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Antimicrobial Resistance among Respiratory Pathogens in Spain: Latest Data and Changes over 11 Years (1996-1997 to 2006-2007)▽

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A nationwide multicenter susceptibility surveillance study (Susceptibility to the Antimicrobials Used in the Community in España [SAUCE] project), SAUCE-4, including 2,559 *Streptococcus pneumoniae*, 2,287 *Streptococcus pyogenes*, and 2,736 *Haemophilus influenzae* isolates was carried out from May 2006 to June 2007 in 34 Spanish hospitals. Then, the results from SAUCE-4 were compared to those from all three previous SAUCE studies carried out in 1996-1997, 1998-1999, and 2001-2002 to assess the temporal trends in resistance and the phenotypes of resistance over the 11-year period. In SAUCE-4, on the basis of the CLSI breakpoints, penicillin (parenteral, nonmeningitis breakpoint) and cefotaxime were the antimicrobials that were the most active against *S. pneumoniae* (99.8% and 99.6%, respectively). Only 0.9% of isolates had a penicillin MIC of ≥2 μg/ml. In *S. pyogenes*, nonsusceptibility to erythromycin was observed in 19.4% of isolates. Among the *H. influenzae* isolates, a β-lactamase-positive prevalence of 15.7% was found. A statistically significant temporal decreasing trend over the 11-year period was observed for nonsusceptibility (from 60.0% to 22.9%) and resistance (from 36.5% to 0.9%) to penicillin and for the proportion of erythromycin-resistant isolates of *S. pneumoniae* of the macrolide-lincosamide-streptogramin B (MLS_B) phenotype (from 98.4% to 81.3%). A similar trend was observed for the prevalence of ampicillin resistance (from 37.6% to 16.1%), β-lactamase production (from 25.7% to 15.7%), and β-lactamase-negative ampicillin resistance (BLNAR) in *H. influenzae* (from 13.5% to 0.7%). Among erythromycin-resistant isolates of *S. pyogenes*, a significant increasing trend in the prevalence of MLS_B was observed (from 7.0% to 35.5%). SAUCE-4 confirms a generalized decline in the resistance of the main respiratory pathogens to the antimicrobials as well as a shift in their resistance phenotypes.

Continuing surveillance for the antibiotic resistance of respiratory pathogens is a recognized public health need, particularly in those countries with high resistance rates, since initial antimicrobial treatment for patients with bacterial community-acquired respiratory tract infections (CARTIs) is usually selected empirically and should provide appropriate coverage against the most common causative organisms (35).

Streptococcus pneumoniae, *Streptococcus pyogenes*, and *Haemophilus influenzae* are of particular concern since these are among the most prevalent bacteria involved in CARTIs and because of their frequent development of resistance to several frequently used antibiotics observed in recent decades, thus jeopardizing the selection of an effective antibiotic therapy (13).

Following the widespread use of the new conjugated heptavalent pneumococcal vaccine (PCV-7), a decrease in antibiotic resistance in pediatric pneumococcal isolates causing disease has been reported in several countries (5, 38), not only in vaccinated individuals but also in unvaccinated individuals, as a result of the herd immunity effect (16). Nevertheless, some recent studies have observed an increase in the prevalence of drug-resistant *S. pneumoniae* as a result of an increase in the prevalence of the more resistant nonvaccine serotypes (27).

Although in *S. pyogenes* resistance to penicillin has not been reported to date, high macrolide resistance rates have been observed in some countries over the last years, causing concern. In Spain, the prevalence of macrolide resistance has remained stable since the 1990s, although an increase in the macrolide-lincosamide-streptogramin B (MLS_B) constitutive phenotype has been reported (39).

β-Lactamase production by *H. influenzae* is a well-known predictor of treatment failure in CARTIs (11). In addition, *H. influenzae* isolates carrying amino acid substitutions in the *ftsI* gene (encoding PBP 3) are phenotypically recognized as BLNAR, which leads to the loss of susceptibility to aminopenicillin and some cephalosporins.

The Susceptibility to the Antimicrobials Used in the Community in España (Spain) (SAUCE) project is a longitudinal surveillance study designed to evaluate the antimicrobial susceptibilities of respiratory bacterial pathogens to the antibiotics most commonly used in the community. Between the 1996-1997 and 2006-2007 seasons, four prevalence studies were carried out. This paper reports on the antimicrobial susceptibilities and phenotypes of resistance for isolates collected in

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SAUCE-4 (2006-2007 season) and also analyzes the temporal trends in resistance and the phenotypes of resistance over the 11-year period.

MATERIALS AND METHODS

Isolates from the four prevalence studies (1996-1997, 1998-1999, 2001-2002, and 2006-2007) were collected from a total of 14 to 34 microbiology laboratories (34 in the 2006-2007 study) all over Spain. The results of the three first three studies were published previously (3, 4, 21, 34, 36, 37).

Isolates, susceptibility testing, and phenotypes of resistance (SAUCE-4). The centers were requested to collect each month the first 15 isolates of *S. pneumoniae*, *S. pyogenes*, and *H. influenzae* from clinically significant specimens corresponding to community-acquired infections (acute pharyngitis for *S. pyogenes* and acute otitis media, acute exacerbations of chronic bronchitis, and pneumonia for *S. pneumoniae* and *H. influenzae*) during a 1-year period (May 2006 to June 2007). The following data were collected: specimen source, hospital ward, unit of care, and date of sample collection.

After isolation, identification, and susceptibility testing at the participating laboratory, the isolates were stored at −70°C in a frozen medium containing 1% vegetable peptone and 8% glycerol. The samples were thereafter shipped once a month to an UNE-EN ISO 15189- and UNE-EN ISO 17025-acreditated central laboratory (Instituto Valenciano de Microbiología, Valencia, Spain), where the compliance of the isolates with the inclusion criteria in the study was verified. Confirmation of the identities of the isolates was provided by a positive bile solubility test and inhibition by optochin for *S. pneumoniae*; inhibition by bacitracin, the pyrrolidonyl arylamidase test, and serogroup A agglutination (Streptex; Murex, Chantillon, France) for *S. pyogenes*; and catalase, X- and V-factor requirements, and hemolysis on horse blood for *H. influenzae.* Capsular serotyping of *H. influenzae* was performed by slide agglutination with specific antisera against capsular antigen (Difco Laboratories). The isolates were kept frozen at −70°C in duplicate for further antimicrobial susceptibility testing and were recovered by the hot-loop touching method to avoid repeated thaw and freezing.

MICs were determined using the Clinical Laboratory Standards Institute (CLSI) broth microdilution method active in the year of study and were interpreted according to CLSI M100-S19 (8). The *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247 and ATCC 49766, and *Escherichia coli* ATCC 35218 strains were used as controls. *H. influenzae* ATCC 10211 was used to verify the growth-supporting properties of each *Haemophilus* test medium (HTM).

As there is no ciprofloxacin MIC interpretive standard described by the CLSI for *Streptococcus pneumoniae*, a 4-μg/ml MIC for *S. pneumoniae* was considered for resistance, as defined by the European Committee on Antimicrobial Susceptibility Testing (15). Pharmacokinetic (PK)/pharmacodynamic (PD) breakpoints (the maximum MIC value complying with the adequate value for the predictive PD parameter) are based on the PK/PD relationships of the agents that result in successful clinical outcomes. Thus, use of the PK/PD breakpoints overcomes most of the limitations associated with use of the CLSI breakpoints (1). Because PK/PD breakpoints are predictive of clinical and/or microbiological success in the treatment of infection, we also used the PK/PD breakpoints (23, 28, 37) for interpretation of the MICs.

Erythromycin-nonsusceptible pneumococcal and *S. pyogenes* isolates (MICs ≥ 0.5 μg/ml) were tested by the double-disk method to detect the constitutive, inducible, or efflux phenotype (40). β-Lactamase production was determined using the chromogenic cephalosporin method (nitrocefin; Becton Dickinson). β-Lactamase-negative isolates with ampicillin MICs of ≥2 μg/ml were categorized as BLNAR isolates. In order to detect low-BLNAR *H. influenzae* isolates, those isolates with ampicillin and amoxicillin-clavulanate MICs of 0.5 to 1 μg/ml and ≥2 μg/ml, respectively, were searched for the presence of amino acid substitutions in the *ftsI* gene by PCR. Amplification of the *ftsI* gene between nucleotide positions 936 and 1640 was carried out using the primers already described (9) and sequencing to detect amino acid substitutions. Strains with mutations in the *ftsI* gene were classified according to previously defined BLNAR groups (9).

Statistical analyses. Differences in the prevalence of antibiotic resistance between different groups were assessed by the chi-square or Fisher exact test. Associations were determined by calculation of odd ratios (ORs) with their corresponding 95% confidence intervals (CIs). For assessing temporal trends over the 11-year period, Spearman nonparametric correlation coefficients were calculated. Simple linear regression analysis was also performed to determine the temporal trends in resistance and the phenotypes of resistance. A significance level of <0.05 was specified for all analyses. Statistical analyses were performed using the G-Stat (version 2.0) and the SPSS (version 14) programs.

RESULTS

Over the last 1-year study period (May 2006 to June 2007), a total of 7,582 valid isolates were recovered: 2,559 of *S. pneumoniae*, 2,287 of *S. pyogenes* and 2,736 of *H. influenzae*.

***Streptococcus pneumoniae*.** The *S. pneumoniae* isolates were obtained from the following sources: potentially contaminated respiratory samples in 1,389 cases (54.3%), blood in 707 cases (27.6%), otic samples in 304 cases (11.9%), and samples from other potentially sterile sites, such as pleural fluid and bronchial telescoped catheters, in 159 cases (6.2%). Four hundred thirty isolates (16.8%) were collected from pediatric samples.

Table 1 summarizes the microbiological and PK/PD antimicrobial susceptibilities as well as the MIC_{50} and MIC_{90} for every antibiotic tested. On the basis of the CLSI breakpoints, penicillin (parenteral administration, nonmeningitis breakpoint) and cefotaxime were the most active antimicrobials (99.8% and 99.6%, respectively), with the rates of susceptibility to levofloxacin, amoxicillin-clavulanate, and cefuroxime axetil being the highest among the oral antimicrobials (97.6%, 94.8%, and 94.5%, respectively). On the contrary, azithromycin, clarithromycin, erythromycin, cefaclor, and penicillin (oral administration breakpoint) were the least active antibiotics, with less than 80% of isolates being susceptible. Regarding cefditoren, there is no CLSI breakpoint, and the MIC interpretative standards defined by FDA (http://www.fda.gov) and by the Spanish Regulatory Agency (http://www.aemps.es) are different. Applying the FDA susceptible breakpoint (≤0.125 μg/ml), 94.9% of the isolates would be considered susceptible to cefditoren, whereas applying the Spanish susceptible breakpoint (≤0.5 μg/ml), 99.6% of the isolates would be considered susceptible.

Only 0.9% of the isolates had a penicillin MIC of ≥2 μg/ml (18 isolates with a MIC of 2 μg/ml and 6 isolates with a MIC of 4 μg/ml). The MIC_{90}s for all three macrolides tested were ≥128 μg/ml.

The prevalence of multidrug-resistant pneumococci defined by resistance to three or more drug classes (represented by penicillin [MIC ≥ 2 μg/ml], erythromycin [MIC ≥ 1 μg/ml], and levofloxacin [MIC ≥ 8 μg/ml]) was 0%. Of the 24 penicillin-resistant pneumococcal (PRP) isolates, 13 showed resistance to erythromycin. There was no isolate concurrently resistant to penicillin and levofloxacin.

The risk for macrolide resistance and ciprofloxacin resistance was higher among PNP isolates than among penicillin-susceptible isolates (OR = 5.1, 95% CI = 4.1 to 6.3, and $P < 0.001$ for erythromycin; OR = 4.0, 95% CI = 2.3 to 7.1, and $P < 0.001$ for ciprofloxacin).

The rates of penicillin nonsusceptibility (based on oral penicillin breakpoints) and erythromycin resistance were slightly higher in children than in adults (26.7% versus 22.2% [$P = 0.039$] for penicillin nonsusceptibility; 25.3% versus 20.3% [$P = 0.019$] for erythromycin resistance). On the contrary, the rate of ciprofloxacin resistance was lower in children than in adults (0.2% versus 2.6%; $P = 0.004$). Significant differences regarding penicillin and erythromycin nonsusceptibility were observed when isolates from blood were compared with isolates from respiratory samples (17.5% versus 25.3% [$P < 0.001$] for penicillin; 17.3% versus 23.0% [$P = 0.001$] for erythromycin). Both in adults and in children, the highest penicillin

TABLE 1. *In vitro* activities of 12 antibiotics against 2,559 *S. pneumoniae* clinical isolates collected in 2006-2007[a]

Antibiotic	MIC (μg/ml)		CLSI susceptibility[b] (% of isolates)			PK/PD	
	50%	90%	Susceptible	Intermediate	Resistant	Susceptibility (% of isolates)	Breakpoint (μg/ml)
Penicillin (O)	≤0.015	0.5	77.1	22.0	0.9	NA	
Penicillin (P)	≤0.015	0.5	99.8	0.2	0.0	NA	
Ampicillin	≤0.015	2	NA	NA	NA	93.4	≤2
Amoxicillin-clavulanate	≤0.015	1	94.8	4.0	1.2	94.8	≤2
Amoxicillin-clavulanate[c]			NA	NA	NA	98.8	≤4
Cefuroxime (P)	≤0.015	1	83.8	10.7	5.5	99.3	≤4
Cefuroxime (O)	≤0.015	1	94.5	4.3	1.3	94.5	≤1
Cefaclor	0.125	16	79.1	2.0	19.0	75.9	≤0.5
Cefditoren	≤0.015	0.125	NA	NA	NA	94.9	≤0.12
Cefotaxime	≤0.015	0.25	99.6	0.2	0.2	99.6	≤2
Erythromycin	≤0.015	≥128	78.9	0.0	21.1	78.9	≤0.25
Clarithromycin	≤0.015	≥128	78.2	0.9	20.9	78.2	≤0.25
Azithromycin	≤0.015	≥128	77.6	1.6	20.8	74.3	≤0.12
Ciprofloxacin	0.25	0.5	NA	NA	2.2[d]	97.0	≤1
Levofloxacin	0.125	0.25	97.6	1.9	0.5	97.7	≤2

[a] Abbreviations: NA, not applicable (no CLSI breakpoint criteria); O, oral; P, parenteral.
[b] The CLSI breakpoints used were 0.06 μg/ml (susceptible), 0.12 to 1 μg/ml (intermediate), and 2 μg/ml (resistant) for oral penicillin; 2 μg/ml (susceptible), 4 μg/ml (intermediate), and 8 μg/ml (resistant) for parenteral penicillin, amoxicillin-clavulanate, and levofloxacin; 0.5 μg/ml (susceptible), 1 μg/ml (intermediate), and ≥2 μg/ml (resistant) for parenteral cefuroxime and azithromycin; ≤1 μg/ml (susceptible), 2 μg/ml (intermediate), and 4 μg/ml (resistant) for cefuroxime axetil (oral), cefaclor and cefotaxime; and 0.25 μg/ml (susceptible), 0.5 μg/ml (intermediate), and 1 μg/ml (resistant) for erythromycin and clarithromycin.
[c] High dose of amoxicillin-clavulanate (sustained release, 2,000/125-mg formulation).
[d] A resistance breakpoint of ≥4 μg/ml for ciprofloxacin was considered (15).

and erythromycin nonsusceptibility rates were found among isolates from middle ear samples (28.7% and 33%, respectively, for adults; 30.7% and 32.3%, respectively, for children).

Among the 541 erythromycin-resistant pneumococcal isolates, the most frequent phenotype of resistance was MLS_B (81.3%), with only 2 isolates showing an inducible MLS_B phenotype. The remaining 101 (18.7%) isolates showed the M phenotype. The MLS_B phenotype was present in 97.0% and 79.2% of isolates showing nonsusceptibility to erythromycin in children and adults, respectively (OR 2.4; 95% CI = 1.2 to 5.0; $P = 0.008$).

***Streptococcus pyogenes*.** MIC_{50}s, MIC_{90}s, and the percentages of susceptible, intermediate, and resistant isolates are provided in Table 2. Up to 19.0% of isolates were resistant to erythromycin. Ten isolates (0.4%) were intermediate to erythromycin.

The prevalence of erythromycin-nonsusceptible isolates was significantly higher in adults than in children (21.9% versus 17.8%; $P = 0.015$).

Among the 445 isolates showing nonsusceptibility to erythromycin, the predominant phenotype of resistance was the M phenotype (64.5%). Of the isolates nonsusceptible to erythromycin, 35.5% had the MLS_B phenotype. Of these, 150 (94.9%) had a constitutive phenotype and 8 (5.1%) had an inducible phenotype. There was no difference between the distribution of phenotypes of resistance in adults and children.

***Haemophilus influenzae*.** A total of 2,272 samples (83.0%) were collected from the lower respiratory tract (mostly sputa), 415 (15.2%) from middle ear exudates, 41 (1.5%) from blood, and 8 (0.3%) from pleural fluid.

Table 3 shows the microbiological and PK/PD susceptibilities of *H. influenzae* to the antimicrobials tested. Using the CLSI breakpoints, almost all antimicrobials tested presented very high susceptibility rates (>97%). The only one that showed a lower susceptibility rate was ampicillin (83.9%). Using the PK/PD breakpoints, the rates of susceptibility of *H. influenzae* to clarithromycin, azithromycin, and cefaclor shifted from 99.3%, 100%, and 97.8%, respectively, by applying the CLSI breakpoints to 5.1%, 23.8%, and 25.8%, respectively, by applying the PK/PD breakpoints.

Four hundred twenty-nine isolates (15.7%) were β-lactamase producers, with the production of β-lactamase being more frequent in children than in adults (20.8% versus 14.6%; $P = 0.007$). Eighteen isolates (0.7%) exhibited a BLNAR phenotype (β-lactamase negative, ampicillin MIC ≥ 2 μg/ml). Nevertheless, when 134 isolates exhibiting an ampicillin MIC between 0.5 and 1 μg/ml and an amoxicillin-clavulanate MIC

TABLE 2. *In vitro* susceptibilities to different antimicrobial agents of 2,287 *S. pyogenes* clinical isolates collected in 2006-2007

Antibiotic	MIC μg/ml		CLSI susceptibility[a] (% of isolates)		
	50%	90%	Susceptible	Intermediate	Resistant
Penicillin	≤0.015	≤0.015	100	0	0
Ampicillin	≤0.015	≤0.015	100	0	0
Amoxicillin-clavulanate	≤0.015	≤0.015	NA[b]	NA	NA
Cefuroxime	≤0.015	≤0.015	NA	NA	NA
Cefaclor	0.03	≤0.06	NA	NA	NA
Cefditoren	≤0.015	≤0.015	NA	NA	NA
Cefotaxime	≤0.015	≤0.015	100	0	0
Erythromycin	≤0.015	2	80.6	0.4	19.0
Clarithromycin	≤0.015	2	80.9	2.6	16.5
Azithromycin	≤0.015	2	81.1	7.3	11.6
Ciprofloxacin	0.06	0.125	NA	NA	NA
Levofloxacin	0.125	0.250	100	0.0	0.0

[a] The CLSI breakpoints used were 0.12 μg/ml (susceptible) for penicillin and ampicillin; 0.5 μg/ml (susceptible) for cefotaxime; 0.25 μg/ml (susceptible), 0.5 μg/ml (intermediate), and 1 μg/ml (resistant) for erythromycin and clarithromycin; 0.5 μg/ml (susceptible), 1 μg/ml (intermediate), and 2 μg/ml (resistant) for azithromycin; and 2 μg/ml (susceptible), 4 μg/ml (intermediate), and 8 μg/ml (resistant) for levofloxacin.
[b] NA, not applicable (no CLSI breakpoint criteria).

TABLE 3. *In vitro* susceptibilities of 2,736 *H. influenzae* clinical isolates collected in Spain in 2006-2007[a]

Antibiotic	MIC (μg/ml)		CLSI susceptibility[b] (%)			PK/PD	
	50%	90%	Susceptible	Intermediate	Resistant	Susceptibility (% of isolates)	Breakpoint (μg/ml)
Ampicillin	0.12	4	83.9	2.9	13.3	86.7	≤2
Amoxicillin-clavulanate	0.25	1	99.7	0.0	0.3	97.5	≤2
Amoxicillin-clavulanate[c]			NA	NA	NA	99.7	≤4
Cefuroxime (O)	0.5	1	99.3	0.6	0.1	91.4	≤1
Cefaclor	1	4	97.8	2.2	0.0	25.8	≤0.5
Cefditoren	≤0.015	≤0.015	NA	NA	NA	99.6	≤0.12
Cefotaxime	≤0.015	≤0.015	99.9	0.0	0.1	99.9	≤2
Erythromycin	1	4	NA	NA	NA	32.5	≤0.25
Clarithromycin	2	4	99.3	0.7	0.0	5.1	≤0.25
Azithromycin	0.25	1	100	0.0	0.0	23.8	≤0.12
Ciprofloxacin	≤0.015	≤0.015	99.8	0.0	0.2	99.8	≤1
Levofloxacin	≤0.015	≤0.015	99.9	0.0	0.1	99.9	≤2

[a] Abbreviations: NA, not applicable (no CLSI breakpoint criteria); O, oral.
[b] The CLSI breakpoints used were 1 μg/ml (susceptible), 2 μg/ml (intermediate), and 4 μg/ml (resistant) for ampicillin; 4 μg/ml (susceptible), and 8 μg/ml (resistant) for amoxicillin-clavulanate; 4 μg/ml (susceptible), 8 μg/ml (intermediate), and 16 μg/ml (resistant) for cefuroxime; ≤8 μg/ml (susceptible), 16 μg/ml (intermediate), and 32 μg/ml (resistant) for cefaclor; 2 μg/ml (susceptible) for cefotaxime and levofloxacin; 8 μg/ml (susceptible), 16 μg/ml (intermediate), and 32 μg/ml (resistant) for clarithromycin; 1 μg/ml (susceptible) for ciprofloxacin; and 4 μg/ml (susceptible) for azithromycin.
[c] High dose of amoxicillin-clavulanate (sustained release, 2,000/125-mg formulation).

of ≥2 μg/ml were investigated for the presence of low-BLNAR *H. influenzae* isolates, all 134 isolates (4.9%) had mutations in the *ftsI* gene. The most frequent BLNAR groups (*H. influenzae* isolates with mutations in the *ftsI* gene, as previously defined) among those isolates were groups IIb and IIc (43.1% and 37.2%, respectively) followed by group IIa (11.8%), with one-third of the isolates belonging to one simple pattern (350N, 377I, 502V, 526K). Seven isolates (0.3%) were β-lactamase positive and amoxicillin-clavulanate resistant (BLPACR).

The majority of isolates (99.3%) were noncapsulated, whereas serogroups a, b, d, and f represented 0.6%, 0.6%, 0.2%, and 0.1% of the isolates, respectively. There was one isolate belonging to serogroup c. Thirty-eight noncapsulated isolates were obtained from blood and eight from pleural fluid.

Temporal trends over the 11-year period (SAUCE-1 to SAUCE-4). The prevalence of resistance to several antimicrobials as well as the frequency of the most relevant resistant phenotypes were compared among the four SAUCE studies carried out over a 11-year period.

The temporal trends in the prevalence of nonsusceptibility and resistance to oral penicillin, resistance to erythromycin, and resistance to ciprofloxacin for *S. pneumoniae* across the four SAUCE studies are shown in the Fig. 1. A statistically significant temporal decreasing trend was observed both for nonsusceptibility to penicillin ($R^2 = 0.985$; β regression coefficient [β] = −2.575; $P = 0.007$) and for resistance to penicillin ($R^2 = 0.895$; β = −0.964; $P = 0.036$). For resistance to erythromycin and ciprofloxacin, the model showed a decreasing temporal trend, although it did not reach statistical significance ($P = 0.141$ and $P = 0.155$, respectively). The proportion of isolates with the MLS_B phenotype among erythromycin-resistant isolates was significantly lower in SAUCE-4 (81.3%) than in the previous SAUCE studies (98.4%, 93.7%, and 89.9% for SAUCE studies 1 to 3, respectively) ($P < 0.001$), showing a temporal decreasing trend across the years of the study ($R^2 = 0.989$; β = −0.966; $P = 0.004$). A temporal decreasing trend for penicillin nonsusceptibility correlated with a temporal trend for the MLS_B phenotype ($r = 0.999$; $P < 0.001$).

The rate of erythromycin resistance and the frequency of the MLS_B phenotype in *S. pyogenes* isolates across the all four SAUCE studies are shown in Fig. 2. The erythromycin resistance rate in the most recent study (SAUCE-4) was lower than that seen in the 2001-2002 season in SAUCE-3 (19.0% versus 24.3%; $P < 0.001$). Nevertheless, there was no significant temporal trend in the prevalence of erythromycin resistance during the 11-year period ($P = 0.309$). Among erythromycin-resistant isolates, a significant temporal increasing trend was observed in the prevalence of the MLS_B phenotype ($R^2 = 0.929$; β = −0.964; $P = 0.036$).

Figure 3 illustrates the progression of the prevalence of ampicillin resistance and the phenotypes of resistance in *H. influenzae* in the all four SAUCE studies. A decrease in the prevalence of ampicillin resistance was observed in the

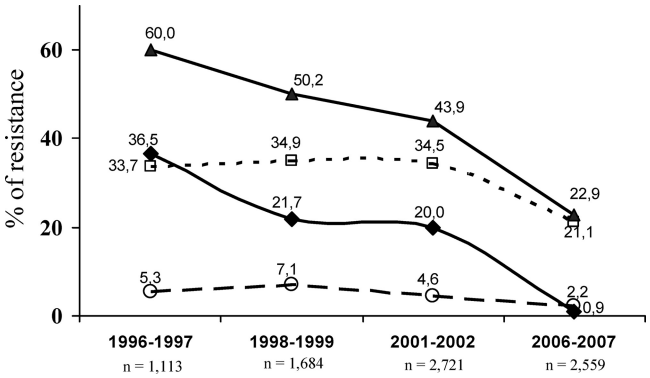

FIG. 1. Temporal trends in penicillin resistance, erythromycin resistance, and ciprofloxacin resistance in *S. pneumoniae* in Spain (percentage of clinical isolates). Symbols: solid line with filled triangles, nonsusceptibility to penicillin; dashed line with open squares, resistance to erythromycin; solid lines with filled diamonds, resistance to penicillin (MIC ≥ 2 μg/ml); dashed lines with open circles, resistance to ciprofloxacin. SAUCE-1, 1996-1997; SAUCE-2, 1998-1999; SAUCE-3, 2001-2002; SAUCE-4, 2006-2007. $P < 0.001$ for all comparisons of SAUCE-4 with previous SAUCE studies.

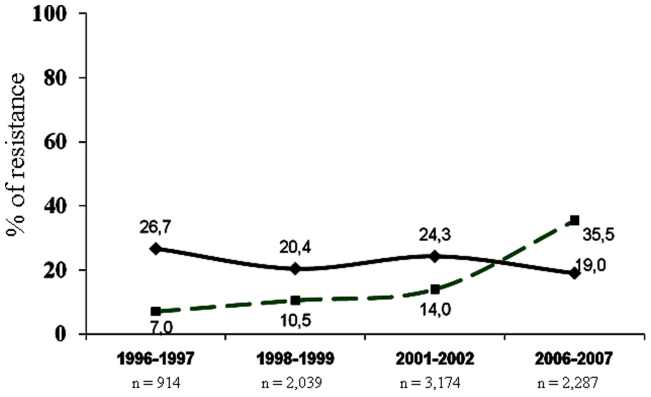

FIG. 2. Temporal trends in erythromycin resistance and the MLS_B phenotype in *S. pyogenes* in Spain (percentage of clinical isolates). Symbols: dashed line with squares, percentage of erythromycin-resistant isolates with the MLS_B phenotype; solid line with diamonds, resistance to erythromycin. SAUCE-1, 1996-1997; SAUCE-2, 1998-1999; SAUCE-3, 2001-2002; SAUCE-4, 2006-2007. $P < 0.001$ for comparisons of SAUCE-4 with SAUCE-3.

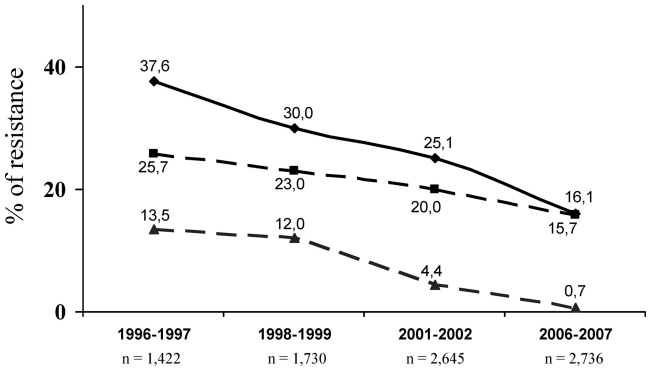

FIG. 3. Temporal trends in prevalence of resistance to ampicillin and phenotypes of resistance in *H. influenzae* in Spain (percentage of clinical isolates). Symbols: solid line with diamonds, resistance to ampicillin; dashed line with squares, β-lactamase production; dashed line with triangles, BLNAR (ampicillin MIC ≥ 2 μg/ml). In SAUCE-4, there were 134 isolates (4.9%) with mutations in the *ftsI* gene exhibiting ampicillin MICs between 0.5 and 1 μg/ml and amoxicillin-clavulanate MICs of ≥2 μg/ml. SAUCE-1, 1996-1997; SAUCE-2, 1998-1999; SAUCE-3, 2001-2002; SAUCE-4, 2006-2007. $P < 0.001$ for all comparisons of SAUCE-4 with previous SAUCE studies.

SAUCE-4 study with respect to the prevalence in the preceding SAUCE study (16.1% versus 25.1%; $P < 0.001$). A similar decrease in the prevalence of β-lactamase production and BLNAR was observed ($P < 0.001$ for both comparisons). A statistically significant temporal decreasing trend was also found for ampicillin resistance ($R^2 = 0.957$; $\beta = -0.986$; $P = 0.014$), for β-lactamase production ($R^2 = 0.991$; $\beta = -0.996$; $P = 0.004$), and for the prevalence of BLNAR ($R^2 = 0.934$; $\beta = -0.966$; $P = 0.034$). A temporal decreasing trend for ampicillin resistance in *H. influenzae* correlated strongly with a temporal trend for penicillin nonsusceptibility in *S. pneumoniae* ($r = 0.998$; $P < 0.001$).

DISCUSSION

The SAUCE project is an extensive national multicenter surveillance study with a very large sample size in relation to the Spanish population that provides more reliable information on resistance than other studies with fewer centers and fewer numbers of isolates.

The present study shows a generalized decline in the rates of resistance of the main respiratory pathogens to the antimicrobials as well as a shift in their resistance phenotypes.

Of special interest is the marked decrease, observed in the 2006-2007 study, of the prevalence of resistance of *S. pneumoniae* to oral and parenteral penicillins, with only 0.9% and 0.2% of isolates having MICs of ≥2 and ≥4 μg/ml, respectively, figures that can be considered a historic milestone in a country like Spain that has traditionally been a hot spot for PRP. Such figures had not been reported in Spain since the 1970s (32).

For erythromycin, despite a close relationship between penicillin nonsusceptibility and erythromycin resistance being shown, the decrease in the prevalence of resistance over the 11-year period did not exhibit a significant temporal decreasing trend, although a significant decrease was observed from SAUCE-3 (2001-2002) to SAUCE-4 (2006-2007). These different patterns in the temporal variation of erythromycin resistance make it evident that not all drugs have the same capabilities for the selection of resistance and that resistance to all drugs is not developed to the same extent or that the decrease in the rate of resistance is not influenced by external factors to the same extent.

Interestingly, ciprofloxacin resistance in *S. pneumoniae* in the SAUCE-4 study was significantly lower than that seen in previous SAUCE studies. Perhaps the replacement of ciprofloxacin by more potent antipneumococcal quinolones (levofloxacin and moxifloxacin) could be a potential explanation for this finding. Indeed, a study carried out in our country found an inverse and paradoxical correlation between the regional consumption of quinolones and resistance to ciprofloxacin (20); those regions with higher levels of consumption of quinolones had a lower prevalence of resistance to ciprofloxacin in *S. pneumoniae*. In another study, De-la-Campa et al. observed stabilization in the rates of fluoroquinolone resistance from 2002 to 2006 (12).

The decline in the prevalence of resistance of *S. pneumoniae* to penicillin and, to a lesser degree, to erythromycin is in accordance with other reports (14, 16, 17, 38). Several factors could have influenced the decrease in the prevalence of nonsusceptibility and resistance to penicillin observed in this study. First, after the introduction of PCV-7 in Spain (16, 17, 38) and other countries (6, 22), significant reductions in the rates of penicillin resistance, erythromycin resistance, and multiresistance have been observed. The shift in the pneumococcal resistant population affects not only vaccinated children but also unvaccinated children and adults, as a result of a herd immunity effect (10, 25, 31), probably due to the fact that children are mainly responsible for the transmission of drug-resistant *S. pneumoniae* in the community (10, 33). Since the launch of PVC-7 in June 2001 in our country, vaccine use increased, with the vaccine coverage rate estimated to be close to 50% before 2006 in children under 2 years of age (24). Second, since the decrease in penicillin resistance was observed before the introduction of PCV-7, other factors should also have been in-

volved. The volume of antimicrobial use is the major selection pressure driving changes in the frequency of antibiotic resistance (2, 30). However, not all antimicrobials have the same efficiency at selecting for resistance. Rather, different antibiotics seem to have different accountabilities for the given rates of resistance in a given bacterial species. Third, other potential or even not yet well-identified factors (i.e., clonal spread and clonal turnover) could be involved in the selection and spread of resistance.

Although no resistance to β-lactams among *S. pyogenes* isolates has been reported, macrolide resistance is of concern in some areas. In the present study, up to 19% of isolates were resistant to erythromycin. When this figure is compared with those from previous SAUCE studies, considerable fluctuations in the rates of resistance to macrolides can be observed. Unlike penicillin resistance in *S. pneumoniae* and ampicillin resistance in *H. influenzae*, there was no temporal decreasing trend over the 11 years in the prevalence of erythromycin resistance in *S. pyogenes*, although a decrease compared to the rate in a previous SAUCE study (2001-2002) was observed. The level of macrolide consumption in Spain decreased from 2002 to 2005 (7), and the decrease in the erythromycin resistance rate observed in SAUCE-4 could reflect the decrease in macrolide consumption during those years. Nevertheless, whether decreasing antibiotic use in the community would have a sustained impact on resistance rates is unclear. Mathematical models as well as empirical data suggest that after a reduction in prescribing, resistance takes longer to decline than it took for resistance to rise after excessive antibiotic use (2).

The temporal increasing trend in the prevalence of the MLS_B phenotype in *S. pyogenes* (from 14% in 2001-2002 to 35.5% in 2006-2007) is probably due to the replacement of macrolide-resistant clones.

Concerning *H. influenzae*, the main findings were the decrease in the prevalence of isolates with phenotypes of resistance (β-lactamase production and BLNAR) and, as a result of this, the decrease in the prevalence of ampicillin resistance. The proportion of *H. influenzae* isolates producing β-lactamase decreased by 10% over 11 years and by 4.3% over the last 6 years. This decreasing trend is in accordance with other reports from Spain, Europe, and the United States (18, 26, 29). With regard to BNLAR isolates, up to 4.9% of isolates showing susceptibility to ampicillin (MIC of 0.5 to 1 μg/ml) had mutations in the *fstI* gene encoding PBP 3, confirming the findings from other authors about the fact that current ampicillin breakpoints (8) may fail to detect a significant number of low-BLNAR isolates (19).

When PK/PD breakpoints were used for *H. influenzae*, large discrepancies were observed in terms of susceptibility, mainly in macrolides and cefaclor. So, for instance, the rate of susceptibility to clarithromycin shifted from 99.3% (by use of the CLSI breakpoints) to 5.1% (by use of the PK/PD breakpoints). Some studies of acute otitis media have reported that *H. influenzae* behaves clinically as a macrolide-resistant organism, because bacteriological failures occur in patients infected with *H. influenzae*, despite the *in vitro* susceptibility claimed by use of the CLSI breakpoints (11). Perhaps the CLSI breakpoints for macrolides and cefaclor should be reviewed.

Interestingly, 38 noncapsulated *H. influenzae* isolates were collected from blood (92.7% of overall blood isolates) and 8 from pleural fluid (all pleural fluid isolates), which highlights the importance of noncapsulated isolates as a cause of invasive disease and the potential role of the new 10-valent pneumococcal nontypeable *H. influenzae* protein D conjugate vaccine (PHiD-CV).

In summary, this study shows a decreasing trend in the prevalence of resistance of the three most common pathogens involved in CARTIs. Over the last years, health authorities and scientific societies have implemented nationwide campaigns and made recommendations for a rational use of antibiotics, initiatives that should continue.

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