion of other types. Deployment of PCV7 was followed by increased prevalence of serotype 19A isolates, many of them multidrug-resistant, and, although serotype 19A is now covered by PCV13, a niche may be created for yet further types. Internationally, several groups have remarked on the increased prevalence of multidrug-resistant serotype 15A and 35B isolates [14-21] and a recent PHE analysis of invasive pneumococcal infections, using the data series of Table 3, noted 15A to be among several serotypes now increasing in numbers and proportion in the UK [13]. The present analysis extends these findings, confirming that serotype 15A *S. pneumoniae* are of growing importance, as also shown (i) in the BSAC bacteraemia series (Table 1), which overlaps the PHE series but also includes Scotland and Ireland, (ii) the BSAC series LRTI (Table 2), which is the sole UK surveillance to test *S. pneumoniae* from their predominant disease setting, and (iii) among PHE reference submissions, which provide a rolling snapshot of resistance phenotypes causing concern to microbiologists at sending laboratories, which are predominantly in England, Wales and Northern Ireland, although with a few isolates received from elsewhere (Table 5). By 2013 and 2014, serotype 15A was consistently (i) among the top 10 serotypes in both the PHE and BSAC surveillances of invasive *S. pneumoniae* (Tables 1 and 3), (ii) was the top serotype among respiratory isolates (Table 2) and (iii) accounted for almost one third of all the *S. pneumoniae* sent for reference investigation as ‘unusually’ resistant. Critically, and unlike other rising pneumococcal serotypes (8, 10A, 11A, 12F, and 24F – see Tables 1, 3 and ref [13]) serotype 15A isolates were commonly resistant or non-susceptible to multiple antibiotics, including macrolides, clindamycin, tetracycline and penicillin. While none of the surveillances captures clinical outcomes, the fact that serotype 15A is rising in invasive infections implies that these organisms are virulent.

Around one third of serotype 15A isolates had ‘triple resistance’ (i.e. to macrolides and tetracycline together with intermediate penicillin resistance), a higher proportion than for other serotypes (Table 4). This proportion did not change substantially over time (although assessment is complicated by small total numbers of isolates in the earlier years), indicating that the serotype was gaining prominence both generally and as a resistant type, again implying that the surface polysaccharides of serotype 15A support virulence.

Triple resistance among serotype 15A isolates was strongly associated (p < 0.0001, Fisher’s exact of chi-squared tests) with ST63 and its variants and extremely rare among serotype 15A isolates belonging to other

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**Table 6**

Sequence types in relation to resistance of serotype 15A *Streptococcus pneumoniae* subjected to genomic sequencing (n = 156)

| Number (%) non-susceptible (intermediate or resistant) | Erythromycin | Tetracycline | Penicillin | Triple resistance |
|--------------------------------------------------------|--------------|--------------|------------|-------------------|
| ST63                                                   | 61           | 61 (100%)    |            | 35 (57.4%)        |
| ST63 SLV and DLV                                        | 17           | 17 (100%)    | 17 (100%)  | 14 (82.4%)        |
| Other 15A×                                              | 78           | 2 (2.6%)     | 3 (3.8%)   | 1 (1.3%)          |

SLV: single locus variant; ST: sequence type.

× Includes 21 ST58 and SLVs, 19 ST3811, 11 ST73 and SLVs and 27 isolates belonging to sequence types with four representatives or fewer.
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Conflict of interest

DML has shares in Pfizer and GSK, who make pneumococcal conjugate vaccines, and occasionally lectures and does contract and consultancy work for both companies. Other authors declare no conflict of interest.

Authors’ contributions

CS, MK: molecular characterisation of isolates; NF/TH: Public Health England reference surveillance and typing of S. pneumoniae, on which this analysis is predicated; RR/SM/RJ: British Society for Antimicrobial Chemotherapy’s Resistance Surveillance Standing Committee and to the members of the British Society for Antimicrobial Chemotherapy; DP: Public Health England reference investigation of resistant S. pneumoniae, on which this analysis is predicated; RP, RH, NW: reference investigation of resistant S. pneumoniae on which analysis is predicated; PS: extraction and consolidation of data series; MD: Bioinformatic analysis of sequence data; DML: primary observation of rise of 15A S. pneumoniae, wrote manuscript. All authors commented upon and contributed to improving the manuscript.

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