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Lack of correlation between reduced outpatient consumption of macrolides and macrolide resistance of invasive *Streptococcus pneumoniae* isolates in Slovenia during 1997–2017

Tamara Kastrin[a,*], Metka Paragi[a], Vanja Erčulj[b], Tjaša Žohar Čretnik[c], on behalf of the Slovenian Meningitidis Study Group[1], Tom Bajec[d], Milan Čižman[e]

[a] *National Laboratory of Health, Environment and Food, Department for Public Health Microbiology, Grablovičeva 44, 1000 Ljubljana, Slovenia*
[b] *Rho Sigma Research & Statistics, Ljubljana, Slovenia*
[c] *National Laboratory of Health, Environment and Food, Centre for Medical Microbiology, Prvomajska ulica 1, 2000 Maribor, Slovenia*
[d] *Tomtim d.o.o, Ljubljana, Slovenia*
[e] *University Medical Centre Ljubljana, Department of Infectious Diseases, Japljeva 2, 1525 Ljubljana, Slovenia*

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ABSTRACT

Objectives: The objective of this study was to investigate the correlation between decreased national consumption of macrolides and resistance of invasive *Streptococcus pneumoniae* isolates in Slovenia during 1997–2017.

Methods: A total of 4241 invasive *S. pneumoniae* isolates were collected in Slovenia from 1997 to 2017. The presence of *erm*(B), *mef*(E), *mef*(A) and *erm*(TR) genes was determined by PCR in 612 erythromycin-resistant isolates. Selected isolates carrying the *mef*(A) gene were further examined by pulsed-field gel electrophoresis (PFGE). Multilocus sequence typing (MLST) was performed for 161 erythromycin-resistant isolates from 2004 to 2009.

Results: Consumption of macrolides decreased by 42.5% between 1997 and 2017, and by 57.0% from the highest consumption during 1999 to 2017. Resistance of *S. pneumoniae* increased by 120.7% in the same period, from 5.8% in 1997 to 12.8% in 2017. The most prevalent serotypes among macrolide-resistant isolates were 14 (54.9%), 19A (9.0%), 19F (8.3%), 6B (7.2%), 6A (5.2%) and 9V (19; 3.0%). The most prevalent determinant of macrolide resistance in the observed period was *erm*(B) (43.0%; 263/612), followed by *mef*(A) (36.3%; 222/612) and *mef*(E) (14.9%; 91/612). During the study period, an increasing trend in serotype 14, *mef*(A)-carrying isolates was observed, with a peak in 2011 ($P < 0.001$); 63/71 isolates (88.7%) with the *mef*(A) gene were clonally related and were related to the international England^{14}-9 clonal cluster.

Conclusions: The reason for the observed increase in macrolide resistance among invasive *S. pneumoniae* in Slovenia despite decreased macrolide consumption was spread of the England^{14}-9 clonal cluster.

1. Introduction

Macrolides are commonly used antibiotics in outpatients. In 2012, overall consumption of macrolides varied in European Union/European Economic Area (EU/EEA) countries by a factor of 25, from 0.3 defined daily doses (DDD) per 1000 inhabitants per day (DID) (Sweden) to 7.4 DID (Greece), with a population-weighted mean consumption of 2.8 DID [1]. Consumption of macrolides varied from 2–23% of total consumption of all antibacterials for systemic use. Intermediate-acting macrolides accounted for 61% of the total macrolide consumption, followed by long-and short-acting macrolides [1]. As in previous years, wide intercountry variations in macrolide susceptibility of invasive *Streptococcus pneumoniae* were noted in 2016 [2]. In 2016, the national percentage of isolates from invasive pneumococcal diseases with non-susceptibility to macrolides ranged between 0.0% (Iceland) and 60% (Cyprus) [2]. A significant decreasing trend was observed during the period 2013–2016 in only two countries (Finland and Belgium). A study by Goossens showed that macrolide use is the single most important driver of the emergence of macrolide resistance in vivo [3]. If usage of antimicrobials is the main driver of resistance, it seems logical to reduce antimicrobial

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[*] Corresponding author.
E-mail address: tamara.kastrin@nlzoh.si (T. Kastrin).

[1] Members of the Slovenian Meningitidis Study Group are listed in Acknowledgments.

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resistance by reducing their usage. Evidence of positive correlations are mixed [4–7]. No study has reported the complete reversal of acquired drug resistance in bacterial populations that are no longer exposed to specific antimicrobial drugs.

The objective of this study was to evaluate the national long-term correlation between consumption of macrolides by outpatients and macrolide resistance among invasive *S. pneumoniae* isolates in Slovenia.

2. Materials and methods

2.1. Use of antibiotics

Ambulatory care data were collected for the period 1997–2017 using the Anatomical Therapeutic Chemical Classification System with Defined Daily Doses (ATC/DDD) (WHO version 2017), with the results expressed in DID [8].

2.2. Bacterial isolates and serotyping

Surveillance of invasive diseases caused by *S. pneumoniae* has been performed constantly in Slovenia in children since 1993 and in adults since 1997 [9]. *S. pneumoniae* isolates recovered from blood cultures and cerebrospinal fluid from all Slovenian microbiological laboratories were collected at the Department for Public Health Microbiology, National Laboratory of Health, Environment and Food (Ljubljana, Slovenia). Isolates were identified by typical colony morphology and haemolysis on blood agar and were further tested for optochin sensitivity using an optochin disk (Oxoid Ltd., Basingstoke, UK) and bile solubility. Altogether, 4241 *S. pneumoniae* isolates were collected during 1997–2017, of which 992 (23.4%) were from children (aged 0–14 years) and 3249 (76.6%) were from adult patients. Isolates were serotyped by the Quellung (or Neufeld) reaction using antisera provided by the Statens Serum Institut (Copenhagen, Denmark).

2.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed by the disk diffusion method for oxacillin (screening test), erythromycin, clindamycin, vancomycin and rifampicin and by Etest (bioMérieux, Marcy-l'Étoile, France) for the determination of minimum inhibitory concentrations (MICs) of penicillin, cefotaxime, ceftriaxone, cefuroxime, erythromycin, tetracycline, chloramphenicol and trimethoprim/sulfamethoxazole (SXT). The recommendations of the Clinical and Laboratory Standards Institute (CLSI) were followed for isolates from 1997 to 2014 [10], whilst for isolates from 2015 the European Committee on Antimicrobial Susceptibility Testing (EUCAST) recommendations were followed [11]. *Streptococcus pneumoniae* ATCC 49619 was used as a quality control strain.

2.4. Bacterial DNA isolation and detection of resistance determinants

Resistance determinants were analysed for 612 erythromycin-resistant *S. pneumoniae* isolated from 1997 to 2017. Isolation of DNA for PCR was performed using a MagNA Pure instrument and MagNA Pure LC DNA Isolation Kit III (Roche Diagnostics GmbH, Manheim, Germany) according to the manufacturer's instructions. Each macrolide-resistant isolate was tested for the presence of the *erm*(B), *erm*(TR) and *mef* genes by PCR with sequence-specific primers and reaction conditions according to Sutcliffe et al. [12]. Distinction between *mef*(A) and *mef*(E) was made by PCR–restriction fragment length polymorphism (PCR-RFLP) as described by Del Grosso et al. [13].

2.5. Pulsed-field gel electrophoresis (PFGE)

Genomic DNA of the selected isolates was digested with the restriction enzyme *Sma*I. Fragments were separated by PFGE in 0.5× TBE [tris–borate–ethylene diamine tetra–acetic acid (EDTA)] buffer on 1% agarose (Bio-Rad, Hercules, CA) in GenePath programme 12 (18.5 h, 14 °C at 6 V/cm with a ramped pulse time of 1 s–17 s). Following electrophoresis, gels were stained for 30 min with 0.5 mg/L ethidium bromide. DNA bands were visualised on a Gel Doc XR system with the Quantity One Software programme (Bio-Rad). BioNumerics software (Applied-Maths, Sint-Martens-Latem, Belgium) was used to generate UPGMA (unweighted pair-group method with arithmetic mean) dendrograms of fragment patterns with the Dice coefficient (optimisation and position tolerance settings of 1.0% and 1.5%). PFGE-based clusters were defined as isolates with $\geq$80% genetic relatedness. *Staphylococcus aureus* NCTC 8352 strain was used for quality control. PFGE patterns were compared with 13 representative international pneumococcal clones from the Pneumococcal Molecular Epidemiology Network (PMEN).

2.6. Multilocus sequence typing (MLST)

MLST was performed as described by Enright and Spratt [14] with modified primers (http://www.cdc.gov/streplab/alt-mlst-primers.html). Briefly, the internal fragments of seven housekeeping genes (*aroE*, *gdh*, *gki*, *recP*, *spi*, *xpt* and *ddl*) of the pneumococcal genome were amplified and sequenced and the sequence types (STs) were then determined by comparing the sequences with alleles from the pneumococcal MLST database (http://pubmlst.org/spneumoniae/). New alleles and STs were submitted to the database curator for assignment.

2.7. Statistical analysis

Separate time series analysis was done for the resistance of *S. pneumoniae* to erythromycin and consumption of macrolides to establish whether a linear trend was present in the data. The correlation between the two time series (resistance and consumption) was done by calculating the Pearson correlation coefficient. Before calculating the Pearson correlation coefficient, each time series was examined for stationarity via partial autocorrelation plot. None of the series was stationary, therefore the change from one period to another (first difference) was calculated. By differencing, the time series became stationary and correlation between then could be calculated. Separate time series analysis on resistance to probe for linear trend in the data was also done on the subgroup of children and adults, for each of isolates with *mef*(E), *erm*(B), *mef*(A) genes as well as for each serotype. IBM SPSS Statistics v.24.0. (IBM Corp., Armonk, NY) was used for statistical analysis. *P*-values of <0.05 were considered statistically significant [15].

3. Results

3.1. Consumption of macrolides and resistance of S. pneumoniae to erythromycin

In 1997 consumption of macrolides was 2.85 DID, which increased to the highest consumption of 3.81 DID in 1999 and then slowly decreased, with temporary increases, to 1.64 DID in 2017 (Table 1). Consumption of macrolides decreased by 42.5% from 2.85 DID in 1997 to 1.64 DID in 2017, and by 57.0% from the highest consumption of 3.81 DID during 1999–2017. On average, consumption decreased by 0.11 DID ($P < 0.001$). Resistance of *S. pneumoniae* increased from 5.8% in 1997 to 24.6% in 2011 and then decreased slowly to 12.8% in 2017 (Table 1). Overall, a statistically

Table 1
Erythromycin resistance in invasive *Streptococcus pneumoniae* in children and adults, and use of macrolides in Slovenia, 1997–2017.

Year	Children		Adults		All		Macrolide use (DID)[a]
	n	%R	*n*	%R	*n*	%R	
1997	31	6.5	72	5.6	103	5.8	2.85
1998	46	4.3	59	6.8	105	5.7	3.53
1999	32	3.1	77	3.9	109	4.6	3.81
2000	41	12.2	70	5.7	111	8.1	3.58
2001	43	14.0	105	11.4	148	12.2	3.17
2002	30	10.0	62	16.1	92	14.1	2.82
2003	38	18.4	115	4.3	153	7.8	2.99
2004	34	17.6	137	10.2	171	11.1	3.02
2005	39	17.9	172	9.9	211	11.3	3.04
2006	31	22.6	126	11.1	157	13.4	2.31
2007	65	24.6	126	12.7	191	16.8	2.44
2008	59	30.5	144	12.5	203	17.7	2.23
2009	71	28.2	182	13.7	253	17.8	2.12
2010	52	34.6	180	13.9	232	18.5	1.89
2011	63	46.0	197	17.8	260	24.6	1.85
2012	60	38.3	185	15.1	245	20.8	1.72
2013	48	20.8	226	8.4	274	10.6	1.72
2014	77	29.9	212	16.5	289	20.1	1.64
2015	47	29.8	285	16.8	332	18.7	1.79
2016	46	21.7	235	13.6	281	14.9	1.51
2017	39	12.8	282	12.8	321	12.8	1.64
Total	992		3249		4241		

n, total number of isolates; %R, percentage of resistant isolates; DID, defined daily doses (DDD) per 1000 inhabitants per day.

[a] Using the Anatomical Therapeutic Chemical Classification System with Defined Daily Doses (ATC/DDD) [8].

significant positive trend in the resistance of *S. pneumoniae* was observed (reg. coeff. = 0.6; $P < 0.001$). This trend was stronger in the case of isolates from children (reg. coeff. = 1.2; $P = 0.001$), where the highest resistance rate was 46.0% in 2011, followed by a decreasing trend to 12.8% in 2017.

In 2013, a substantial decrease in resistance (to 10.6%) was observed. During the 21-year period (1997–2017), azithromycin was the most commonly prescribed macrolide, except for 4 years (2000, 2003–2005), followed by clarithromycin (Fig. 1). During the analysed years we ceased to prescribe erythromycin, roxithromycin and dirithromycin, and the consumption of midecamycin, azithromycin and miocamycin decreased by 86%, 40% and 84%, respectively. Consumption of clarithromycin increased by 104% from 0.28 DID to 0.57 DID (Fig. 1).

3.2. Correlation between consumption of macrolides and resistance of S. pneumoniae *to erythromycin*

A partial autocorrelation plot of consumption of macrolides and resistance of *S. pneumoniae* to erythromycin showed that the time series was not stationary. To make the time series stationary, the first difference (change from one period to another) of each time series was computed. No statistically significant correlation between the two stationary time series was found ($r = -0.20$; $P = 0.408$). Probing different time lags of macrolide consumption revealed no statistically significant correlation with *S. pneumoniae* resistance to erythromycin.

3.3. Resistance genotypes

Of 4241 invasive *S. pneumoniae* isolates collected during the years 1997–2017 in Slovenia, 636 (15.0%) showed resistance to erythromycin (MIC ≥ 1 mg/L), among which 232 were isolated from children (aged 0–14 years) and 404 were from adults (>14 years). Excluding 24 isolates that did not grow, the molecular basis of macrolide resistance was determined for 612 erythromycin-resistant isolates from 1997 to 2017, of which 263 (43.0%) had the *erm*(B) gene, 222 (36.3%) had the *mef*(A) gene, 91 (14.9%) had the *mef*(E) gene and 36 (5.9%) did not carry any of the tested genes. The *erm*(TR) gene was negative for all tested isolates. No dual mechanism of resistance was detected.

Erythromycin MICs were ≥256 mg/L for *erm*(B)-positive isolates, 4–32 mg/L for *mef*(A)-positive isolates and 2–32 mg/L for *mef* (E)-positive isolates. During the observed period, the number of isolates with the *erm*(B) gene was constantly increasing (reg. coeff. = 0.85; $P < 0.001$), the increase in the number of isolates with the *mef*(E) gene was lower (reg. coeff. = 0.21; $P = 0.003$), whereas the number of isolates with the *mef*(A) gene increased greatly (reg. coeff. = 1.41; $P < 0.001$) and reached a peak in 2011 (Fig. 2).

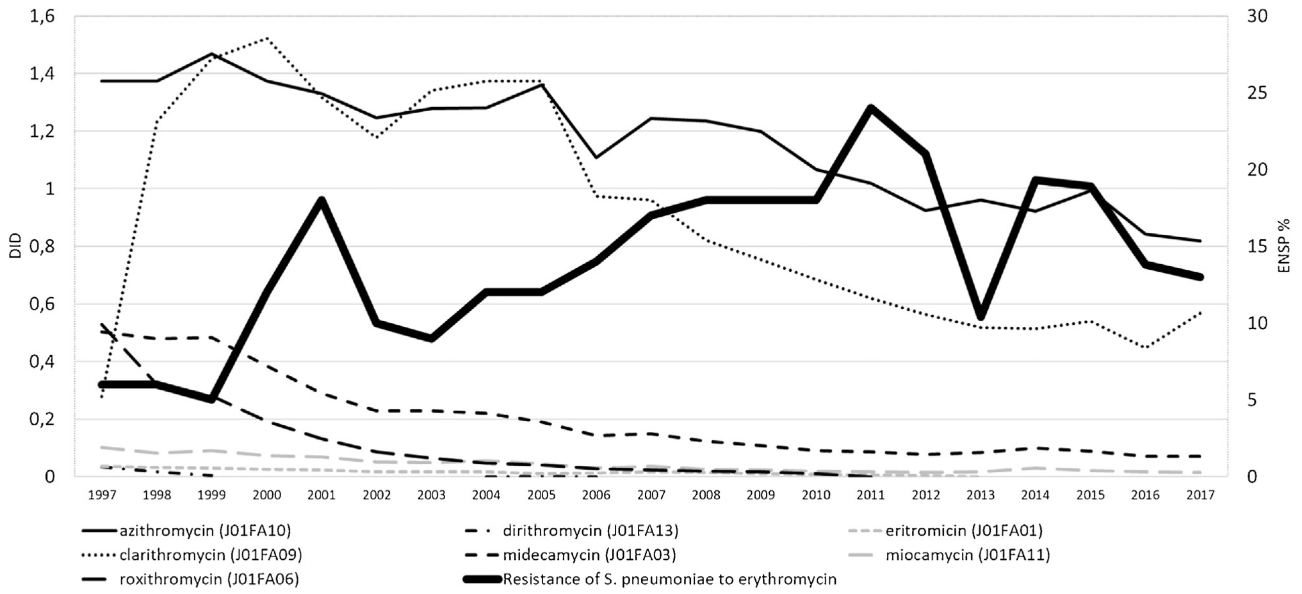


Fig. 1. Outpatient consumption of macrolides in defined daily doses (DDD) per 1000 inhabitants per day (DID) (left-hand axis) and erythromycin resistance of invasive *Streptococcus pneumoniae* (right-hand axis) in Slovenia, 1997–2017. ENSP, erythromycin-non-susceptible *S. pneumoniae.*

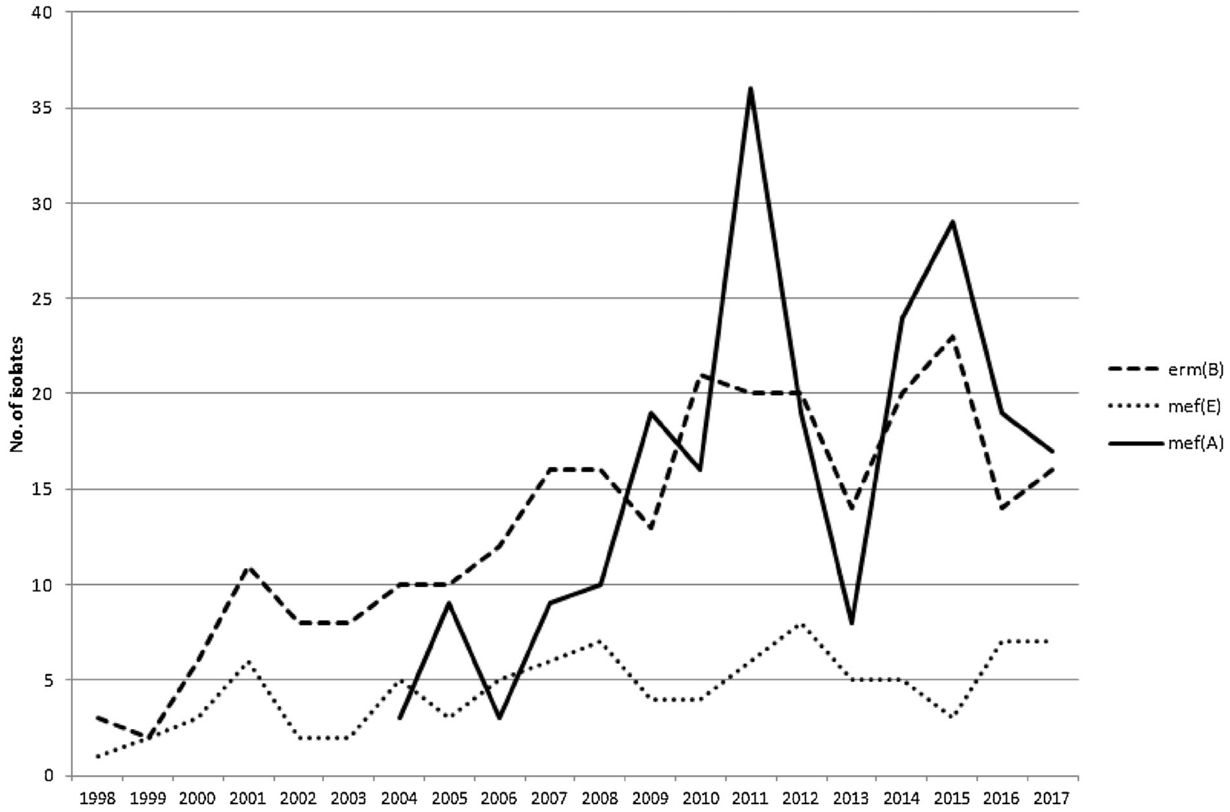


Fig. 2. Number of isolates carrying macrolide resistance genes in invasive *Streptococcus pneumoniae* in Slovenia, 1997–2017.

3.4. Co-resistance in macrolide-resistant S. pneumoniae

Table 2 shows the pattern of co-resistance of the *S. pneumoniae* isolates to other antimicrobials. Co-resistance to one or more commonly used antibiotics was detected in 57.2% (350/612) of the erythromycin-resistant isolates from 1997 to 2017, more often in *erm*(B)-positive strains. Of 263 isolates with the *erm*(B) gene, 248 (94.3%) were also resistant to at least one other antibiotic. Isolates harbouring the *erm*(B) gene were multidrug-resistant (resistant to at least three separate classes of antibiotics) in 58.2% of cases (153/263). The most frequent co-resistance pattern in the isolates with *erm*(B) was resistance to penicillin, tetracycline and SXT, which was the case for 91 of 263 isolates with the *erm*(B) gene. The *mef*(E) gene was also associated with multidrug resistance. Of 91 isolates with *mef*(E), 50 (54.9%) were multidrug-resistant. The most frequent co-resistance pattern in isolates with *mef*(E) was resistance to penicillin and SXT (42 of 91 isolates). All except one isolate with *mef*(A) were susceptible to all of the tested antibiotics. In 2017, one isolate with *mef*(A) that was serotype 19A and was penicillin-non-susceptible was detected.

Table 2
Distribution of erythromycin-resistant strains of invasive *Streptococcus pneumoniae* according to defined resistance genotypes and co-resistances isolated in Slovenia, 1997–2017.

Resistance phenotype	Resistance genotype[a]			
	erm(B)	*mef*(E)	*mef*(A)	No gene detected
No co-resistance	15	18	**221**	8
ERY/TET	85	2	0	7
ERY/PEN	4	19	1	2
ERY/SXT	6	2	0	5
ERY/PEN/TET	37	4	0	6
ERY/PEN/SXT	4	**42**	0	3
ERY/TET/SXT	8	0	0	0
ERY/TET/CHL	2	0	0	0
ERY/PEN/TET/SXT	**91**	3	0	3
ERY/PEN/SXT/CHL	1	0	0	0
ERY/TET/SXT/CHL	5	0	0	1
ERY/PEN/TET/SXT/CHL	5	1	0	1
Total	263	91	222	36

ERY, erythromycin; TET, tetracycline; PEN, penicillin; SXT, trimethoprim/sulfamethoxazole; CHL, chloramphenicol.

[a] Most common resistance pattern for each resistance genotype in bold.

3.5. Distribution of serotypes in macrolide-resistant S. pneumoniae

A small number of serotypes predominated among the macrolide-resistant isolates. The most prevalent serotypes were 14 (349; 54.9%), 19A (57; 9.0%), 19F (53; 8.3%), 6B (46; 7.2%), 6A (33; 5.2%) and 9V (19; 3.0%); in addition, serotypes 23F, 33F, 15A, 24F, 6C, 1, 3, 4, 16F, 18C, 35B, 9N, 23B, 15B, 22F, 23A, 27, 31, 38 and 7F were also found. In the observed period, an increase in serotype 14 was noticed among macrolide-resistant isolates (reg. coeff. = 1.91; $P < 0.001$). Serotypes 19A and 6A also showed an increasing trend (reg. coeff. = 0.30; $P < 0.001$; and reg coeff. = 0.18; $P = 0.001$, respectively) (Fig. 3). The *mef*(A) gene was present in isolates of serotype 14 (219 cases), with three exceptions; in two cases it was present in serotype 9V and in 2017 it was detected one case in serotype 19A.

3.6. Genetic relatedness

PFGE analysis was performed for 71 isolates carrying the *mef*(A) gene isolated from 2010 to 2012, at the peak of macrolide resistance and when the increase in the *mef*(A) gene was the greatest. The isolates showed high genetic homogeneity (Fig. 4). Among the 71 isolates, 63 (88.7%) were closely related (similarity $\geq$80%). The isolates were related to the international clone defined as England^{14}-9 clone by the PMEN.

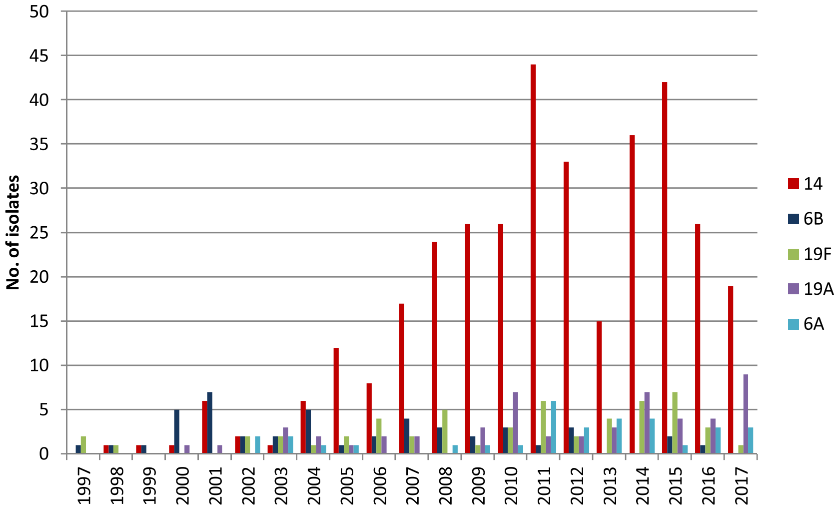


Fig. 3. Prevalence of the five most common serotypes in invasive macrolide-resistant *Streptococcus pneumoniae* in Slovenia, 1997–2017.

3.7. Multilocus sequence typing

A total of 161 isolates were investigated by MLST to determine the genetic population structure of invasive macrolide-resistant pneumococci. MLST data showed that isolates carrying the *mef*(A) gene showed a high degree of homogeneity; 49 (92.5%) of 53 belonged to ST9, and three other STs were detected (ST162, ST6054 and ST6047). Isolates carrying the *erm*(B) gene were the most heterogenous with 17 different STs. Seven different STs were detected in isolates carrying the *mef*(E) gene.

4. Discussion

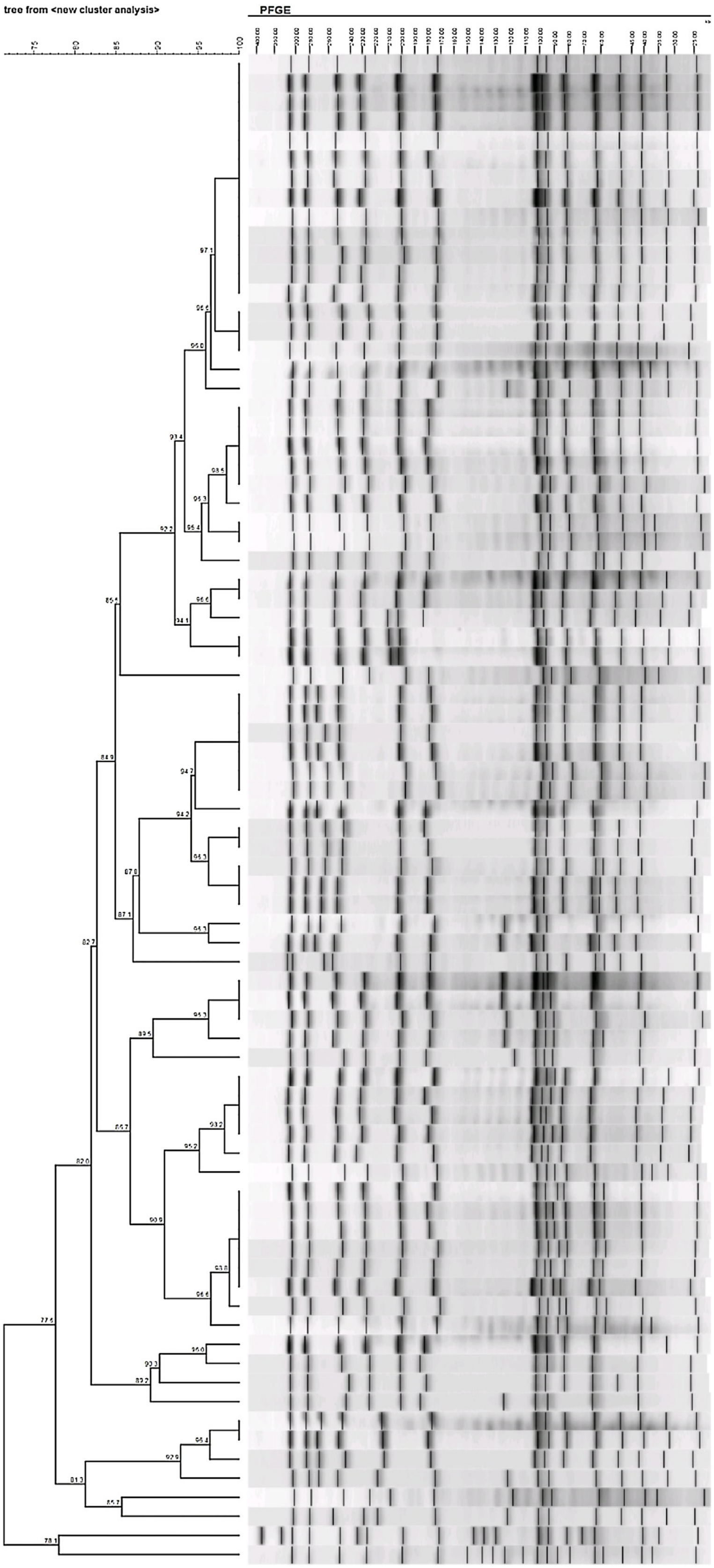
In Slovenia, macrolide consumption decreased by 42.5% during the 21-year period from 1997 to 2017 and, in the same period, resistance of invasive *S. pneumoniae* isolates increased from 5.8% in 1997 to 12.8% in 2017. Consumption of midecamycin, azithromycin and miocamycin decreased by 86%, 40% and 84%, respectively, but the consumption of clarithromycin doubled. In Slovenia, macrolides accounted for 15.6% in 1997 and fell in 2017 to 10.6% of all outpatient antibiotics for systemic use. In the community, five large studies have shown the possibility of decreasing antimicrobial resistance through a decrease in macrolide use [7,16–19]. In Japan, a decreased percentage of macrolide consumption among total antibiotic consumption was associated with lower resistance of *Streptococcus pyogenes* [16]. In Finland and South Korea, the decline in a macrolide resistant clone explained the correlation between decreased macrolide use and decreased resistance of *S. pyogenes* to macrolides [7,18,20]. In Belgium, an increase in the number of *S. pyogenes* with a low fitness cost and maintaining drug use below a critical threshold explained the correlation between decreased resistance of *S. pyogenes* and decreased consumption of macrolides [17]. In an Ethiopian community following cessation of mass distribution of azithromycin for trachoma, the resistance of nasopharyngeal *S. pneumoniae* isolates significantly ($P < 0.001$) decreased 12 months and 24 months after the last treatment [19].

Resistant pneumococcal clones present before mass azithromycin treatments (5%) increased in frequency after treatment (15%), consistent with the theory that antibiotic selection pressure results in clonal expansion of existing resistant strains [21]. The question that remains is why some studies show a reduction in resistance in response to prescribing restriction while others do not. The key parameter influencing the reduction of antimicrobial resistance are antibiotic selection pressure, clonality of resistant clones, co-selection and the fitness cost of resistance [4]. In Slovenia, selective pressure was decreased by decreased use of macrolides and co-selection with decreased use of other classes of antibiotics. Consumption of co-selective antibiotics such as SXT, tetracyclines and penicillins decreased by 34.3%, 67.8% and 7.6%, respectively. In our previous study, we already demonstrated that multiresistance was very common among macrolide-resistant invasive pneumococci in Slovenia, where 53.8% were resistant to three or more different classes of antibiotics [22]. In the present study, we confirmed the previous finding, as 57.2% of erythromycin-resistant isolates from 1997–2017 in Slovenia were co-resistant to one or more tested antibiotics.

The most common macrolide resistance mechanism in invasive pneumococci isolated from 1997 to 2017 in Slovenia was ribosomal methylation by the *erm*(B) gene (43.0% of the macrolide-resistant isolates). All of the isolates had an MIC $\geq$ 256 mg/L, indicating their clinical importance. In other European countries, *erm*(B) is also reported to be the predominant mechanism of macrolide resistance, although the percentages vary widely between countries [2,12,23–26]. The *mef*(A) gene was observed in 36.3% of macrolide-resistant strains and *mef*(E) in 14.9%. As expected, presence of the efflux mechanism conferred a lower level of resistance. The *mef*(E) and *mef*(A) genes were differentiated, and indeed, the isolates showed different characteristics. Isolates carrying *mef*(A) were serotype 14 (except for two with serotype 9V and one with serotype 19A) and were susceptible (except one isolate) to all tested antibiotics. The efflux mechanism predominates in the USA, Canada, the UK, Germany and Norway [26].

Multiresistance is very common among macrolide-resistant invasive pneumococci in Slovenia. Of the 612 erythromycin-resistant invasive pneumococci isolated in Slovenia from 1997 to 2017, 350 (57.2%) were resistant to two or more different classes of antibiotics. Strains carrying the *erm*(B) gene were multiresistant in 58.2% of cases, whereas strains with the *mef*(E) gene were associated with multidrug resistance in 54.9% of cases. Strains carrying the *mef*(A) gene were susceptible to all tested antibiotics, except in one case.

In this study, clonal spread of the cluster of serotype 14 carrying the *mef*(A) gene, related to the international clone England^{14}-9, was also demonstrated. The isolates showed high genetic homogeneity. The first case of this clone was detected in Slovenia in 2002, and then 36 cases were counted in 2011, when it also became the most common gene responsible for macrolide resistance. The international clone England^{14}-9 was described in



	ATCC 700676			
mef(A)	1938Po	ERY	14	2011
mef(A)	1939Po	ERY	14	2011
mef(A)	1944Po	ERY	14	2011
	ATCC 700676			
mef(A)	1815Po	ERY	14	2011
mef(A)	1728Po	ERY	14	2010
mef(A)	1938Po	ERY	14	2011
	ATCC 700676			
	ATCC 700676			
mef(A)	1985Po	ERY	14	2012
mef(A)	2050Po	ERY	14	2012
	ATCC 700676			
	ATCC 700676			
	ATCC 700676			
	ATCC 700676			
mef(A)	1726Po	ERY	14	2010
mef(A)	1656Po	ERY	14	2010
mef(A)	661P	ERY	14	2010
mef(A)	672P	ERY	14	2010
mef(A)	1833Po	ERY	14	2011
mef(A)	1588Po	ERY	14	2010
mef(A)	753P	ERY	14	2011
mef(A)	1753Po	ERY	14	2010
mef(A)	747P	ERY	14	2011
mef(A)	748P	ERY	14	2011
mef(A)	767P	ERY	14	2012
mef(A)	1945Po	ERY	14	2011
mef(A)	743P	ERY	14	2011
mef(A)	730P	ERY	14	2011
mef(A)	1795Po	ERY	14	2011
mef(A)	1915Po	ERY	14	2011
mef(A)	751P	ERY	14	2011
mef(A)	645P	ERY	14	2010
mef(A)	648P	ERY	14	2010
mef(A)	791P	ERY		2012
mef(A)	1757Po	ERY	14	2011
mef(A)	752P	ERY	14	2011
mef(A)	754P	ERY	14	2011
mef(A)	1843Po	ERY	14	2011
mef(A)	794P	ERY	14	2012
mef(A)	2099Po	ERY	14	2012
mef(A)	2020Po	ERY	14	2012
mef(A)	735P	ERY	14	2011
mef(A)	736P	ERY	14	2011
mef(A)	1798Po	ERY	14	2011
mef(A)	711P	ERY	14	2011
mef(A)	763P	ERY	14	2012
mef(A)	1941Po	ERY	14	2011
mef(A)	684P	ERY	14	2010
mef(A)	788P	ERY	14	2012
mef(A)	701P	ERY	14	2011
mef(A)	2125Po	ERY	14	2012
mef(A)	1787Po	ERY	14	2011
mef(A)	703P	ERY	14	2011
mef(A)	704P	ERY	14	2011
mef(A)	717P	ERY	14	2011
mef(A)	734P	ERY	14	2011
mef(A)	1585Po	ERY	14	2010
mef(A)	1842Po	ERY	14	2011
mef(A)	1594Po	ERY	14	2010
mef(A)	1639Po	ERY	14	2010
mef(A)	768P	ERY	14	2012
mef(A)	785P	ERY	14	2012
mef(A)	738P	ERY	14	2011
mef(A)	789P	ERY	14	2012
mef(A)	1910Po	ERY	14	2011
mef(A)	1851Po	ERY	14	2011
mef(A)	809P	ERY	14	2012
mef(A)	808P	ERY	14	2012
mef(A)	802P	ERY	14	2012
mef(A)	659P	ERY	14	2010
mef(A)	1926Po	ERY	14	2011
mef(A)	799P	ERY	14	2012
mef(A)	2011Po	ERY	14	2012
mef(A)	750P	ERY	14	2011
mef(A)	761P	ERY	14	2012
mef(A)	1738Po	ERY	14	2010
mef(A)	2012Po	ERY	14	2012

Fig. 4. Dendrogram based on pulsed-field gel electrophoresis (PFGE) *Sma*I restriction pattern of selected invasive pneumococcal isolates carrying the *mef*(A) gene. From left to right: presence of the *mef*(A) gene; isolate name; resistance to erythromycin (ERY); serotype; and year of isolation.

the UK primarily associated as an erythromycin-resistant serotype 14 pneumococci. The clone has spread intercontinentally and was one of the major serotype 14 clones associated with macrolide resistance in the USA prior to vaccine introduction [27]. Clonal spread of England^{14}-9 was also documented in Italy, Germany, the UK and Norway [23,28–30]. Spread of a clone resistant to erythromycin only explains the increase of macrolide resistance among invasive *S. pneumoniae* isolates in Slovenia.

The 10-valent pneumococcal conjugate vaccine (PCV-10) was introduced in Slovenia during 2015, using a 2 + 1 schedule. It is recommended and free of charge (for all children born after 1 October 2014). In Slovenia, pneumococcal vaccination coverage was 48.8% in 2015, 49.4% in 2016 and 55.2% in 2017. Because of the low vaccination coverage, no significant influence on invasive pneumococcal diseases was noticed.

Despite vaccination, we must strive further to decrease the consumption of antibiotics to which pathogens might be resistant. Laboratory surveillance is needed to monitor further evolution of this important bacterium and the response to the vaccination programme.

This study showed that reduced consumption of antibiotics is not enough to reverse antibiotic resistance in the community. Emergence and clonal expansion of resistant clones can compromise the benefits of restricted prescribing.

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Competing interests

None declared.

Ethical approval

Not required.

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