ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2001, p. 3226–3228 Vol. 45, No. 11
0066-4804/01/$04.00+0 DOI: 10.1128/AAC.45.11.3226–3228.2001

Antimicrobial Susceptibilities of 1,730 *Haemophilus influenzae* Respiratory Tract Isolates in Spain in 1998-1999

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Received 28 December 2000/Returned for modification 25 June 2001/Accepted 8 August 2001

A β-lactamase prevalence of 23% was found among 1,730 *Haemophilus influenzae* isolates. Ampicillin susceptibility was 70%, and 12% of β-lactamase-negative strains presented diminished susceptibility to ampicillin (BLNAR phenotype). Susceptibility of 90% was found for cefaclor and clarithromycin, whereas it was nearly 100% for cefotaxime, cefixime, azithromycin, and cefuroxime. Ciprofloxacin-resistant (0.1%) and β-lactamase-positive amoxicillin/clavulanate-resistant (BLPACR) phenotypes (0.1%) are anecdotal so far.

The aim of the present study is to describe the current susceptibility patterns of *Haemophilus influenzae* to first-line antibiotics used for respiratory tract infections, as well as to describe the prevalence of the new phenotypes of antibiotic resistance in Spain.

A total of 1,730 consecutive clinical isolates of *H. influenzae* obtained from community-acquired respiratory tract infections, collected between November 1998 and October 1999, were included in this prospective surveillance study involving 17 hospitals selected on the basis of geographic location. Strains were sent to a central laboratory (Instituto Valenciano de Microbiología, Valencia, Spain) to confirm the identification by colony morphology, Gram staining, growth in chocolate but not in blood agar, catalase test, and X and V factor requirements. In addition, a β-lactamase test (nitrocefin; Becton Dickinson) was performed (9). Susceptibility testing was performed following the National Committee for Clinical Laboratory Standards (NCCLS) 1999 guidelines using a semiautomated microdilution (7) method (Sensititre; Trek Diagnostics Inc., Westlake, Ohio) against antimicrobials commonly used as empiric therapy of respiratory tract infections in Spain. These were penicillin, amoxicillin, ampicillin, amoxicillin-clavulanate, cefaclor, cefuroxime, cefixime, cefotaxime, erythromycin, clarithromycin, azithromycin, and ciprofloxacin.

H. influenzae ATCC 49247, *H. influenzae* ATCC 49766, and *Escherichia coli* ATCC 35218 were used as quality control strains following NCCLS recommendations (7). BLNAR strains were defined as those strains failing to detect β-lactamase production and presenting with either a high (≥4 μg/ml) or intermediate (2 μg/ml) ampicillin MIC (3). On the other hand, β-lactamase-positive amoxicillin-clavulanate-resistant (BLPACR) strains were defined as those with an MIC to amoxicillin-clavulanate of ≥8 μg/ml that rendered positive with the nitrocefin disk test (3). Differences between β-lactamase-positive and β-lactamase-negative strains with respect to the prevalence of susceptibility were calculated using comparison of proportions with the chi square test. Estimation of 95% confidence intervals (95%CI) for population proportions was made by the exact binomial method. Epi-Info version 6.04 was used for statistical calculations.

A total of 1,534 samples (88.6%) were collected from the lower respiratory tract (sputum and bronchoalveolar lavage), 26 (1.5%) were from hemoculture, and 170 (9.8%) were from middle ear exudate. The vast majority of isolates (97.3%) were not typeable, whereas serogroups b, d, e, and f represented just 1.2, 0.2, 0.2, and 1%, respectively. The different production of β-lactamase in hemoculture (30.7%) in comparison with middle ear samples (23.5%) and lower respiratory tract samples (22.8%) did not show a significant statistical difference.

Prevalence of susceptibility, and MICs for 50 and 90% of strains (MIC_{50} and MIC_{90}) are shown in Table 1. According to NCCLS breakpoints, all antibiotics tested exhibited susceptibility rates equal to or higher than 99% except for ampicillin (70%), clarithromycin (89%), and cefaclor (91%). The antibi-

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otics with lowest intrinsic potency (as measured by MIC_{90}) happened to be penicillin, amoxicillin, ampicillin, cefaclor, erythromycin, and clarithromycin, all of them displaying an MIC_{90} of ≥8 μg/ml. The remaining antibiotics for which there are NCCLS breakpoints presented a far better intrinsic potency, and as expected, third-generation cephalosporins and ciprofloxacin showed the lowest MIC_{90}.

Nearly one of every four isolates (399 of 1,730) was a β-lactamase producer (23%; 95%CI, 21 to 25%), a figure very much like that found in our previous surveillance study (25.7%) (4), but significantly lower than that reported in Spain by other national (10) and multinational (11) studies, around 30 to 35%. Considering geographical differences in our 17 centers, β-lactamase production ranged from 15.8 to 30.4%, but no statistical significance was reached. The β-lactamase production status of the isolates did not influence their susceptibility to the antibiotics tested except for ampicillin (9.7% for β-lactamase positive versus 87.9% for β-lactamase negative; $P < 0.0001$) and cefaclor (70.4% for β-lactamase positive versus 97.7% for β-lactamase negative; $P < 0.0001$). Cefaclor was the sole oral cephalosporin for which β-lactamase production influenced the prevalence of susceptibility.

Concerning β-lactamase-negative isolates, 87 (6.5%) and 74 (5.5%) strains were found to be intermediate and resistant to ampicillin, respectively, and were therefore categorized as BLNAR. This means that 9.3% of the whole *H. influenzae* population of the study were BLNAR (95% CI, 8 to 10.7%), suggesting an increase compared with previous reports in Spain of around 5% (3,10). Again, no significant differences in the prevalence of BLNAR strains were seen on a geographical basis.

Macrolide nonsusceptibility was around 1% for azithromycin and 10% for clarithromycin, most of this being nonsusceptibility categorized as intermediate resistance. In any case, clarithromycin was significantly less active than azithromycin in terms of MIC_{90} values (16 μg/ml versus 2 μg/ml).

Regarding the extremely rare ciprofloxacin resistance phenotype, we found only two isolates (0.1%) with MICs of 2 and 4 μg/ml, which is in accordance with other authors (1). Likewise, only two isolates (0.1%) were found to be BLPACR, which is less than reported elsewhere (3).

H. influenzae is one of the most prevalent isolates in community-acquired respiratory tract infections. Multicenter surveillances have had key importance in ascertaining β-lactamase production (2,4,5,8,10), decreasing susceptibility to certain macrolides (4), and increasing intrinsic resistance to ampicillin mediated by altered penicillin-binding proteins (PBP) (6), the so-called β-lactamase-negative ampicillin resistance (BLNAR). Likewise, surveys have revealed novel resistance phenotypes as very rare ciprofloxacin-resistant (1) and β-lactamase-positive and amoxicillin-clavulanate-resistant (BLPACR) isolates (3).

Our survey shows that β-lactamase production in Spain (23%) is homogeneously distributed and seems to be decreasing compared with other reports, but ampicillin susceptibility hardly changes (69.9%; 95% CI, 67.7 to 72%). However, the prevalence of the BLNAR phenotype seems to be increasing (9.3%), and sharp differences in the activity of macrolides are also evident. Both BLPACR and ciprofloxacin-resistant phenotypes constitute to date just anecdotal events (0.1%), but

TABLE 1. MIC_{50}, MIC_{90}, range of MICs, and susceptibility of 1,730 clinical isolates of *H. influenzae*[a]

Antimicrobial agent	Overall (n = 1,730)						β-Lactamase positive (n = 399)						β-Lactamase negative (n = 1,331)					
	MIC (μg/ml)			No. (%) of strains			MICs (μg/ml)		No. (%) of strains				MICs (μg/ml)		No. (%) of strains			
	MIC_{50}	MIC_{90}	Range of MICs	Susceptible	Intermediate	Resistant	MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant		MIC_{50}	MIC_{90}	Susceptible	Intermediate	Resistant	
Penicillin	1	≥16	≤0.015–≥16	NA	NA	NA	≥16	≥16	NA	NA	NA		0.5	4	NA	NA	NA	
Amoxicillin	1	≥16	≤0.015–≥16	NA	NA	NA	≥16	≥16	NA	NA	NA		1	4	NA	NA	NA	
Ampicillin	≤0.5	≥8	≤0.5–≥8	1,209 (69.9)	96 (5.5)	425 (24.6)	≥8	≥8	39 (9.7)	9 (2.3)	351 (88)		≤0.5	2	1,170 (87.9)	87 (6.5)[b]	74 (5.6)[b]	
Amox-clav[c]	1	2	≤0.015–8	1,722 (99.5)	NA	8 (0.5)	1	4	397 (99.5)	NA	2 (0.5)		0.5	2	1,325 (99.5)	NA	6 (0.5)	
Cefaclor	4	8	≤1–≥64	1,581 (91.4)	99 (5.7)	50 (2.9)	8	≥64	281 (70.4)	75 (18.8)	43 (10.8)		4	8	1,699 (97.7)	24 (1.8)	7 (0.5)	
Cefuroxime	1	4	≤0.25–8	1,718 (99.3)	12 (0.7)	0	1	4	392 (98.2)	7 (1.8)	0		1	4	1,725 (99.6)	5 (0.4)	0	
Cefixime	≤0.25	≤0.25	≤0.25–≥8	1,727 (99.8)	NA	NA	≤0.25	≤0.25	397 (99.5)	NA	NA		≤0.25	≤0.25	1,330 (99.9)	NA	NA	
Cefotaxime	≤0.25	≤0.25	≤0.25–8	1,729 (99.9)	NA	NA	≤0.25	≤0.25	399 (100)	NA	NA		≤0.25	≤0.25	1,330 (99.9)	NA	NA	
Erythromycin	4	8	≤0.12–≥64	NA	NA	NA	4	8	NA	NA	NA		4	8	NA	NA	NA	
Clarithromycin	4	16	≤0.25–≥64	1,546 (89.4)	159 (9.2)	25 (1.4)	8	16	353 (88.5)	40 (10)	6 (1.5)		4	16	1,193 (89.7)	119 (8.9)	19 (1.4)	
Azithromycin	0.5	2	≤0.12–≥64	1,712 (98.9)	NA	NA	0.5	2	393 (98.5)	NA	NA		0.5	2	1,319 (99.1)	NA	NA	
Ciprofloxacin	≤0.5	≤0.5	≤0.5–4	1,728 (99.9)	NA	≤0.5	≤0.5	≤0.5	399 (100)	NA	NA		≤0.5	≤0.5	1,329 (99.8)	NA	NA	

[a] NCCLS 2000 breakpoint criteria concentrations are expressed in micrograms per milliliter for susceptible, intermediate, and resistant categories are: penicillin, NA/NA/NA (NA, no NCCLS breakpoint criteria); amoxicillin, NA/NA/NA (but the results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin); ampicillin, ≤1/2/≥4; amoxicillin-clavulanate, ≤4/NA/≥8; cefaclor, ≤8/16/≥32; cefuroxime, ≤4/8/≥16; cefixime, ≤1/NA/NA; cefotaxime, ≤2/NA/NA; erythromycin, NA/NA/NA; clarithromycin, ≤8/16/≥32; azithromycin, ≤4/NA/NA; ciprofloxacin, ≤1/NA/NA.

[b] According to NCCLS, BLNAR strains should be considered resistant to amoxicillin-clavulanate, cefaclor, and cefuroxime despite apparent in vitro susceptibility of some BLNAR strains to these agents.

[c] Amox-clav; amoxicillin-clavulanate (2:1); concentrations refer to amoxicillin.

they must be monitored. Regarding BLNAR strains and following NCCLS, the question is open as to what extent ampicillin-intermediate strains should be considered resistant to the remaining β-lactams antibiotics within the family.

Differences in susceptibility patterns between different surveillances over time due to the increasing prevalence of certain phenotypes of resistance or the emergence of new ones make national multicenter surveys a must. Most of the multicenter surveillances of resistance carried out so far have as a main criticism that they make their estimations based either on a relatively small number of isolates from a single country or on a large number of isolates from many different countries. Both attitudes cannot but lead to a somewhat inadequate representation of the complex reality of the resistance dynamics for a given country. That is why extensive national multicenter surveillances provide more reliable information, ensuring a better estimation for the country whose specific information on resistance is sought.

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