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Resistance patterns of selected respiratory tract pathogens in Poland

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ABSTRACT

This study presents the results of a survey of the in-vitro susceptibility to antimicrobial agents of major pathogens responsible for community-acquired respiratory tract infections in Poland during 2002–2004. The collection of 1184 bacterial isolates comprised 398 *Streptococcus pneumoniae*, 344 *Haemophilus influenzae*, 302 *Streptococcus pyogenes* and 140 *Moraxella catarrhalis*. Among the pneumococcal isolates, 16.8% were penicillin-non-susceptible (PNSP), of which 80.6% were identified as multidrug-resistant. Overall, 9.0% of *H. influenzae* isolates were β-lactamase-positive, although this percentage increased noticeably in the third year of the study. Based on PCR results, 12.8% of *H. influenzae* isolates were identified as low-level β-lactamase-negative, ampicillin-resistant (BLNAR), and one isolate as low-level β-lactamase-positive, amoxycillin–clavulanic acid-resistant (BLPACR). Pulsed-field gel electrophoresis (PFGE) classified 45 *H. influenzae* isolates with altered penicillin-binding proteins into 15 PFGE types, including two predominant types (with four and six sub-types) containing 15 and ten isolates, respectively. Resistance to tetracycline, erythromycin and clindamycin was found in 20.9%, 8.9% and 4.6% of *S. pyogenes* isolates, respectively. The production of β-lactamase characterised 91.4% of *M. catarrhalis* isolates. In summary, the overall occurrence of PNSP in Poland remains stable, although there was a noticeable increase in the proportion of fully-resistant isolates. A rising trend in the prevalence of β-lactamase producers and low-level BLNAR isolates was observed among Polish isolates of *H. influenzae*.

Keywords *Haemophilus influenzae*, *Moraxella catarrhalis*, respiratory tract pathogens, *Streptococcus pneumoniae*, surveillance, susceptibility

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INTRODUCTION

Community-acquired respiratory tract infections represent one of the major causes of morbidity and mortality worldwide, and remain the most frequent reason for prescription of antibiotics to non-hospitalised patients. *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Moraxella catarrhalis* are the most common bacterial aetiological agents of typical lower respiratory tract infections (LRTIs), whereas *Streptococcus pyogenes* is most commonly responsible for acute pharyngitis [1,2]. Microbiological investigations to guide treatment are often time-consuming and are not feasible for every patient. In addition, the resistance patterns of the major LRTI pathogens differ and may change dramatically over time. This is a major concern with respect to the appropriate selection of antibiotics for empirical therapy. It has also been observed that the prevalence of multidrug-resistant strains varies geographically [3]. Therefore, in order to select the optimal antimicrobial treatment regimen, it is necessary to know the local susceptibility patterns of the most common pathogens responsible for a particular type of infection. The present study describes the results of a surveillance of the in-vitro susceptibility to antimicrobial agents of major pathogens

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responsible for community-acquired respiratory tract infections in Poland during 2002–2004. This study constitutes a follow-up at the national level of the international Alexander Project, which ceased at the end of 2001 [3].

MATERIALS AND METHODS

The study protocol anticipated the collection of 400 isolates per year belonging to four species, namely *H. influenzae*, *S. pneumoniae*, *M. catarrhalis* and *S. pyogenes*, with at least 100 isolates of *S. pneumoniae* and 100 isolates of *H. influenzae*. Between 1 January 2002 and 31 December 2004, 1184 isolates were collected, comprising 398 *S. pneumoniae*, 344 *H. influenzae*, 302 *S. pyogenes* and 140 *M. catarrhalis*. More than 94% of the isolates (n = 1121) were collected from 19, 11, 18 and five centres, respectively, representing all the regions of Poland. The remaining isolates were from centres that sent fewer than three isolates. Except for patients with infections caused by *S. pyogenes*, the study included isolates from both outpatients and hospitalised patients with clinically-proven community-acquired LRTIs (acute exacerbations of chronic bronchitis or pneumonia), with sampling undertaken for the hospitalised patients within 48 h of admission. The samples investigated included sputum (evaluated by the numbers of white blood cells, epithelial cells, and the presence of mucus threads), bronchoalveolar lavage fluid or blood [4]. *S. pyogenes* isolates were isolated from the throat swab cultures of patients with acute pharyngitis/tonsillitis. Only one isolate per patient was included in the study. All isolates were identified to the species level according to standard procedures [5].

For *H. influenzae*, *S. pneumoniae* and *S. pyogenes*, MICs of appropriate antibiotics (see Results) were determined by broth microdilution tests and interpreted according to CLSI guidelines [6]. Additionally, for pneumococci resistant to ciprofloxacin (MIC ≥4 mg/L) [7,8], the MICs of levofloxacin and moxifloxacin and, for isolates resistant to macrolides, the MIC of telithromycin, were determined. All erythromycin-resistant *S. pyogenes* isolates were assigned to specific phenotypes, i.e., constitutive MLS_B ($cMLS_B$), inducible MLS_B ($iMLS_B$) and efflux-mediated resistance (M phenotype) on the basis of double-disk tests with clindamycin and erythromycin (2-μg and 15-μg disks, respectively) [6]. Fisher's exact test was used to analyse the differences in susceptibility frequencies.

Production of β-lactamase by *H. influenzae* and *M. catarrhalis* isolates was determined with the nitrocefin assay, used according to the manufacturer's instructions (Becton Dickinson, Meylan, France). Quality control strains used for antimicrobial susceptibility testing during this study were *H. influenzae* ATCC 49247, ATCC 49766 and ATCC 10211, and *S. pneumoniae* ATCC 49619.

PCR assays were performed for *H. influenzae* isolates with ampicillin MICs ≥1 mg/L and/or amoxycillin–clavulanic acid MICs ≥1 mg/L and/or elevated cephalosporin MICs in order to identify isolates with altered penicillin-binding proteins (PBPs). Such isolates can be classified as β-lactamase-negative, ampicillin-resistant (BLNAR), low-level BLNAR, β-lactamase-positive, amoxycillin–clavulanic acid-resistant (BLPACR), and low-level BLPACR, depending on resistance level, mutation types and concomitant production of β-lactamase. In brief, primer pairs O_1/O_3, HI-1/HI-2, PBP3S-S/PBP3S-R and PBP3BLN-S/PBP3BLN-R were used to produce amplicons characteristic of *H. influenzae*, capsule production, β-lactamase-negative, ampicillin-susceptible (BLNAS) isolates and BLNAR isolates, respectively [9–11]. Isolates yielding a PCR product only with the first pair, or the first two pairs, of primers were identified as low-level BLNAR [11]. For isolates that yielded specific products for capsule genes, PCRs to identify serotypes a–f were performed with primers a1/a2, b1/b2, c1/c2, d1/d2, e1/e2 and f1/f2, respectively [9]. In this study, the term 'ampicillin-resistant' will be used in relation to low-level BLNAR or low-level BLPACR isolates, despite their in-vitro susceptibility to this antibiotic according to CLSI criteria [6]. The relatedness among *H. influenzae* isolates with altered PBPs was evaluated by restriction fragment length polymorphism analysis of *Sma*I-digested chromosomal DNA using pulsed-field gel electrophoresis (PFGE), and was interpreted as described previously [12,13].

RESULTS

The isolates responsible for LRTI, mostly from adult patients, were recovered mainly from sputum (74.4%, n = 656), followed by bronchoalveolar lavage fluid (18.0%, n = 159) and blood (7.6%, n = 67). All *S. pyogenes* isolates were obtained from throat swabs (n = 302). Clinical and demographical data of the patients included in the study are summarised in Table 1.

Table 1. Numbers of isolates of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pyogenes*, grouped according to diagnosis, specimen type, service, gender and age during the period 2002–2004

	S. pneumoniae (n = 398)	*H. influenzae* (n = 344)	*M. catarrhalis* (n = 140)	*S. pyogenes* (n = 302)
No. of isolates/year				
2002/2003/2004	137/118/143	128/115/101	47/33/60	46/163/93
Diagnosis				
AECB	136	219	109	0
Pneumonia	191	83	17	0
Pharyngitis/tonsillitis	0	0	0	302
Unspecified LRTI	71	42	14	0
Specimen				
Sputum	255	278	123	0
BAL	85	58	16	0
Blood	58	8	1	0
Throat swab	0	0	0	302
Service				
Inpatient	275	239	108	61
Outpatient	81	39	17	68
Unknown	42	66	15	173
Gender				
Female	112	100	50	154
Male	266	238	88	114
Unspecified	20	6	2	34
Age group (years)				
≤16	47	17	8	138
17–30	27	17	3	54
31–45	33	29	8	47
46–60	97	84	31	11
61–75	120	132	59	6
>75	61	60	30	1
Unknown	13	5	1	45

AECB, acute exacerbations of chronic bronchitis; BAL, bronchoalveolar lavage; LRTI, lower respiratory tract infection.

S. pneumoniae

The in-vitro antimicrobial susceptibilities and MIC_{50}/MIC_{90} values for 398 *S. pneumoniae* isolates are shown in Table 2. In total, 16.8% were non-susceptible to penicillin (penicillin non-susceptible pneumococci; PNSP), with 5.0% (n = 20) of isolates being intermediately-susceptible to penicillin (MIC 0.12–1.0 mg/L), and 11.8% (n = 47) being penicillin-resistant pneumococci (PRP) (MIC ≥ 2 mg/L). During the 3-year study period, the proportion of PNSP remained at a similar level, with 17.5% in 2002, 16.1% in 2003, and 16.8% in 2004. However, among the isolates of PNSP, the proportion of PRP was higher in the second (78.9%; p 0.06) and third (83.3%; p 0.03) years than in the first year (50%) of the study.

All penicillin-susceptible pneumococci were also susceptible to amoxycillin and ceftriaxone. Only two isolates were resistant to cefuroxime, and two and three isolates were resistant and intermediately-susceptible, respectively, to cefaclor. All penicillin intermediately-susceptible isolates were fully-susceptible only to amoxycillin. Except for two isolates, erythromycin-resistant pneumococci were always resistant to azithromycin and clarithromycin. Noteworthy differences in susceptibility to anti-pneumococcal drugs were observed among isolates of penicillin-susceptible pneumococci and PNSP (Table 2). Among PNSP, 80.6% of isolates were multidrug-resistant, i.e., resistant at least to three groups of antibiotics, most frequently including co-trimoxazole, tetracyclines, macrolides and various generations of cephalosporins. Resistance to ciprofloxacin was found in 26 (6.6%) isolates, all of which were susceptible to moxifloxacin, and all but two of which were susceptible to levofloxacin, with MICs ranging from 0.06 to 0.25 mg/L and from 1.0 to 4.0 mg/L, respectively. Among pneumococci resistant to erythromycin (n = 54), three had intermediate resistance to telithromycin, with MICs of 2.0 mg/L.

H. influenzae

The results of susceptibility testing of *H. influenzae* isolates are summarised in Table 3. All *H. influenzae* isolates were susceptible to amoxycillin–clavulanic acid, ceftriaxone, ciprofloxacin, azithromycin and chloramphenicol, whereas 92.4%, 97.4% and 58.1% were susceptible to clarithromycin, tetracycline and co-trimoxazole, respectively. Of 344 isolates, 31 (9.0%) were identified as β-lactamase-positive, amoxycillin-resistant (BLPAR). During the first 2 years of the study (2002–2003), the proportions of BLPAR isolates were similar, i.e., 6.3% and 5.2%, respectively. However, this percentage increased noticeably to 16.8% in 2004.

In total, 123 isolates met the criteria required for further testing for altered PBPs. Of these, 44 (12.8% of all *H. influenzae* isolates) were identified as non-typeable low-level BLNAR isolates, based on the PCR assay used. One isolate of serotype b (Hib) possessed two mechanisms of β-lactam resistance, being both a β-lactamase producer and having changes in PBPs (BLPACR). The proportion of isolates with modified PBPs during the first year of the study was 7.8%, and this

Table 2. In-vitro activities of antibiotics against *Streptococcus pneumoniae* isolates

	All isolates (n = 398)			PSP (n = 331)			PIP (n = 20)			PRP (n = 47)		
Antibiotic	**%I**	**%R**	**MIC_{50}/MIC_{90}[a]**	**%I**	**%R**	**MIC_{50}/MIC_{90}[a]**	**%I**	**%R**	**MIC_{50}/MIC_{90}[a]**	**%I**	**%R**	**MIC_{50}/MIC_{90}[a]**
Penicillin	5.0	11.8	0.015/2.0	0	0	0.015/0.015	100	0	0.25/1.0	0	100	2.0/8.0
Amoxycillin	0	2.5	≤0.015/1.0	0	0	≤0.0075/0.03	0	0	0.25/1.0	0	21.7	2.0/8.0
Cefaclor	2.5	14.6	0.5/>8.0	0.9	0.6	0.5/1.0	35.0	45.0	2.0/>8.0	0	100	>8.0/>8.0
Cefuroxime	0.5	13.6	≤0.015/4.0	0	0.6	≤0.015/0.03	5.0	30.0	0.25/4.0	2.1	97.9	8.0/8.0
Ceftriaxone	5.0	0.8	0.015/1.0	0	0	0.015/0.03	10.0	0	0.12/1.0	38.3	6.4	1.0/2.0
Erythromycin	0.3	13.3	0.03/16.0	0.3	5.4	0.03/0.12	0	45.0	0.06/>16.0	0	55.3	8.0/>16.0
Clarithromycin	0.3	12.5	0.03/>8.0	0	5.1	0.03/0.06	0	40.0	0.03/>8.0	2.1	53.2	>8.0/>8.0
Azithromycin	0.3	12.5	0.06/>16.0	0	5.1	0.03/0.12	0	40.0	0.06/>16.0	2.1	53.2	>16.0/>16.0
Clindamycin	0	12.5	0.03/8.0	0	4.5	0.03/0.12	0	45.0	0.06/>8.0	0	55.3	>8.0/>8.0
Ciprofloxacin[b]	0	6.5	2.0/2.0	0	4.5	2.0/2.0	0	10.0	2.0/2.0	0	19.1	2.0/>4.0
Tetracycline	0.7	23.4	0.25/32.0	0.9	17.5	0.25/16.0	0	55.0	16.0/>32.0	0	51.1	16.0/>32.0
Co-trimoxazole	17.0	30.7	0.5/16.0	19.0	21.2	0.5/8.0	10.0	70.0	8.0/>16.0	6.3	80.9	8.0/>16.0
Rifampicin	0.5	0.5	0.03/0.12	0.3	0	0.03/0.12	0	10.0	0.03/0.12	2.1	0	0.03/0.12

PSP, penicillin-susceptible pneumococci; PIP, penicillin intermediately-susceptible pneumococci; PRP, penicillin-resistant pneumococci; %I, percentage of intermediately-susceptible isolates; %R, percentage of resistant isolates.
[a]MIC_{50}/MIC_{90} (mg/L).
[b]Pneumococci with ciprofloxacin MICs ≥4 mg/L were regarded as resistant to this antibiotic.

Table 3. MIC distributions (mg/L) of β-lactam antibiotics for *Haemophilus influenzae* isolates (n = 344)

Antibiotics	≤0.0037	0.0075	0.015	≤0.03	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	%S[a]
Ampicillin																
BLNAS (n = 269)					2	6	79	117	42	23						100
BLPAR (n = 31[a])													2	9	20	0
PBPs-mod (n = 45)									17	27					1	97.8
Amox/clav																
BLNAS				5		9	22	101	89	20	18	5				100
BLPAR									14	15	2					100
PBPs-mod							2	2	12	12	9	8				100
Cefaclor																
BLNAS									11	59	99	72	20	2	6	97.0
BLPAR									1	2	9	14	2	1	2	90.3
PBPs-mod											2	19	16	5	3	82.2
Cefuroxime																
BLNAS							3	34	147	64	19	2				100
BLPAR							1	6	14	8	2					100
PBPs-mod									3	16	21	4	1			97.5
Ceftriaxone																
BLNAS	161	13	94		1											100
BLPAR	23	2	6													100
PBPs-mod	6	25	7		2	4		1								100

%S, percentage of susceptible isolates; BLNAS, β-lactamase-negative ampicillin-susceptible isolates; BLPAR, β-lactamase-positive ampicillin-resistant isolates; PBPs-mod, isolates with modified penicillin-binding proteins, including 44 low-BLNAR (β-lactamase-negative ampicillin-resistant isolates) and one low-BLPACR isolate (N.B. the low-level BLPACR isolate appears in two categories, i.e. BLPAR and PBPs-mod); Amox/clav, amoxycillin–clavulanic acid.
[a]Including one low-level BLPACR (β-lactamase-positive, amoxycillin–clavulanic acid-resistant) isolate.

increased to 15.6% and 16.8% in 2003 and 2004, respectively. There were differences in levels of resistance to other antibiotics among BLNAS isolates, BLPAR isolates and isolates with modified PBPs. For example, the MIC_{50} and MIC_{90} values of cefuroxime and amoxycillin–clavulanic acid for isolates with modified PBPs were four-fold higher than those for BLNAS isolates. These isolates were also less susceptible to co-trimoxazole (22.2%) than were BLNAS isolates (61.3%; $p < 0.0001$) or BLPAR isolates (80.7%; $p < 0.0001$). Resistance to tetracycline was found more often among BLPAR isolates (22.6%) than among BLNAS isolates (0.7%; $p < 0.0001$) or among isolates with modified PBPs (2.2%; $p < 0.001$).

PFGE analysis classified 45 isolates with altered PBPs into 15 PFGE types, including eight types with one representative, four types with two representatives, and one type with three representatives. Two predominant PFGE types (A and C), with four and six sub-types, contained 15 and ten representatives, respectively. Eleven isolates belonging to three sub-types of PFGE type A were isolated in a single centre during 2003–2004, although other PFGE types were also present. The BLPACR Hib isolate had a unique PFGE pattern.

S. pyogenes

The highest proportion (45.7%) of *S. pyogenes* isolates was cultured from patients aged <17 years. However, most infections in this group affected children aged 3–8 years (n = 87, 63.0%), followed by children aged 9–16 years (31.2%) and children aged <3 years (5.8%). All 302 isolates of *S. pyogenes* tested were susceptible to penicillin, with MIC_{50} and MIC_{90} values of 0.0075 and 0.015 mg/L, respectively. Of these, 20.9%, 8.9% and 4.6% were resistant to tetracycline, erythromycin and clindamycin, respectively. In the group of 27 erythromycin-resistant isolates, 14 (51.9%) had the $cMLS_B$ phenotype, nine (33.3%) had the $iMLS_B$ phenotype, and four (14.8%) had the M phenotype. Resistance to tetracycline was more prevalent in the $iMLS_B$ group (88.9%) than in the $cMLS_B$ group (14.3%), whereas all isolates with the M phenotype were sensitive to tetracycline. Resistance to clindamycin was observed exclusively in $cMLS_B$ isolates.

M. catarrhalis

In the group of *M. catarrhalis* isolates, 91.4% were found to produce β-lactamase and this proportion was similar in all 3 years studied.

DISCUSSION

During the past decade, increasing numbers of PRP in some countries have resulted in the limited use of penicillin as the antibiotic of choice for the empirical treatment of diseases in which pneumococci are strongly implicated as an

aetiological agent [3,14]. In the present study, 16.8% of pneumococci were found to be PNSP, which is a proportion similar to that reported in previous studies from Poland [15,16], but slightly higher than in Polish LRTIs isolates reported to the Alexander Project between 1998 and 2000 (12.6%) [3]. The prevalence of PNSP in Poland is higher than that in some neighbouring countries, e.g., Germany (5.9%), the Czech Republic (6.2%) and Russia (6.2%), but lower than that in Slovakia (35.4%) and generally in western Europe (22.2%) and the USA (37.0%) [3]. Although the percentage of PNSP was stable during the study period, there was a considerable increase in the proportion of PRP among PNSP, from 50% to 83.3%, a finding that has also been reported from other studies [17–19]. This change in proportions is of great concern, since LRTIs caused by PRP, in contrast to those caused by penicillin intermediately-resistant pneumococci, cannot be treated effectively by penicillins [6]. Additionally, PRP are often multidrug-resistant, and this phenomenon further limits the available therapeutic options [14,20]. In the present study, an increase in resistance to other β-lactams, macrolides, clindamycin, co-trimoxazole, ciprofloxacin and newer quinolones was found in comparison with previous studies [3,21], and this rise was associated with an increasing proportion of PRP. The study revealed that the least active antimicrobial agent against pneumococci and *H. influenzae* was co-trimoxazole. This may reflect the very high consumption of this compound as a first-line drug for the empirical therapy of respiratory tract infections in Poland (unpublished data from the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products).

The most unexpected findings concerned the *H. influenzae* isolates, in which resistance to β-lactams may be associated with either β-lactamase production (most commonly) or altered PBPs, or both [22]. Isolates with altered PBPs have been observed increasingly, despite being difficult to detect in the laboratory [23–26]. Production of β-lactamase in the current 3-year study was found in 9.0% of the isolates, which is only slightly higher than the proportion reported previously [27]. This overall percentage places Poland among countries with low levels of ampicillin resistance, which is typical for eastern Europe (6.4%) [3]. However, there was an increase in BLPAR isolates to 16.8% during the last year of the study. Recently, a higher proportion of BLPAR isolates recovered from respiratory tract infections in Polish children (24%) was also reported, although this encompassed isolates from only one region [16]. Although, globally, β-lactamase production remains the most important mechanism of resistance to amino-penicillins in *H. influenzae*, isolates with modifications in PBPs were more prevalent in the present study (13.1%). An even higher proportion of BLNAR isolates was detected during the study conducted by Fluit *et al*. [23], in which Poland had the highest rate of BLNAR isolates from respiratory tract infections in Europe (20%). Moreover, this previous report, albeit based on a small number of isolates, showed an increase in the prevalence of BLNAR isolates in Poland, from 4.0% in 1997–1998 to 20% in 2002–2003 [23]. A rising trend was also observed during the present study, with the prevalence of isolates with altered PBPs having increased three-fold by the third year. There have also been reports from other countries, e.g., France, Spain and, especially, Japan (meningitis isolates), in which 18.9%, 9.3% and 55.4% of *H. influenzae* isolates, respectively, had changes in PBPs, with or without production of β-lactamase [24–26].

The present data indicated that all isolates with altered PBPs were of the low-level BLNAR or low-level BLPACR types, and their detection was possible only by PCR. Although elevated MIC_{50}/MIC_{90} values for low-level BLNAR isolates, in comparison with BLNAS isolates, were observed for all β-lactams tested, all low-level BLNAR isolates had ampicillin MICs ≤1 mg/L and, as such, were classified as susceptible to this antibiotic according to CLSI criteria. However, it is known that some isolates of *H. influenzae* with ampicillin MICs of 0.5–2.0 mg/L also exhibit changes in PBPs [26,28]. Additionally, even if MIC values are the same as those of BLNAS isolates, the bactericidal effect of β-lactams against low-level BLNAR strains possessing altered PBP genes may be decreased [11]. Therefore, it is important to monitor both BLNAR and low-level BLNAR isolates that, despite their apparent susceptible phenotype, demonstrate changes in PBPs, indicating a stepwise acquisition of resistance similar to that seen in *S. pneumoniae* [28]. It is also necessary to reconsider changes in laboratory procedures to include PCR or any other methods that allow identification of BLNAR, low-level BLNAR, BLPACR and

low-level BLPACR isolates, and to re-evaluate the interpretation of susceptibility tests in order to minimise the risk of treatment failure.

PFGE analysis revealed that the population of isolates with altered PBPs in Poland is diverse, as has been described previously for non-typeable BLNAR isolates [29–31]. However, the identification of isolates with the same or very similar PFGE patterns, not only from patients attending the same hospital, but also from different parts of the country, suggests possible clonal spread of isolates with altered PBPs, as has been observed previously [32].

Currently, *S. pyogenes* isolates are universally susceptible to penicillin and, as expected, all isolates of *S. pyogenes* tested during this survey were highly susceptible to this antibiotic. Tetracycline was the least active antibiotic against *S. pyogenes*, as was also found in a previous study of *S. pyogenes* isolates in Poland, although the proportion of resistant isolates was only half that in the present study [35]. However, this discrepancy is a consequence of the fact that only about 50% of isolates were recovered from throat swabs in the previous study [33]. The present study found that <9% of isolates were resistant to erythromycin, but among these, isolates with the $cMLS_B$ phenotype were most common. Previously, erythromycin resistance reached 12%, but with $iMLS_B$ as the most common phenotype [33]. Thus, it seems that the level of erythromycin resistance in Poland is lower than in some other countries, e.g., Spain (21.7%) and Greece (24%), but is higher than in the USA (6.8%) [34–36].

The prevalence of β-lactamase-producing *M. catarrhalis* isolates was >90% and did not change during the study period. A similar proportion was also found in a previous Polish study [27], in *M. catarrhalis* isolates collected as part of the Alexander Project (92.1%) in 1998–2000, and in Canadian isolates (92.4%) collected in 1997–2002 [3,37].

Following its initiation in 1992, the Alexander Project was pivotal in defining the role of global surveillance of antimicrobial susceptibility patterns among common respiratory pathogens [38]. However, only one, or very few, national centres are involved in large international projects, and therefore local and regional variations may not be described adequately. For this reason, national and local multicentre surveys, such as the present study, are still required.

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