J Antimicrob Chemother 2018; **73** Suppl 5: v28–v35
doi:10.1093/jac/dky069

Journal of Antimicrobial Chemotherapy

Results from the Survey of Antibiotic Resistance (SOAR) 2014–16 in Ukraine and the Slovak Republic

D. Torumkuney[1]*, T. Pertseva[2], E. Bratus[3], A. Dziublik[4], V. Yachnyk[4], A. Liskova[5], O. Sopko[6], K. Malynovska[6] and I. Morrissey[7]

[1]GlaxoSmithKline, 980 Great West Road, Brentford, Middlesex, TW8 9GS, UK; [2]Dnipropetrovsk State Medical Academy, Soborna Square 4, 49027, Dnipro, Ukraine; [3]Dnipropetrovsk State Medical Academy, Diagnostic Center, Soborna Square 4, 49027, Dnipropetrovsk, Kyiv, Ukraine; [4]State Organization National Institute of Phthisiology and Pulmonology named after F. G. Yanovsky, National Academy of Medical Sciences of Ukraine, Amosova Str. 10, 03680, Kiev, Ukraine; [5]Nitra Teaching Hospital, Department of Clinical Microbiology, Spitalska 6, 950 01 Nitra, Slovak Republic; [6]GlaxoSmithKline, Pavla Tychyny avenue, 1-V, Kyiv, 02152, Ukraine; [7]IHMA Europe Sàrl, Route de l'Ile-au-Bois 1A, 1870 Monthey/VS, Switzerland

*Corresponding author. Tel: +44 20 8047 1671; E-mail: didem.x.torumkuney@gsk.com

Objectives: To determine antibiotic susceptibility in isolates of *Streptococcus pneumoniae* and *Haemophilus influenzae* collected in 2014–16 from Ukraine and the Slovak Republic.

Methods: MICs were determined by CLSI broth microdilution and susceptibility was assessed using CLSI, EUCAST and pharmacokinetic/pharmacodynamic (PK/PD) breakpoints.

Results: *S. pneumoniae* isolates collected in Ukraine ($n = 100$) showed susceptibility rates ≥97% for amoxicillin, amoxicillin/clavulanic acid, penicillin [intravenous (iv) non-meningitis] and fluoroquinolones, between 83% and 86% for oral penicillin, macrolides and cefaclor, and 75% for trimethoprim/sulfamethoxazole. Susceptibility was substantially lower in the Slovak Republic ($n = 95$). All isolates were susceptible to the fluoroquinolones, but susceptibility to penicillin, amoxicillin, amoxicillin/clavulanic acid, cefuroxime and trimethoprim/sulfamethoxazole varied between 61% and 64%, with only 44% of isolates susceptible to the macrolides. Susceptibility of *H. influenzae* was more homogeneous, with susceptibility to amoxicillin/clavulanic acid, ceftriaxone, cefuroxime, azithromycin and the fluoroquinolones seen in >90% of isolates by CLSI criteria in both countries. Much greater variability was seen across breakpoints, especially for azithromycin, cefaclor and cefuroxime. The β-lactamase rate was 5.1% (5/98) in the Slovak Republic and 7.3% (7/96) in Ukraine, but the Slovak Republic also had a relatively high rate of β-lactamase-negative-ampicillin-resistant (BLNAR) isolates (7.1%; 7/98).

Conclusions: The variability found across these two neighbouring countries illustrates the need to monitor and publish national and local resistance patterns. This information is not only critical for effective empirical therapy but can also be used to help shape and support antimicrobial stewardship efforts in order to limit antibiotic resistance.

Introduction

In 2015, lower respiratory infections remained the most deadly communicable disease and the third leading cause of death worldwide with 3.2 million deaths.[1] Even in Europe, where mortality due to communicable diseases is much lower than in many other regions of the world, these infections still represent the sixth most common cause of death.[1] It is therefore critical that decisions regarding antimicrobial therapy, which is typically empirical for lower respiratory infections, are supported by solid surveillance data of local antimicrobial resistance patterns. This is especially important since surveillance studies have shown that resistance levels can vary substantially across countries and even between institutions in the same country.[2–5] Monitoring and reporting of local resistance rates can be undertaken by individual hospitals and laboratories as well as through larger surveillance studies that can facilitate comparative analyses across regions and longitudinal analyses over time. The Survey of Antibiotic Resistance (SOAR) is an international antimicrobial resistance surveillance study that focuses on key respiratory pathogens and has been running since 2002 in the Middle East, Africa, Latin America, Asia-Pacific and Commonwealth of Independent States countries. For this report, recent SOAR data from hospitals in the Slovak Republic and Ukraine were analysed to provide a picture of the current state of antimicrobial susceptibility of *Streptococcus pneumoniae* and *Haemophilus influenzae*, two important bacterial pathogens associated with lower respiratory tract infections.

Table 1. MIC breakpoints (mg/L) used for *S. pneumoniae* and *H. influenzae* isolates

	S. pneumoniae						*H. influenzae*						
	CLSI			EUCAST			CLSI			EUCAST			
Antimicrobial	S	I	R	S	I	R	S	I	R	S	I	R	PK/PD (S only)
Amoxicillin	≤2	4	≥8	NA	NA	NA	NA	NA	NA	≤2	–	≥4	≤2 (≤4)
AMC[a]	≤2	4	≥8	NA	NA	NA	≤4	–	≥8	≤2	–	≥4	≤2 (≤4)
Ampicillin	NA	NA	NA	NA	NA	NA	≤1	2	≥4	≤1	–	≥2	NA
Azithromycin	≤0.5	1	≥2	≤0.25	0.5	≥1	≤4	–	–	≤0.12	0.25–4	≥8	≤0.12
Cefaclor	≤1	2	≥4	≤0.03	0.06–0.5	≥1	≤8	16	≥32	NA	NA	NA	≤0.5
Ceftriaxone	≤1	2	≥4	≤0.5	1–2	≥4	≤2	–	–	≤0.12	–	≥0.25	≤1
Cefuroxime[b]	≤1	2	≥4	≤0.25	0.5	≥1	≤4	8	≥16	≤0.12	0.25–1	≥2	≤1
Clarithromycin	≤0.25	0.5	≥1	≤0.25	0.5	≥1	≤8	16	≥32	≤1	2–32	≥64	≤0.25
Erythromycin	≤0.25	0.5	≥1	≤0.25	0.5	≥1	NA	NA	NA	NA	NA	NA	≤0.25
Levofloxacin	≤2	4	≥8	≤2	–	≥4	≤2	–	–	≤0.06	–	≥0.12	≤2
Moxifloxacin	≤1	2	≥4	≤0.5	–	≥1	≤1	–	–	≤0.12	–	≥0.25	≤2
Penicillin (iv non-meningitis)	≤2	4	≥8	NA[c]	NA[c]	NA[c]	NA	NA	NA	NA	NA	NA	NA
Penicillin (oral)	≤0.06	0.12–1	≥2	≤0.06	0.12–2	≥4	NA	NA	NA	NA	NA	NA	NA
SXT[d]	≤0.5	1–2	≥4	≤1	2	≥4	≤0.5	1–2	≥4	≤0.5	1	≥2	≤0.5

AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole; S, susceptible; I, intermediate; R, resistant; NA, not applicable.
[a]Amoxicillin/clavulanic acid was tested at a 2:1 amoxicillin to clavulanic acid ratio; breakpoints are expressed as the amoxicillin component. PK/PD breakpoints based on high dose (4 g of amoxicillin with 250 mg of clavulanate per day for adults) shown in parentheses,[9] which is the same as CLSI for *H. influenzae* and one dilution higher for *S. pneumoniae*.
[b]Breakpoints used are for cefuroxime axetil.
[c]EUCAST do not give iv breakpoints but dose-specific-susceptible breakpoints are noted for pneumonia: 1.2 g × 4, ≤0.5 mg/L; 2.4 g × 4 (or 1.2 g × 6), ≤1 mg/L and 2.4 g × 6, ≤2 mg/L.
[d]Trimethoprim/sulfamethoxazole was tested at a 1:19 trimethoprim to sulfamethoxazole ratio; breakpoints are expressed as the trimethoprim component.

Materials and methods

Collaborating centres

The following centres took part in the study: one from the Slovak Republic (Hospital Nitra, Nitra) and two from Ukraine (Diagnostic Center of Medical Academy, Dnipropetrovsk, and National Institute of Phthisiology and Pulmonology, Kiev).

Isolates of *H. influenzae* and *S. pneumoniae* from community-acquired respiratory tract infections were sent to a central laboratory (International Health Management Associates, Inc., Switzerland) where they were subcultured and re-identified. *H. influenzae* were re-identified by MALDI-TOF MS methodology and *S. pneumoniae* identity was confirmed by optochin susceptibility and bile solubility. β-Lactamase production was determined for each *H. influenzae* isolate by a chromogenic cephalosporin (nitrocefin) disc method. Duplicate isolates from the same patient were not accepted.

Susceptibility testing

Isolates were evaluated for antibiotic susceptibility using broth microdilution methodology recommended by the Clinical and Laboratory Standards Institute (CLSI).[6]

Both pathogens were assessed for susceptibility to amoxicillin, amoxicillin/clavulanic acid (2:1), azithromycin, cefaclor, ceftriaxone, cefuroxime, clarithromycin, erythromycin, levofloxacin, moxifloxacin and trimethoprim/sulfamethoxazole (1:19). *S. pneumoniae* was also tested for susceptibility to penicillin whereas *H. influenzae* was additionally tested for susceptibility to ampicillin.

Susceptibility to the study drugs was calculated based on CLSI breakpoints, EUCAST breakpoints and pharmacokinetic/pharmacodynamic (PK/PD) breakpoints.[7–9] These breakpoints are shown in Table 1.

Quality control and data analysis

Quality control strains *S. pneumoniae* ATCC 49619, *Escherichia coli* ATCC 25922, *H. influenzae* ATCC 49247, *H. influenzae* ATCC 49766 and *E. coli* ATCC 32518 were included on each day of testing. Results of susceptibility testing were accepted if the results of the control strains were within published limits. Differences in susceptibility (using CLSI criteria) across age groups, source of infection and penicillin susceptibility (*S. pneumoniae* only) were assessed for statistical significance (where $n \geq 20$) with Fisher's exact test using XLSTAT version 2011.1.05. A P value <0.05 was considered statistically significant.

Results

S. pneumoniae isolates

A total of 195 *S. pneumoniae* isolates were collected from three centres in the Slovak Republic and Ukraine from 2014 to 2016. Most pneumococci came from sputum ($n = 60$; 30.8%) and bronchoalveolar lavage ($n = 56$; 28.7%). Less frequently, isolates were from sinuses ($n = 35$; 17.9%), middle ear effusion ($n = 20$; 10.3%), endotracheal aspirate ($n = 5$; 2.6%) and blood ($n = 3$; 1.5%). Sixteen isolates were from an undisclosed respiratory source

Table 2. MIC and susceptibility data for *S. pneumoniae* isolates

						Susceptibility using indicated breakpoints						
		MIC (mg/L)				CLSI			PK/PD	EUCAST		
Antimicrobial	*n*	50%	90%	min	max	%S	%I	%R	%S	%S	%I	%R
Slovak Republic												
amoxicillin[a]	95	0.03	8	≤0.015	>8	64.2	1.1	34.7	64.2 (65.3)	NA	NA	NA
AMC[a]	95	≤0.015	8	≤0.015	8	64.2	15.8	20.0	64.2 (80.0)	NA	NA	NA
azithromycin	95	>2	>2	0.06	>2	44.2	0.0	55.8	41.1	44.2	0.0	55.8
cefaclor	95	1	>4	0.5	>4	57.9	3.2	39.0	6.3	0.0	6.3	93.7
ceftriaxone	95	0.12	2	0.03	2	82.1	17.9	0.0	82.1	63.2	36.8	0.0
cefuroxime	95	≤0.12	>4	≤0.12	>4	63.2	0.0	36.8	63.2	56.8	6.3	36.8
clarithromycin	95	>1	>1	≤0.015	>1	44.2	0.0	55.8	44.2	44.2	0.0	55.8
erythromycin	95	>0.5	>0.5	0.03	>0.5	44.2	0.0	55.8	44.2	44.2	0.0	55.8
levofloxacin	95	1	1	0.5	2	100	0.0	0.0	100	100	–	0.0
moxifloxacin	95	0.12	0.25	0.06	0.25	100	0.0	0.0	100	100	–	0.0
penicillin (oral)	95	≤0.06	4	≤0.06	>4	61.1	3.2	35.8	NA	61.1	3.2	35.8
penicillin (iv non-meningitis)	95	≤0.06	4	≤0.06	>4	64.2	31.6	4.2	NA	NA	NA	NA
SXT	95	0.12	>2	≤0.06	>2	64.2	1.1	34.7	64.2	64.2	1.1	34.7
Ukraine												
amoxicillin[a]	100	≤0.015	1	≤0.015	8	97.0	0.0	3.0	97.0 (97.0)	NA	NA	NA
AMC[a]	100	≤0.015	0.5	≤0.015	8	97.0	2.0	1.0	97.0 (99.0)	NA	NA	NA
azithromycin	100	0.12	>2	≤0.03	>2	84.0	0.0	16.0	76.0	84.0	0.0	16.0
cefaclor	100	1	4	0.25	>4	86.0	1.0	13.0	28.0	0.0	28.0	72.0
ceftriaxone	100	0.03	0.5	≤0.015	2	98.0	2.0	0.0	98.0	90.0	10.0	0.0
cefuroxime	100	≤0.12	2	≤0.12	>4	89.0	2.0	9.0	89.0	88.0	0.0	12.0
clarithromycin	100	≤0.015	>1	≤0.015	>1	84.0	1.0	15.0	84.0	84.0	1.0	15.0
erythromycin	100	0.03	>0.5	0.03	>0.5	83.0	0.0	17.0	83.0	83.0	0.0	17.0
levofloxacin	100	1	1	1	2	100	0.0	0.0	100	100	–	0.0
moxifloxacin	100	0.12	0.25	0.06	0.5	100	0.0	0.0	100	100	–	0.0
penicillin (oral)	100	≤0.06	1	≤0.06	4	83.0	9.0	8.0	NA	83.0	14.0	3.0
penicillin (iv non-meningitis)	100	≤0.06	1	≤0.06	4	97.0	3.0	0.0	NA	NA	NA	NA
SXT	100	0.25	>2	0.12	>2	75.0	13.0	12.0	75.0	75.0	13.0	12.0

AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole; S, susceptible; I, intermediate; R, resistant; NA, not applicable.
[a]Amoxicillin and amoxicillin/clavulanic acid PK/PD susceptibility at high dose shown in parentheses.

(8.2%). Most isolates ($n = 98$; 50.3%) came from adult patients (13–64 years old), 69 (35.4%) were from children (aged ≤12 years) and 28 (14.4%) were from elderly patients (aged ≥65 years).

Summary MIC and susceptibility data for all 195 *S. pneumoniae* isolates broken down by country of origin are shown in Table 2. MIC distribution data are given in Table 3.

S. pneumoniae susceptibility in the Slovak Republic

Of the 95 pneumococci collected in the Slovak Republic, 64.2% were penicillin susceptible by CLSI penicillin intravenous (iv) (non-meningitis) breakpoints and 61.1% by CLSI penicillin oral breakpoints or EUCAST breakpoints. All the *S. pneumoniae* were susceptible to levofloxacin and moxifloxacin by all breakpoints. Susceptibility to ceftriaxone was 82.1% by CLSI and PK/PD breakpoints, but only 63.2% by EUCAST breakpoints. Susceptibility to amoxicillin and amoxicillin/clavulanic acid was 65.3% and 80.0%, respectively, at high-dose PK/PD breakpoints, but reduced to 64.2% by CLSI and low-dose PK/PD breakpoints. Susceptibility to trimethoprim/sulfamethoxazole was 64.2% by all breakpoints, and to cefuroxime it was 63.2% by CLSI and PK/PD criteria and 56.8% by EUCAST standards. The other antimicrobials showed susceptibility rates of <60% (Table 2).

Insufficient isolates were obtained to analyse antimicrobial susceptibility by specimen source. There was no significant difference in antimicrobial susceptibility based on patient age (data not shown).

S. pneumoniae susceptibility in Ukraine

Of the pneumococcal isolates from Ukraine, 97.0% were susceptible to penicillin by CLSI penicillin iv (non-meningitis) breakpoints and 83.0% were susceptible by CLSI penicillin oral breakpoints or EUCAST breakpoints. All were susceptible to levofloxacin and moxifloxacin by all breakpoints. Ninety-eight percent were also susceptible to ceftriaxone by CLSI and PK/PD breakpoints, with a reduction to 90.0% by EUCAST breakpoints. Susceptibility to amoxicillin and amoxicillin/clavulanic acid was 97.0% by CLSI and PK/PD

Table 3. MIC distribution data for *S. pneumoniae* isolates

	Number of isolates at MIC (mg/L)																	
Antimicrobial	≤0.015	≤0.03	0.03	≤0.06	0.06	≤0.12	0.12	0.25	0.5	>0.5	1	>1	2	>2	4	>4	8	>8
Slovak Republic ($n = 95$)																		
amoxicillin	15		36		7			2			1				1		32	1
AMC	52		5		1		1	1			1				15		19	
azithromycin					1		38	3						53				
cefaclor									6		49		3		1	36		
ceftriaxone			34		1		22	2	1		18		17					
cefuroxime						52		2	6						2	33		
clarithromycin	35		7									53						
erythromycin			41		1					53								
levofloxacin									1		90		4					
moxifloxacin					1		59	35										
penicillin				58				1	1		1				30	4		
SXT				2			53	5	1				1	33				
Ukraine ($n = 100$)																		
amoxicillin	51		32		2		3	1			6		2				3	
AMC	84		2		1		1	1	1		7				2		1	
azithromycin		4			13		59	8						16				
cefaclor								2	26		58		1		5	8		
ceftriaxone	3		78		3		3	1	2		8		2					
cefuroxime						88					1		2		6	3		
clarithromycin	73		10					1	1		2	13						
erythromycin			79		4					17								
levofloxacin											91		9					
moxifloxacin					21		58	20	1									
penicillin				83			2	3	1		3		5		3			
SXT							49	11	15				13	12				

AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole.

low-dose breakpoints; using high-dose PK/PD criteria susceptibility to amoxicillin remained at 97.0%, whereas susceptibility to amoxicillin/clavulanic acid increased to 99.0%. Susceptibility by CLSI breakpoints to the oral cephalosporins and the macrolides ranged from 83.0% for erythromycin to 89.0% for cefuroxime. Susceptibility was the same or similar by PK/PD and EUCAST breakpoints except for susceptibility of isolates to cefaclor, which showed values of 28.0% using PK/PD criteria and 0% using EUCAST standards. Trimethoprim/sulfamethoxazole was the only agent to which <80% of isolates were susceptible (75.0% by all three breakpoints) (Table 2).

Antimicrobial susceptibility using CLSI criteria was compared across specimen sources and age groups. The only statistically significant difference observed was for the macrolides between isolates from paediatric patients ($n = 27$; 70.4% susceptible to all three macrolides) and adults ($n = 65$; 89.2% susceptible, $P = 0.03$) and for cefaclor between isolates from paediatric patients (70.4% susceptible) and adults (90.8% susceptible, $P = 0.02$).

Antimicrobial susceptibility of S. pneumoniae by penicillin susceptibility

Among the 195 *S. pneumoniae* isolates from both countries, 141 (72.3%) were penicillin susceptible (PSSP), 12 (6.2%) were penicillin intermediate (PISP) and 42 (21.5%) were penicillin resistant (PRSP) according to CLSI oral breakpoints. PSSP and PRSP sub-groups had a sample size >20 and were compared for susceptibility to other antimicrobial agents (Figure 1). Among PSSP isolates, susceptibility to the fluoroquinolones, amoxicillin, amoxicillin/clavulanic acid, cefuroxime and ceftriaxone was 100%, to cefaclor it was 97.9%, to trimethoprim/sulfamethoxazole 90.8% and to the macrolides 80.9% (using CLSI criteria). The fluoroquinolones also maintained 100% activity against PRSP isolates, but susceptibility to the other agents was significantly lower than in PSSP isolates ($P < 0.0001$), with rates ranging from 0% to 12% to all remaining agents except ceftriaxone (54.8%).

H. influenzae isolates

A total of 194 *H. influenzae* isolates were collected from the three centres in the Slovak Republic and Ukraine from 2014 to 2016. Specimen origins of the isolates included sputum ($n = 91$; 46.9%), sinuses ($n = 43$; 22.2%), bronchoalveolar lavage ($n = 30$; 15.5%), endotracheal aspirate ($n = 14$, 7.2%) and middle ear effusion ($n = 10$, 5.2%). Six isolates (3.1%) were from an undisclosed respiratory source. Most isolates ($n = 116$; 59.8%) came from adult patients, 52 (26.8%) were from children and 26 (13.4%) were from elderly patients. The rate of β-lactamase-positive isolates was

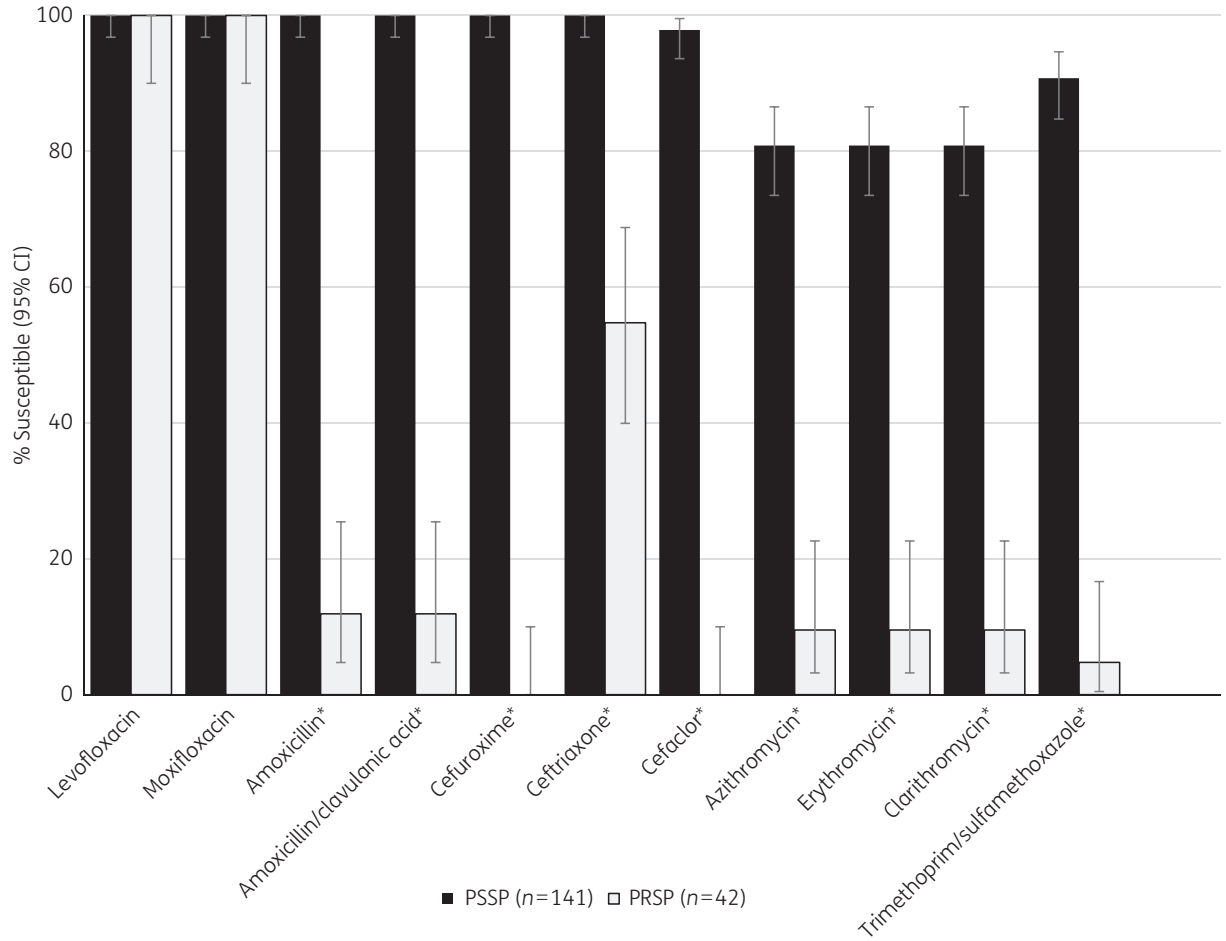

Figure 1. Percentage susceptibility rates (with 95% CI) using CLSI criteria for antimicrobials against all *S. pneumoniae* according to susceptibility to penicillin (oral). *PSSP significantly more susceptible than PRSP isolates ($P < 0.0001$). PSSP, penicillin-susceptible *S. pneumoniae*; PRSP, penicillin-resistant *S. pneumoniae*.

5.1% in the Slovak Republic (5/98) and 7.3% in Ukraine (7/96). Seven isolates from the Slovak Republic (7.1%) and one from Ukraine (1.0%) were β-lactamase-negative-ampicillin-resistant (BLNAR) by CLSI breakpoints (ampicillin MIC $\geq$4 mg/L), and eight from the Slovak Republic (8.2%) and one from Ukraine (1.0%) were BLNAR by EUCAST breakpoints (ampicillin MIC $\geq$2 mg/L).

Summary MIC and susceptibility data for all 194 *H. influenzae* isolates broken down by country of origin are shown in Table 4. MIC distribution data are given in Table 5.

H. influenzae susceptibility in the Slovak Republic

All isolates of *H. influenzae* from the Slovak Republic were susceptible to levofloxacin and moxifloxacin by all three breakpoint guidelines as well as to ceftriaxone by CLSI and PK/PD breakpoints (but susceptibility was reduced to 96.9% by EUCAST criteria). Susceptibility testing also resulted in 100% susceptibility to amoxicillin/clavulanic acid using CLSI and PK/PD high-dose breakpoints. However, CLSI guidelines state that BLNAR strains should be considered resistant to amoxicillin/clavulanic acid, cefuroxime and cefaclor, even if they appear susceptible *in vitro*.[7] This reduces the percentage susceptible to amoxicillin/clavulanic acid to 92.9%, the same rate as obtained using PK/PD low-dose and EUCAST breakpoints. Similarly, susceptibility to cefuroxime was reduced from 100% to 92.9% by CLSI criteria after adjusting for BLNAR, and susceptibility to cefaclor was reduced from 89.8% to 86.7%. Susceptibility to both agents was dramatically lower using the other breakpoints (82.7% and 1.0% by PK/PD and EUCAST criteria, respectively, for cefuroxime, and 0% by PK/PD breakpoints for cefaclor). Susceptibility to ampicillin and amoxicillin was 86.7%, to trimethoprim/sulfamethoxazole it was 77.6% by all available breakpoints; and to clarithromycin 34.7% by CLSI standards and 0% by PK/PD and EUCAST criteria.

Antimicrobial susceptibility was compared by specimen source and age group with no significant difference observed (data not shown).

H. influenzae susceptibility in Ukraine

In Ukraine, susceptibility of *H. influenzae* ($n = 96$) was 100% to ceftriaxone, levofloxacin and moxifloxacin by all three breakpoint

Table 4. MIC and susceptibility data for *H. influenzae* isolates

						Susceptibility using indicated breakpoints						
		MIC (mg/L)				CLSI			PK/PD	EUCAST		
Antimicrobial	*n*	50%	90%	min	max	%S	%I	%R	%S	%S	%I	%R
Slovak Republic												
amoxicillin[a]	98	≤2	8	≤2	>128	NA	NA	NA	86.7 (89.8)	86.7	–	13.3
AMC[a,b]	98	0.5	2	0.25	4	100 (92.9)	–	0.0 (7.1)	92.9 (100)	92.9	–	7.1
ampicillin	98	0.25	4	≤0.06	>128	86.7	1.0	12.2	–	86.7	–	13.3
azithromycin	98	2	2	1	4	100	–	–	0.0	0.0	100	0.0
cefaclor[b]	98	4	16	1	16	89.8 (86.7)	10.2 (6.1)	0.0 (7.1)	0.0	NA	NA	NA
ceftriaxone	98	≤0.03	0.06	≤0.03	0.25	100	–	–	100	96.9	–	3.1
cefuroxime[b]	98	0.5	2	0.06	4	100 (92.9)	0.0	0.0 (7.1)	82.7	1.0	81.6	17.4
clarithromycin	98	16	16	8	>16	34.7	59.2	6.1	0.0	0.0	100	0.0
levofloxacin	98	0.015	0.03	0.008	0.06	100	–	–	100	100	–	0.0
moxifloxacin	98	0.015	0.015	0.008	0.06	100	–	–	100	100	–	0.0
SXT	98	0.25	>2	0.03	>2	77.6	3.1	19.4	77.6	77.6	0.0	22.5
Ukraine												
amoxicillin[a]	96	≤2	4	≤2	>128	NA	NA	NA	87.5 (91.7)	87.5	–	12.5
AMC[a,b]	96	0.5	2	0.25	4	100 (99.0)	–	0.0 (1.0)	95.8 (100)	95.8	–	4.2
ampicillin	96	0.25	1	0.12	>128	91.7	0.0	8.3	–	91.7	–	8.3
azithromycin	96	2	2	0.12	8	97.9	–	–	2.1	2.1	95.8	2.1
cefaclor[b]	96	4	16	1	32	87.5	6.3	6.3	0.0	NA	NA	NA
ceftriaxone	96	≤0.03	≤0.03	≤0.03	0.12	100	–	–	100	100	–	0.0
cefuroxime[b]	96	1	4	0.25	8	97.9 (96.9)	2.1	0.0 (1.0)	70.8	0.0	70.8	29.2
clarithromycin	96	8	16	≤1	>16	61.5	37.5	1.0	0.0	2.1	97.9	0.0
levofloxacin	96	0.015	0.03	0.008	0.06	100	–	–	100	100	–	0.0
moxifloxacin	96	0.015	0.03	0.008	0.12	100	–	–	100	100	–	0.0
SXT	96	0.25	>2	≤0.015	>2	60.4	2.1	37.5	60.4	60.4	0.0	39.6

AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole; S, susceptible; I, intermediate; R, resistant; NA, not applicable; min, minimum; max, maximum.

[a]Amoxicillin and amoxicillin/clavulanic acid PK/PD susceptibility at high dose shown in parentheses.

[b]In clinical settings, isolates of BLNAR are considered resistant to amoxicillin/clavulanic acid, cefaclor and cefuroxime. If clinical susceptibility to these agents was reduced due to BLNAR, data are shown in parentheses.

guidelines. Susceptibility to amoxicillin/clavulanic acid *in vitro* was also 100% by CLSI breakpoints and high-dose PK/PD breakpoints, but because one isolate was a BLNAR strain, susceptibility according to CLSI standards was slightly reduced to 99.0%. Susceptibility was 95.8% using low-dose PK/PD or EUCAST breakpoints. Similarly, susceptibility to cefuroxime was slightly reduced from 97.9% to 96.9% after adjusting for BLNAR using CLSI criteria; susceptibility was reduced much further using the other breakpoint criteria (70.8% by PK/PD and 0% by EUCAST standards). Susceptibility to cefaclor by CLSI criteria did not change as one BLNAR isolate had already tested resistant to cefaclor (87.5%), but susceptibility was 0% using the PK/PD breakpoint. A similarly dramatic difference between breakpoints was again seen for clarithromycin (61.5% by CLSI criteria, 0% by PK/PD and 2.1% by EUCAST). Susceptibility to trimethoprim/sulfamethoxazole was seen in 60.4% of isolates by all breakpoints.

Insufficient isolates were obtained to analyse antimicrobial susceptibility by specimen source or age.

Discussion

The countries in eastern Europe studied for this report showed substantial variability in the susceptibility of *S. pneumoniae*, similar to findings in other regions of the world that have been studied with the SOAR programme.[2–5] *S. pneumoniae* isolates collected in Ukraine ($n = 100$) showed higher susceptibility than those in the Slovak Republic, with rates ≥97% for all studied agents except oral penicillin, macrolides and cefaclor (between 83% and 86% susceptible) as well as cefuroxime (89%) and trimethoprim/sulfamethoxazole (75%). In the Slovak Republic, the fluoroquinolones remained 100% active, but of the other tested agents only ceftriaxone exceeded 80% susceptibility by CLSI criteria. Penicillin (oral and iv), amoxicillin, amoxicillin/clavulanic acid, cefaclor, cefuroxime and trimethoprim/sulfamethoxazole showed susceptibility between 58% and 64%, and the macrolides only 44%. There was significantly lower antimicrobial susceptibility (CLSI breakpoints) seen with isolates from the Slovak Republic compared with

Table 5. MIC distribution data for all *H. influenzae* isolates

	Number of isolates at MIC (mg/L)																								
Antimicrobial	≤0.004	0.008	≤0.015	0.015	≤0.03	0.03	≤0.06	0.06	0.12	0.25	≤0.5	0.5	≤1	1	≤2	2	>2	4	8	16	>16	32	>32	64	128	>128
Slovak Republic ($n = 98$)																										
amoxicillin															85			3	5							5
AMC										11		50		20		10		7								
ampicillin							1		12	54				18		1		7		1					1	3
azithromycin														16		78		4								
cefaclor														5		41		30	12	10						
ceftriaxone					88			1	6	3																
cefuroxime								1		1		54		25		8		9								
clarithromycin																			34	58	6					
levofloxacin	47			12		35		4																		
moxifloxacin	18			73		6		1																		
SXT						8		29	10	29						3	19									
Ukraine ($n = 96$)																										
amoxicillin															84			4	1		1		4			2
AMC										10		39		20		23		4								
ampicillin									7	55				26				1		1			1	2		3
azithromycin									2	2		8		36		43		3	2							
cefaclor														5		22		36	21	6		6				
ceftriaxone					93			2	1																	
cefuroxime										3		32		33		18		8	2							
clarithromycin													2					6	51	36	1					
levofloxacin	9			59		21		7																		
moxifloxacin	20			55		16		4	1																	
SXT			1			7		15	14	21						2	36									

isolates from Ukraine to penicillin (iv), amoxicillin, amoxicillin/clavulanic acid, cefuroxime, cefaclor or macrolides ($P < 0.0001$), ceftriaxone ($P = 0.002$) and oral penicillin ($P = 0.001$).

Few data on antimicrobial susceptibility have been published for these countries. The most recent data available were generated through the European Antimicrobial Resistance Surveillance Network (EARS-Net);[10] however, only the Slovak Republic participated and only invasive (blood and CSF) isolates are included in EARS-Net surveillance. Nevertheless the EARS-Net data of 22.2% penicillin non-susceptibility in 2015[10] confirmed our findings of low susceptibility to penicillin in the Slovak Republic even though the non-susceptibility rate was higher in the current study of respiratory isolates (38.9%). Interestingly, Marchese and Schito described high resistance rates among respiratory *S. pneumoniae* isolates from the Slovak Republic over 15 years ago: data from the Alexander Project from 1997 showed penicillin resistance of 26.7% and macrolide resistance of 13.3%.[11] Riedel *et al.*[12] also reported high non-susceptibility rates to penicillin (52.3%) and erythromycin (36.4%) in isolates from community-acquired respiratory tract infections collected in 2004–05 in the Slovak Republic. The same study showed that antimicrobial use in the Slovak Republic between 1998 and 2004 was third highest among 15 European countries, thus providing evidence for the association between antimicrobial resistance and increased use of antibiotics.[12,13] In a more recent study of antibiotic consumption in 2011 in the broader European region, the Slovak Republic also ranked high (15th among 42 countries) in total antibiotic use.[14]

Ukraine was not included in any of these studies, but data from an earlier SOAR report are available for comparison.[15] Susceptibility among the *S. pneumoniae* isolates from 2014 to 2016 included in the current study was similar to the *S. pneumoniae* isolates collected in 2011–13, with only a small decrease of between 2 and 5 percentage points for most antimicrobial agents. For example, amoxicillin/clavulanic acid showed susceptibility by CLSI criteria of 100% in 2011–13 compared with 97.0% in the current study, and the respective values for oral penicillin were 87.3% and 83.0%. The exception is trimethoprim/sulfamethoxazole, where susceptibility was very low in 2011–13 (9.7%),[11] but 75% in the current study for 2014–16. It would be interesting to monitor these fluctuations in future studies.

In contrast to *S. pneumoniae*, antimicrobial activity against *H. influenzae* was fairly homogeneous between the two countries. Susceptibility to amoxicillin/clavulanic acid, ceftriaxone, cefuroxime, azithromycin and the fluoroquinolones was >90% by CLSI criteria. Susceptibility to clarithromycin was lower and more variable (35% in the Slovak Republic compared with 61.5% in Ukraine), as was trimethoprim/sulfamethoxazole (77.6% in the Slovak Republic compared with 60.4% in Ukraine). The ampicillin resistance rate was 12.2% in the Slovak Republic, where the β-lactamase rate was 5.1% and an additional 7.1% of isolates were BLNAR. Ampicillin resistance was lower in Ukraine (8.3%) due to a lower β-lactamase rate (7.3%) and a low rate of BLNAR isolates (1.0%). These rates were also low in Ukraine in 2011–13, with a β-lactamase rate of 4.5% and no BLNAR isolates.[15] Furthermore, susceptibility rates of

H. influenzae in Ukraine were similar in the current study compared with the results in 2011–13, with the activity of most antimicrobial agents either still at 100% or only about 2 percentage points lower than in the earlier study. The exception is clarithromycin, where susceptibility was 61.5% using CSLI criteria in 2014–16 but 98.5% in 2011–13.[15] Information on *H. influenzae* is sparse in the literature for the Slovak Republic. Interestingly, β-lactamase rates found through the Alexander Project 20 years ago were similar to those in our current study (5% in 1996 and 1997 in the Slovak Republic).[11] Furthermore, the Slovak Republic, which had the highest BLNAR rate in the current study, was one of only four European countries where such isolates were found in 1997 (albeit at a very low rate).[11]

Although antimicrobial activity against *H. influenzae* was fairly homogeneous in the two countries, this was not the case when comparing susceptibility rates obtained using different breakpoints. For example, whereas >97% of isolates were susceptible to azithromycin using CLSI breakpoints, only ≤2.1% were susceptible using PK/PD and EUCAST criteria. Similarly, large differences were found for cefaclor between CLSI and PK/PD breakpoints and for cefuroxime between CLSI/PK/PD and EUCAST standards. Furthermore, although differences between breakpoints were small for most antimicrobial agents for *S. pneumoniae*, they were substantial for cefaclor. For example, in Ukraine 86.0% of *S. pneumoniae* isolates were susceptible by CLSI criteria, 28.0% by PK/PD and 0% using EUCAST standards. These differences are confusing for clinicians, but also hamper the use of surveillance data for research and public health efforts like antimicrobial stewardship.

This study's main limitation stems from having to draw conclusions about a country's antimicrobial susceptibility levels using data from just one or two hospital sites. It is reassuring that the results for Ukraine were very similar in the current study, which used two sites, to the earlier SOAR report, with eight sites. Nevertheless, more solid susceptibility rates can no doubt be achieved if surveillance is undertaken at more hospitals in each country, whether independently or as part of a national, regional or even international surveillance programme. Larger surveillance studies, such as SOAR, have the advantage of being able to employ a consistent methodology, which facilitates comparison between regions as well as trend analyses over time, but even surveillance at a single hospital can help local clinicians in selecting antibiotics for empirical therapy. In this study, data from just one or two hospitals per country were able to confirm a pattern of resistance already seen many years ago: high resistance of *S. pneumoniae* in the Slovak Republic compared with its neighbour Ukraine to the east. Regular monitoring and publication of such resistance data are not only critical for effective empirical therapy but can also be used to help shape antimicrobial stewardship strategies in an effort to lower historically high resistance rates.

Acknowledgements

We thank Gudrun Maechler and Olena Sopko for reviewing the manuscript and Jana Galisinova and Zuzana Benova for recruiting the centres.

Funding

This study was funded by GlaxoSmithKline.

Transparency declarations

This article forms part of a Supplement sponsored by GlaxoSmithKline. D. Torumkuney is an employee of GlaxoSmithKline and holds shares in GlaxoSmithKline. O. Sopko is an employee of GlaxoSmithKline and does not hold any shares. I. Morrissey is an employee of IHMA, a medical communication and consultancy company, who participated in the exploration, interpretation of the results and preparation of this manuscript on behalf of GlaxoSmithKline. IHMA also provided medical writing support in the form of writing assistance, collating authors' comments, grammatical editing and referencing that was paid for by GlaxoSmithKline. All other authors declare that they have no conflict of interest. Editorial assistance was provided by Tracey Morris, Livewire Editorial Communications.

References

1 World Health Organization. *Top 10 Causes of Death. Situation and Trends*. http://www.who.int/gho/mortality_burden_disease/causes_death/top_10/en/.

2 Soyletir G, Altinkanat G, Gur D *et al*. Results from the Survey of Antibiotic Resistance (SOAR) 2011–13 in Turkey. *J Antimicrob Chemother* 2016; **71** Suppl 1: i71–83.

3 Jamsheer A, Rafay AM, Daoud Z *et al*. Results from the Survey of Antibiotic Resistance (SOAR) 2011–13 in the Gulf States. *J Antimicrob Chemother* 2016; **71** Suppl 1: i45–61.

4 Torumkuney D, Chaiwarith R, Reechaipichitkul W *et al*. Results from the Survey of Antibiotic Resistance (SOAR) 2012–14 in Thailand, India, South Korea and Singapore. *J Antimicrob Chemother* 2016; **71** Suppl 1: i3–19.

5 Kacou-Ndouba A, Revathi G, Mwathi P *et al*. Results from the Survey of Antibiotic Resistance (SOAR) 2011–14 in the Democratic Republic of Congo, Ivory Coast, Republic of Senegal and Kenya. *J Antimicrob Chemother* 2016; **71** Suppl 1: i21–31.

6 Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Tenth Edition: Approved Standard M7-A10*. CLSI, Wayne, PA, USA, 2015.

7 Clinical and Laboratory Standards Institute. *Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Seventh Informational Supplement M100-S27*. CLSI, Wayne, PA, USA, 2017.

8 European Committee on Antimicrobial Susceptibility Testing. *Breakpoint Tables for Interpretation of MICs and Zone Diameters, Version 7.1, 2017*. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf.

9 Anon JB, Jacobs MR, Poole MD *et al*. Antimicrobial treatment guidelines for acute bacterial rhinosinusitis. *Otolaryngol Head Neck Surg* 2004; **130** Suppl 1: 1–45.

10 European Centre for Disease Prevention and Control. *Antimicrobial Resistance Surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net)*. Stockholm: ECDC, 2017.

11 Marchese A, Schito GC. Resistance patterns of lower respiratory tract pathogens in Europe. *Int J Antimicrob Agents* 2000; **16** Suppl 1: S25–9.

12 Riedel S, Beekmann SE, Heilmann KP *et al*. Antimicrobial use in Europe and antimicrobial resistance in *Streptococcus pneumoniae*. *Eur J Clin Microbiol Infect Dis* 2007; **26**: 485–90.

13 Goossens H. Antibiotic consumption and link to resistance. *Clin Microbiol Infect* 2009; **15** Suppl 3: 12–5.

14 Versporten A, Bolokhovets G, Ghazaryan L *et al*. Antibiotic use in eastern Europe: a cross-national database study in coordination with the WHO Regional Office for Europe. *Lancet Infect Dis* 2014; **14**: 381–7.

15 Feshchenko Y, Dzyublik A, Pertseva T *et al*. Results from the Survey of Antibiotic Resistance (SOAR) 2011–13 in Ukraine. *J Antimicrob Chemother* 2016; **71** Suppl 1: i63–9.