INTRODUCTION

Streptococcus pneumoniae (S. pneumoniae) is one of the most common bacterial pathogens in community-acquired pneumonia. However, nosocomial pneumococcal infections are more and more widely observed.

OBJECTIVES

The aim of the study was to analyze nosocomial outbreak in one of the Polish hospitals and to characterize the causative isolates.

PATIENTS AND METHODS

Within 10 days, in 6 patients receiving an antimicrobial therapy due to a primary disease, pneumococcal infections of the lower respiratory tract were identified. All patients, except 1 patient with asthma, were hospitalized due to exacerbation of chronic obstructive pulmonary disease (COPD). The isolates were identified by standard methods. The serotypes of S. pneumoniae were determined by the Pneumotest-Latex kit. The relatedness among isolates was evaluated by restriction fragment length polymorphism followed by pulsed-field gel electrophoresis (RFLP-PFGE). Multilocus sequence typing (MLST) was performed for a representative isolate.

RESULTS

The outbreak was suspected when characteristic multidrug-resistant pneumococci were isolated from patients of a single ward. The outbreak was confirmed by molecular typing techniques. All isolates belonged to serotype 14 and shared the RFLP-PFGE pattern. MLST for a representative isolate revealed that it was a member of the epidemic Spain9V-ST156 clonal complex.

CONCLUSIONS

Timely implementation of infection control measures enabled to eradicate the outbreak. Pneumococcal conjugate vaccine, recently registered for use in adult populations, may have a considerable effect on limiting pneumococcal disease-associated morbidity and mortality. It is especially important for patients with COPD, a disease entity that constitutes a risk factor for the acquisition of multidrug-resistant pneumococci.
PATIENTS AND METHODS Pneumococcal isolates were cultured from sputum (evaluated by the number of white blood cells and epithelial cells, together with the presence of mucus threads) or bronchoalveolar lavage (BALF). All isolates were identified based on typical morphology, Gram stain, susceptibility to optochin (bioMérieux, France), and bile solubility. Sero‐types of S. pneumoniae were determined by latex agglutination using the Pneumotest‐Latex kit (Statens Serum Institut, Denmark). Minimal inhibitory concentrations (MICs) for penicillin, amoxicillin, cefotaxime, erythromycin, azithromycin, clindamycin, ciprofloxacin, levofloxacin, moxifloxacin, tetracycline, rifampicin, chloramphenicol, linezolid, and vancomycin were determined by Etest (AB Biodisk, Sweden) or M.I.C. Evaluators (Oxoid, United Kingdom) according to the manufacturers’ instructions. Susceptibility to telithromycin was evaluated by the disc diffusion method (15 µg, Becton Dickinson, United States). The results were interpreted according to the Clinical and Laboratory Standards Institute guidelines, and isolates with MIC 4 mg/l of ciprofloxacin were considered nonsusceptible.13,14 The quality control strain was S. pneumoniae ATCC 49 619.

The relatedness among isolates was evaluated by pulsed‐field gel electrophoresis (PFGE) of Smal‐digested chromosomal DNA, as previously described.15 A representative of the isolates was characterized by multilocus sequence typing (MLST), multiple loci variable number of tandem repeats analysis (MLVA), and sequencing of the quinolone‐determining regions (QRDRs) in the gyrA, gyrB, parC, and parE genes.16–18

Outbreak description
The outbreak occurred at the Department of Pulmonary Diseases and Respiratory Failure in the Regional Centre of Pulmonology in Bydgoszcz, in a 28‐bed respiratory ward, where from 61% to 100% of beds were occupied at the time of the outbreak. From 2 to 11 January 2009, 6 patients hospitalized due to chronic pulmonary diseases and located in 2 rooms developed new symptoms of acute lower respiratory tract infection (LRTI) after 6 to 25 days of hospitalization (TABLE). Three cases involved male patients sharing the same 3‐bed room, followed by 3 cases in a female 3‐bed room of the same ward. A nosocomial transmission was first suspected when a characteristic multiresistant S. pneumoniae was isolated from the second patient (case 2) located in the same room as the previous patient (index case) 4 days later. In 4 patients, pneumococcal meningitis was recognized based on the X‐ray pictures, 1 patient had an acute exacerbation of chronic obstructive pulmonary disease (COPD), and 1 patient with asthma had LRTI. Patients’ age ranged from 56 to 73 years and all of them had multiple comorbidities such as COPD, diabetes, hypertension, hypothyroidism, obesity, hyperlipidemia, or cardiomyopathy. Patients received extensive antimicrobial treatment before and during the outbreak; however, in most cases, the prior treatment did not cover the susceptibility profile of the infecting S. pneumoniae (TABLE).

Prior to the outbreak, 5 patients were already hospitalized due to acute exacerbation of COPD (III–IV Global Initiative for Chronic Obstructive Lung Disease in respiratory failure) and, in 1 case, due to asthma exacerbation; 4 of these patients were hospitalized at least for 8 days. One male patient (case 2) was previously hospitalized for 25 days and then discharged. However, in the evening of the same day, he returned to the hospital with progressive difficulty in breathing, weakness, and a temperature above 39°C. A similar situation occurred with 1 female patient (case 6), who was readmitted to the same hospital with new symptoms 2 days after discharge. Finally, she died from respiratory failure, 38 days after the appearance of symptoms connected with pneumococcal infection due to a serious primary condition.

Control of the outbreak On January 12, the following infection control measures were implemented to limit the spread of the outbreak: isolation of infected patients, reinforced sanitary regimen (disposable masks and coats for patients and the personnel, use of bactericidal lamps, instruction of patients concerning hand washing and disinfection), limitations for visitors to the ward and enhanced supervision of the compliance with the above recommendations by the hospital infection control team. Beyond that date, the outbreak was eradicated since no further nosocomial transmission of pneumococci was observed. Mutation in gyrA and the S82F mutation in parC (the R6 numbering). Additionally, parC and parE harbored the K140N and the I460V polymorphisms, respectively; no changes were observed in the QRDR of gyrB.

DISCUSSION Infections caused by S. pneumoniae are most prevalent in the extreme age groups and account for more deaths than any other vaccine‐preventable bacterial disease.1,2 Although pneumococcal infections are mostly sporadic community‐acquired cases, they may occur as outbreaks, mainly in institutional settings.4,4 The short‐term outbreak described here was unusual because it encompassed patients already receiving antimicrobial therapy due to the primary disease. However, it did not prevent the outbreak because in most cases it did not cover multidrug‐resistant S. pneumoniae responsible for the infection in all patients. The outbreak was noted due to the vigilance of doctors and microbiologists when new symptoms appeared in the second and further patients of a single ward along with the isolation of characteristic multidrug‐resistant pneumococci.

The outbreak was confirmed by laboratory analyses. Five pneumococcal isolates available for further testing shared the genotype and serotype, and together with the isolate from the index case displayed a very similar drug‐resistance profile, thus strongly indicating their nosocomial
**TABLE**  Clinical details of outbreak patients

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age, y</th>
<th>Sex</th>
<th>Reason for hospitalization</th>
<th>Date of new symptoms (^a)</th>
<th>Date of new symptoms (^b)</th>
<th>Second diagnosis associated with new symptoms</th>
<th>Dates of hospitalization</th>
<th>Antibacterial treatment (iv) (^e)</th>
<th>Comorbidities</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>70</td>
<td>male</td>
<td>AECOPD</td>
<td>2 Jan 2009</td>
<td>23 Dec 2008 – 16 Jan 2009</td>
<td>pneumonia</td>
<td>AMC: 3 × 1.2 g (23.12.08–04.01.09)</td>
<td>CRO: 1 × 2.0 g (05.01.09–15.01.09)</td>
<td>type 2 diabetes, heart failure secondary to hypertensive cardiomyopathy</td>
</tr>
<tr>
<td>3</td>
<td>64</td>
<td>female</td>
<td>asthma</td>
<td>6 Jan 2009</td>
<td>29 Dec 2008 – 19 Jan 2009</td>
<td>LRTI</td>
<td>CLR: 2 × 0.5 g per os (29.12.08)</td>
<td>DOX: 2 × 0.1 g per os (30.12.08–06.01.09)</td>
<td>type 2 diabetes, hypothyroidism, depressive disorders, primary biliary cirrhosis, status post cholecystectomy</td>
</tr>
<tr>
<td>4</td>
<td>73</td>
<td>male</td>
<td>AECOPD</td>
<td>7 Jan 2009</td>
<td>24 Dec 2008 – 21 Jan 2011</td>
<td>pneumonia</td>
<td>AMC: 3 × 1.2 g (24.12.08–28.12.08)</td>
<td>CAZ: 2 × 2.0 g (29.12.08–08.01.09)</td>
<td>type 2 diabetes, mixed hyperlipidemia, coronary artery disease with angina, status post PCI with stent placement to left main coronary artery, ischemic and hypertensive cardiomyopathy without heart failure.</td>
</tr>
<tr>
<td>5</td>
<td>56</td>
<td>female</td>
<td>AECOPD</td>
<td>9 Jan 2009</td>
<td>30 Dec 2008 – 29 Jan 2009</td>
<td>pneumonia</td>
<td>CFP/SUL: 2 × 2.0 g (30.12.08–02.01.09)</td>
<td>CAZ: 2 × 1.0 g (03.01.09–11.01.09)</td>
<td>obesity, arterial hypertension, hypothyroidism</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>female</td>
<td>AECOPD</td>
<td>11 Jan 2009</td>
<td>29 Dec 2008 – 9 Jan 2009;</td>
<td>AECOPD</td>
<td>CFZ: 3 × 1.0 g (11.01.09–13.01.09)</td>
<td>PEN: 3 × 3 ml (17.01.09–22.01.09)</td>
<td>arterial hypertension, osteoporosis</td>
</tr>
</tbody>
</table>

\(^a\) the day of hospitalization when new symptoms developed is given in brackets

\(^b\) after 25 days of hospitalization, the patient was discharged from the hospital; however, in the evening of the same day he returned to the hospital with new symptoms

\(^c\) the patient returned to the hospital with new symptoms 2 days after being discharged

\(^d\) the patient died due to the primary disease

\(^e\) antimicrobials were given intravenously, otherwise as indicated

Antimicrobials used after the detection of new symptoms are marked in bold.

transmission. The isolates belonged to serotype 14, whose representatives are the most common cause of invasive pneumococcal disease in Polish children under 5 years of age and, along with the isolates of serotype 3, in the whole Polish population. Moreover, Polish pneumococci of serotype 14 are typically multidrug-resistant and include, compared with other serotypes, the highest percentage of isolates with penicillin MICs of >0.06 mg/l (70.2%) and cefotaxime MICs of >0.5 mg/l (54.8%).19 MLST and MLVA showed that the outbreak isolates belonged to the clonal complex Spain9V-ST156, representing its variant of serotype 14 (Spain9V-ST156-14). Pneumococci of this complex have global distribution, and in Poland dissemination of its representatives in a relatively short period of time greatly contributed to the increasing prevalence of penicillin-nonsusceptible pneumococci, especially among invasive isolates.20 Originally, the Spain9V-ST156 clone was characterized by susceptibility to macrolides and tetracycline; however, the representatives of this clone, resistant to these compounds, were observed recently.21,22 Isolates belonging to the Spain9V-ST156 clone occurred also among pneumococci nonsusceptible to ciprofloxacin; however, they did not have the alterations in QRDRs as the ones observed among the outbreak isolates and were levofloxacin- and moxifloxacin-susceptible.23 These observations further underline the epidemiological importance of Spain9V-ST156 as being capable of rapid adaptive changes, especially concerning antimicrobial resistance, as observed previously for β-lactams.20

For 2 patients (cases 1 and 6), combined therapy with 2 antimicrobial agents was administered after isolation of S. pneumoniae. The first patient received ceftriaxone with piperacillin/tazobactam because with the new symptoms, Pseudomonas aeruginosa (the same as from the primary culture at the beginning of the hospitalization) together with S. pneumoniae had been isolated from sputum samples. The S. pneumoniae isolate responsible for case 6 was susceptible to penicillin (1 mg/l) but intermediate susceptibility to ceftriaxone (2 mg/l); therefore, an initial therapy with the latter antibiotic was supplemented with penicillin after obtaining the results of susceptibility testing (Table).

Five of 6 patients affected by the outbreak suffered from COPD, a disease that is the major cause of death and disability throughout the world, and is a risk factor for the acquisition of multidrug-resistant pneumococci.9,24 Additionally, isolates of the clone Spain9V-ST156 are often pilated, which helps them to adhere to human epithelial cells and may facilitate nasopharyngeal colonization.25 Polysaccharide pneumococcal vaccine (PPV) has been used for many years to limit pneumococcal infections, especially in the at-risk adult population; however, this type of vaccine does not influence colonization. The results of randomized clinical trials with PPV and their meta-analyses have demonstrated protective effect against invasive pneumococcal disease (IPD) and all-cause-pneumonia among healthy young adults as well as, to a lesser degree, protection against IPD in individuals aged over 65 years.26 A new possibility to reduce the incidence of pneumococcal infections and carriage appeared with pneumococcal conjugate vaccines, which offer long-term protection for small children and bring an indirect effect of creating a herd immunity, observed also in Poland.27 Although conjugated vaccines were formulated for pediatric use, recently one of them was registered also for use in adults, which may contribute to the limitation of IPD-associated morbidity and mortality in this population, especially in cases and outbreaks caused by bacteria with decreased susceptibility to antibiotics. In parallel to that, it is necessary to maintain infection control in hospitals, which is a crucial measure for the outbreak recognition and containment.

Acknowledgements The authors would like to thank Izabela Waśko for performing the RFLP-PFGE analysis. The study was partially supported by the Ministry of Health within the framework of the National Programme of Antibiotic Protection (NPOA, MODUL1 Programme), by the Ministry of Science and Higher Education (Mikrobank 2 Programme), and by the European Union grant HEALTH-F3-2009-223111 with the complementary funding from the Ministry of Science and Higher Education.

REFERENCES


Ognisko zakażeń na oddziale chorób płuc wywołane przez klon Spain9V-ST156-14 Streptococcus pneumoniae

Anna Skoczyńska1, Ewa Sadowy2, Dorota Krawiecka3, Małgorzata Czajkowska-Malinowska4, Anna Ciesielska4, Grzegorz Przybylski5, Renata Żebracka3, Waleria Hryniewicz1

1 Zakład Epidemiologii i Mikrobiologii Klinicznej, Narodowy Instytut Leków, Warszawa
2 Zakład Mikrobiologii Molekularnej, Narodowy Instytut Leków, Warszawa
3 Zakład Mikrobiologii, Kujawsko-Pomorskie Centrum Pulmonologii, Bydgoszcz
4 Oddział Chorób Płuc i Niewydolności Oddychania, Kujawsko-Pomorskie Centrum Pulmonologii, Bydgoszcz
5 Oddział Kliniczny Chorób Płuc, Nowotworów i Gruźlicy, Uniwersytet Mikolaja Kopernika, Collegium Medicum im. Ludwika Rydygiera w Bydgoszczy, Bydgoszcz

SŁOWA KLUCZOWE
Streptococcus pneumoniae, szpitalne ognisko epidemiczne, wielolekooporność

WPROWADZENIE Streptococcus pneumoniae (S. pneumoniae) jest jednym z najważniejszych czynników etiologicznych pozaszpitalnego zapalenia płuc. Jednak coraz częściej obserwuje się zakażenia szpitalne wywoływane przez ten drobnoustrój.

CELE Celem pracy była analiza ogniska epidemicznego zakażeń pneumokokowych w jednym z polskich szpitali i charakterystyka odpowiedzialnych izolatów.


WYNIKI Podejrzenie wystąpienia ogniska epidemicznego nasunęło się po wyhodowaniu od pacjentów jednego oddziału pneumokoków o charakterystycznej wielolekooporności. Wystąpienie ogniska potwierdzono technikami biologii molekularnej. Wszystkie izolaty należały do serotypu 14 i były nierozróżnialne w analizie RFLP-PFGE. Analiza MLST przeprowadzona dla przedstawiciela badanych izolatów wykazała, że należał on do epidemicznego kompleksu klonalnego Spain9V-ST156.

WINIÓSKI Szybkie wdrożenie zaleceń kontroli zakażeń pozwoliło wygasić opisywane ognisko epidemiczne. Niedawno zarejestrowana do stosowania u dorosłych koniugowana szczepionka przeciw pneumokokom może mieć znaczący wpływ na ograniczenie liczby zakażeń pneumokokowych i związaną z nimi śmiertelność. Jest to szczególnie ważne w przypadku pacjentów z POChP, jednostką chorobową będącą czynnikiem ryzyka nabywania wielolekoopornych pneumokoków.