β-Lactamase Production *Haemophilus* spp. and Resistance to Ampicillin in a General Hospital in Porto Alegre City, RS, Brazil (2001-2005)

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In a four-year period (July/2001-June/2005), 410 *Haemophilus* spp. isolates were studied. Those were isolated from sputum at Hospital Nossa Senhora da Conceição (NSC) in Porto Alegre city (RS). β-lactamase enzyme was detected in 113 (27.6%) of isolates through chromogenic cephalosporin method. Fifty-eight (51.3%) of them showed sensibility to ampicillin through disc-diffusion method using *Haemophilus* Test Medium (HTM) by NCCLS criteria. In 297 (72.4%) isolates β-lactamase was not detected by chromogenic cephalosporin method. Five (1.7%) of them were resistant and 1 (0.3%) intermediate to ampicillin using disc-diffusion method. The authors emphasized the importance of *Haemophilus* spp. resistance to ampicillin research in clinical laboratories routine and the use of more than one method for this analysis was proposed, due to different resistance mechanisms in *Haemophilus* spp.
Key-Words: *Haemophilus* spp., β-lactamase, antimicrobial resistance, ampicillin.

In the recent years, the resistance to antimicrobials among pathogens implied in respiratory tract infection (RTI) acquired in community have increased and spread in alarming rates [1]. *Haemophilus influenzae* is an important pathogen in RTI acquired in community, causing signs and symptoms undistinguished from those caused by other pathogens, notoriously *Streptococcus pneumoniae* [2]. *Haemophilus influenzae* is a Gram-negative pathogen and important etiologic agent of morbid-mortality among children under five years old, both in developed countries and in Latin American countries. Among these, *H. influenzae* type B (Hib-capsulated) is considered one of the most important etiologic agent of fatal infections in children [3], whereas encapsulated *H. influenza* is part of superior respiratory tract flora, and it can be considered a protection shield against invasive infections [4]. Since the 1970's, there has been an worldwide increase of *H. influenzae* resistant isolates, mainly to ampicillin. The ampicillin-resistance is mediated by plasmids through transposons (TnA – bacteria mobile chromosome elements) and this mechanism is extremely important to plasmids evolution originating multiple resistance [5]. The resistance of *H. influenzae* to ampicillin is not widely known and discussed [6]. The production of β-lactamase is the main mechanism of ampicillin-resistance and other beta-lactamics [7]. Between 1972 and 1974 the first resistance to ampicillin and chloranphenicol *H. influenzae* was isolated in the United States and Europe, with consequent increasing and diffusion of those strains worldwide [8-10]. The resistance of *H. influenzae* to ampicillin has been described, characterizing β-lactamase extracellular TEM-1 [11], and ROB-1 [12], both acquired through plasmids. Recently another type of ampicillin resistance has been described, mediated by elements other than β-lactamase, once a phenotipic BLNAR (β-lactamase negative ampicillin resistant) characteristic has been shown?[13]. Such resistance has been associated to the presence of altered PBPs (penicillin binding proteins) [14]. The detection of β-lactamase in clinical microbiology laboratories shows a controversial scenary due to the presence of many methods and variable proposes [6,15]. The most acceptable methods are the ones recommended by National Committee for Clinical Laboratory Standards (NCCLS) [16]. The purpose of this study was to describe the consecutive analysis of 410 strains of *Haemophilus* ssp. isolated from sputum between July/2001 and June/2005 and show the importance of the application of more than one analyzes method in the analysis of antimicrobial resistance.

Material and Methods

Hospital

The microbiology department of Hospital Nossa Senhora da Conceição (HNSC), in Porto Alegre, RS, Brazil.

Isolates

Within the period of July 2001 to June 2005, 410 *Haemophilus* spp. were isolated from sputum in the HNSC and were prospectively submitted to resistance tests to antimicrobials. The isolates which were obtained in the period between January and September 2004 have been excluded due to non-commercial availability of the β-lactamase test through chromogenic cephalosporin test, mentioned below.

Isolation

Four hundred and ten *Haemophilus* spp. strains isolated from sputum in chocolate-agar (BioMérieux, Jacarepaguá, Rio de Janeiro) have been tested in microaerophilic condition; increasing condition with low tension production of CO_2 (3 to 5% of CO_2 for *Haemophilus* spp.) by the mean of a candle put inside an appropriate jar.

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Suscetibility Testing

The chromogenic cephalosporin test, also known as nitrocefin (cefinase test) (BD BBL-Becton Dickinson and Company, Sparks, USA) has been applied [17,18]. The cefinase discs contain nitrocephin (chromogenic cephalosporin), and each disc is used to evaluate the production of β-lactamase in a clinic isolated. When the production of this enzyme occurs with the action of bacteria, the discs (with yellowish initial color) are shown in a reddish color in the respective area where the isolated β-lactamase producer was. The second test was the detection of ampicillin resistance using the disc diffusion method, adapted from Kirby-Bauer method to application for *H. influenzae* according to NCCLS (2001 to 2005) recommendations. Haemophilus Test Medium (HTM) was used from Oxoid (Basinstoke, Hampshire, England) and discs of 10 m*g* of ampicillin from Cefar (Cefar Diagnóstica LTDA, São Paulo, SP, Brazil).

Results

Four hundred and ten strains of *Haemophilus* spp. isolated in HNSC from July 2001 to June 2005 were submitted to suscetibility test to antimicrobials through different methods, with phenotipic characteristics according to data shown in Table 1. All the strains and tests were made prospectively. The strains have been consecutively included in the study, except during the period from January to September 2004, when there was not commercial availability for the β-lactamase detection method. Table 1 shows the decreasing of incidences of *Haemophilus* spp. β-lactamase-positive and ampicillin-resistance isolates (BLPAR) during the period of study until 2004. In 2005 the emerge of β-lactamase-negative and ampicillin-resistant (BLNAR) takes place, with following incidence of β-lactamase -negative and ampicillin intermediate (BLNAI) and BLNAR. Table 2 shows that out of the 410 *Haemophilus* spp., 14.1% are BLPAS and signs the emerge of 5 BLNAR cases, which means about 1% of the total isolated ones and 10% of the total ampicillin-resistant (AR), which becomes an alarming fact in the institution.

Discussion

Antibiotic self-administration is a common practice in Latin American countries, which contributes for the increase of many resistant organisms, including the *Haemophilus* spp. ampicillin resistance. This might occur due to the antibiotic administration by patients with respiratory tract infection providing a selective pressure and decrease of suscetibility to β-lactamase [5]. Moreover, the different protocols of recommendation for use of antibiotics in such situation take to a selective pressure in the community, which is observed in different resistant levels in Brazil and other countries [19]. In countries like Brazil, there is some need of better analysis standard for *Haemophilus* spp. resistance in order to assure a better therapy option, mainly where the ampicillin and chloranfenicol combination is used in large scale (taking into account their low cost and effectiveness) [20]. The data shown from the evaluation of results obtained in the ampicillin suscetibility test *versus* β-lactamase test in the cefinase test, present the decrease in the incidence of isolates of positive *Haemophilus* spp. β-lactamase and ampicillin resistance from 2002 on, and the negative β-lactamase ampicillin resistance from 2003 on with some increase in 2005. The importance of the cefinase test is shown in Table 2, where we can see 14% of BLPAS that should induct to ampicillin administration if the cefinase test was not done. Apart from that, the use of isolated cefinase test, in the last 2 years, would not detect those BLNAR strains that correspond to 10% of all the ampicillin resistant. Taking into account all the data shown, the authors reinforce the importance of the use of the cefinase test in clinical microbiology laboratories as a complementary test for *Haemophilus* spp. resistant studies.

Table 1. Distribution of 410 *Haemophilus* spp. isolates from sputum at Hospital Nossa Senhora da Conceição, Porto Alegre (RS)

Years	BLP/AS	BLP/AI	BLP/AR	BLN/AS	BLN/AI	BLN/AR	Total
2001	26	4	20	62	0	0	102
2002	12	2	11	75	0	0	100
2003	8	0	3	84	0	1	96
2004	5	1	1	29	1	0	37
2005	7	2	11	41	0	4	65

The data represent the number of *Haemophilus* spp. by period. BLP=β-lactamase positive; BLN=β-lactamase negative; AS=ampicillin suscetible; AI=ampicillin intermediate; AR=ampicillin resistant.

Table 2. Behavior of 410 *Haemophilus* spp. strains in the β-lactamase and ampicillin disc test

	AS	AI	AR	Total
BLP	58 (14.1%)	9 (2.2%)	46 (11.2%)	113 (27.6%)
BLN	291 (71.0%)	1 (0.24%)	5 (1.21%)	297 (72.4%)
Total	349 (85.1%)	10 (2.4%)	51 (12.4%)	

The data represent the number of *Haemophilus* spp., where the numbers in parentheses represent the percentage corresponding. BLP=b-lactamase-positive; BLN=b-lactamase-negative; AS=ampicillin-suscetible; AI=ampicillin-intermediate; AR=ampicillin-resistant.

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