

# Antimicrobial susceptibility patterns of *Haemophilus* isolates from children in eleven developing nations

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*The antimicrobial susceptibilities of 426 isolates of Haemophilus species, which were collected as part of a worldwide study of the etiology of acute respiratory disease in children in selected developing countries, were determined. Eleven antibiotics were tested using the recently described Haemophilus Test Medium. There was a low prevalence of antibiotic resistance; 6% of strains were resistant to ampicillin, and 1.6% were resistant to chloramphenicol. Strains resistant to both ampicillin and chloramphenicol were recovered only from Thailand. Susceptibility to penicillin G was also determined; the minimum inhibitory concentrations for penicillin and ampicillin were concordant within one 2-fold dilution in 97% of the isolates. Thus, Haemophilus isolates were as susceptible to penicillin G as they were to ampicillin, and penicillin resistance was infrequent overall. These data provide support for the current protocols for the management of acute respiratory infections in children in developing countries, in which penicillin G is a first-line agent.*

## Introduction

Acute respiratory infections are a major cause of morbidity and mortality in children in developing nations (1, 2). A large proportion of the bacterial respiratory infections in children are caused by *Haemophilus influenzae*. Little is known about the patterns of *H. influenzae* antibiotic susceptibility in developing countries; such data are necessary in order to develop rational strategies for therapy of *Haemophilus* infections.

In the past, it has been difficult to compare reports of susceptibility patterns from different countries because of variation in testing methodologies. Susceptibility testing of *Haemophilus* species is complicated by the fastidious nature of these organisms and by their requirement for complex growth media, which in turn affect the accuracy and reproducibility of the susceptibility testing (3, 4). Recently, Jorgensen et al. (5) described the *Haemophilus* Test Medium (HTM). This medium yields rapid growth, is optically clear, and is relatively simple to prepare, thus allowing better standardization of antimicrobial susceptibility testing. This medium has been used in recent surveys of resistance among *H. influenzae* isolates in the United States (6, 7), and is now the test medium recommended for use in the USA by the National Committee for Clinical Laboratory Standards (NCCLS) (8).

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The Board on Science and Technology for International Development (BOSTID) of the United States National Academy of Sciences/National Research Council recently coordinated a multicentre collaborative project to define the etiology and epidemiology of acute respiratory infections in children in 12 developing countries. The method of Jorgensen et al. (5) was used to measure susceptibilities to 11 antibiotics of 426 *Haemophilus* isolates collected from 11 countries participating in the BOSTID project, in order to determine the patterns of resistance among *Haemophilus* isolates from selected areas in the developing world.

## Materials and methods

*Haemophilus* isolates from children with acute respiratory tract infection were identified in each country by standard techniques, and then forwarded to Washington University, St. Louis, Missouri, USA, for further study. Isolates were classified as invasive (those originating from blood, cerebrospinal fluid, pleural fluid, or lung aspirate cultures) or noninvasive (those originating from throat or nasopharyngeal cultures). The identification of all invasive isolates was confirmed as previously described (9). Briefly, *Haemophilus* species were identified by colonial morphology, growth requirements for factors X and V, and porphyrin production. Capsular serotyping was performed by a battery of tests including growth of iridescent colonies with surrounding halos on Levinthal agar containing burro anti-type b antiserum; latex agglutination; and countercurrent immunoelectrophoresis (9). The clinical, epidemiologic, and laboratory methods of the global BOSTID acute respiratory infection studies will be reported elsewhere (J. Bale et al., in preparation). Briefly, blood and throat cultures were obtained routinely from children with acute lower respiratory tract infection attending outpatient clinics or admitted to hospitals. Additionally, in some countries, community-based longitudinal studies were undertaken; at these sites, symptomatic patients provided throat cultures alone. Acute respiratory tract infections were defined by criteria similar to those of the World Health Organization.<sup>a</sup> The parents of all children participating in the BOSTID studies gave informed consent to the individual investigators.

Antibiotic susceptibilities were determined by microbroth dilution using HTM as described (5-8). HTM consists of Mueller-Hinton broth, supplement-

ed with 25 mg/l magnesium, 50 mg/l calcium, 5 g/l yeast extract, 15 mg/l bovine hematin, 15 mg/l  $\beta$ -NAD, and 200 IU/l thymidine phosphorylase. Isolates were grown overnight on enriched chocolate agar, suspended in HTM, and adjusted to a turbidity equivalent to a 0.5 McFarland standard. This suspension was then diluted 1:200 in HTM, and 50  $\mu$ l was added to 50  $\mu$ l of test antibiotic diluted in HTM (to give a final inoculum density in each well of approximately  $5 \times 10^8$  colony forming units/l). Microtitration plates were incubated in an air incubator at 35 °C for 16-20 hours. Endpoints were determined by visual inspection. *Escherichia coli* strain ATCC 25922 and *Staphylococcus aureus* strain ATCC 29213 were included as control strains in each assay. In addition, coded *H. influenzae* type b strains known to be resistant (by standard enzymatic or plate-dilution methods) to ampicillin, chloramphenicol, or rifampicin were included as controls. Minimum inhibitory concentrations (MICs) were interpreted according to the NCCLS guidelines (8). Resistance to each antibiotic was defined according to the following MIC values (all concentrations are given in mg/l): amoxicillin/clavulanic acid,  $\geq 8/4$ ; ampicillin,  $\geq 4$ ; cefaclor,  $\geq 32$ ; cefamandole,  $\geq 16$ ; ceftriaxone,  $\geq 2$ ; chloramphenicol,  $\geq 8$ ; rifampicin  $\geq 4$ ; tetracycline,  $\geq 8$ ; and trimethoprim/sulfamethoxazole (TMP/SMX)  $\geq 4/76$ . Resistance to erythromycin was defined as the presence of an MIC  $\geq 1$  mg/l (5). Because the MIC value defining resistance to penicillin G has not been established, the penicillin G results were expressed in relation to the ampicillin MICs (see Results section). Production of  $\beta$ -lactamase was tested by a rapid acidometric method (10).

## Results

Invasive isolates of *Haemophilus* were recovered from children enrolled in studies in seven of the 11 participating developing countries. A total of 240 children yielded 252 isolates. Of these isolates, 111 (44%) originated from children in Pakistan; 79 (31%) were from Papua New Guinea; and 42 (17%) were from the Philippines. There were 161 (64%) invasive isolates identified as *H. influenzae* type b; 14 (6%) encapsulated, non-type b *H. influenzae* (representing serotypes a, c, d, e, and f) isolates; 65 (26%) nontypable *H. influenzae* isolates; and 12 isolates (5%) identified as *H. parainfluenzae*. The antibiotic susceptibility patterns of these isolates are summarized in Table 1.

Resistance to ampicillin was uncommon among invasive isolates from most countries. Fifteen (6%) of the 252 invasive isolates were resistant to ampicillin (MICs of 4 to 128 mg/l); all 15 produced  $\beta$ -lactamase

<sup>a</sup> World Health Organization. *Case management of acute respiratory infections in children in developing countries*. Report of a Working Group Meeting, Geneva, 3-6 April 1984. Unpublished document WHO/RSD/85 15 Rev 1

Table 1: Antimicrobial susceptibilities of 252 invasive *Haemophilus* isolates

Country	No of isolates	No of isolates resistant <sup>a</sup>					
		Ampicillin or penicillin G	Amoxicillin/clavulanic acid	Chloramphenicol	Erythromycin	Tetracycline	Trimethoprim/sulfamethoxazole
Argentina	4	1	0	0	4	0	0
Guatemala	1	0	0	0	1	0	0
Pakistan	111	8	0	0	96	0	12 <sup>b</sup>
Papua New Guinea	79	1	0	0	79	0	0
Philippines	42	1	1	0	41	0	0
Thailand	11	4	0	4	11	4	1
Uruguay	4	0	0	0	4	0	0

<sup>a</sup> None of the isolates was resistant to cefaclor, cefamandole, ceftriaxone, or rifampicin (see text for MICs that define resistance).

<sup>b</sup> Twenty-five more isolates from Pakistan had intermediate susceptibility to trimethoprim/sulfamethoxazole (trimethoprim MICs of 1 to 2 mg/l)

(Table 1). Six of the 15 isolates were type b *H. influenzae*, and the remaining 9 were non-typable. One of the 15 ampicillin-resistant isolates was also resistant to amoxicillin/clavulanic acid, with an MIC of 64 mg/l; but none of the 15 isolates was resistant to cefaclor, cefamandole, or ceftriaxone.

Among invasive *Haemophilus* isolates, resistance to chloramphenicol, tetracycline, or both was found only in the collection from Thailand (4 of 11 isolates tested from Thailand). Three of these four isolates were resistant to ampicillin as well as to chloramphenicol and tetracycline; the fourth isolate was resistant to chloramphenicol and tetracycline alone. One additional isolate from Thailand was resistant to ampicillin but not to chloramphenicol or tetracycline. Each of these phenotypic patterns has been previously associated with transmissible resistance elements (3, 4, 11, 12).

Resistance to TMP/SMX among invasive isolates was found predominantly in Pakistan (12 of the 13 resistant isolates in the collection). In addition, all 25 of the invasive *Haemophilus* isolates in the collection with intermediate susceptibility to TMP/SMX (trimethoprim MICs of 1 to 2 mg/l) were from Pakistan. Resistance to TMP/SMX in Pakistan was found among invasive non-typable *H. influenzae* isolates (12 resistant, 13 of intermediate susceptibility, 11 susceptible) and invasive *H. parainfluenzae* isolates (all 12 of intermediate susceptibility) but was not detected among the 63 invasive type b *Haemophilus* isolates.

No resistance to rifampicin was found among the invasive isolates of any country studied. In contrast, resistance to erythromycin was found among isolates of all countries studied (94% of isolates resistant. Table 1).

Noninvasive isolates of *Haemophilus* species were provided to us from children enrolled in eight of the 11 studies. A total of 174 isolates from 172 children were examined; 22 (13%) isolates were *H. influenzae* type b (serotypes other than type b were not determined in the remaining noninvasive isolates). The antibiotic susceptibility patterns of these isolates are summarized in Table 2.

In general, the susceptibility patterns of the noninvasive isolates were similar to those of the invasive isolates. Ten (6%) of 174 noninvasive isolates were resistant to ampicillin, and all ten produced  $\beta$ -lactamase. Two of these isolates were also resistant to amoxicillin/clavulanic acid (each had an MIC of 8 mg/l). There were three noninvasive *Haemophilus* isolates resistant to both chloramphenicol and tetracycline as well as ampicillin; all were from Thailand. In addition, one isolate from Thailand had intermediate susceptibility to chloramphenicol and tetracycline, but was susceptible to ampicillin; another isolate (from Colombia) had intermediate susceptibility to chloramphenicol but was resistant to tetracycline and ampicillin.

The susceptibility patterns of all isolates were also determined against penicillin G, for which interpretive breakpoints have not been established. The distribution of MICs of penicillin G and ampicillin against all 426 *Haemophilus* isolates in the collection is shown in Fig. 1. In 97% of isolates, the penicillin and ampicillin MICs were concordant within one 2-fold dilution. In addition, there was a complete concordance between penicillin MICs and the production of  $\beta$ -lactamase; 25/25 isolates with an MIC  $\geq 4$  mg/l produced  $\beta$ -lactamase, while none of 230 isolates tested with an MIC of  $\leq 1$  mg/l produced  $\beta$ -lactamase.

Table 2: Antimicrobial susceptibilities of 174 noninvasive *Haemophilus* isolates

Country	No. of isolates	No of isolates resistant <sup>a</sup>					
		Ampicillin or penicillin G	Amoxicillin/clavulanic acid	Chloramphenicol	Erythromycin	Tetracycline	Trimethoprim/sulfamethoxazole
Argentina	12	1	1	0	11	0	0
Bangladesh	23	0	0	0	22	1	0
Brazil	5	0	0	0	5	0	2
Colombia	52	4	0	0 <sup>b</sup>	48	2 <sup>c</sup>	0
Guatemala	7	0	0	0	7	0	0
Kenya	41	0	0	0	41	0	1
Thailand	29	5	1	3 <sup>b</sup>	25	9 <sup>c</sup>	2 <sup>d</sup>
Uruguay	5	0	0	0	4	0	0

<sup>a</sup> None of the isolates was resistant to cefaclor, cefamandole, ceftriaxone, or rifampicin (see text for MICs that define resistance).

<sup>b</sup> One more isolate each from Colombia and Thailand had intermediate susceptibility to chloramphenicol (MIC = 4 mg/l).

<sup>c</sup> One more isolate from Colombia, and two more isolates from Thailand had intermediate susceptibility to tetracycline (MIC = 4 mg/l).

<sup>d</sup> Two more isolates from Thailand had intermediate susceptibility to trimethoprim/sulfamethoxazole (trimethoprim MICs = 1–2 mg/l).

## Discussion

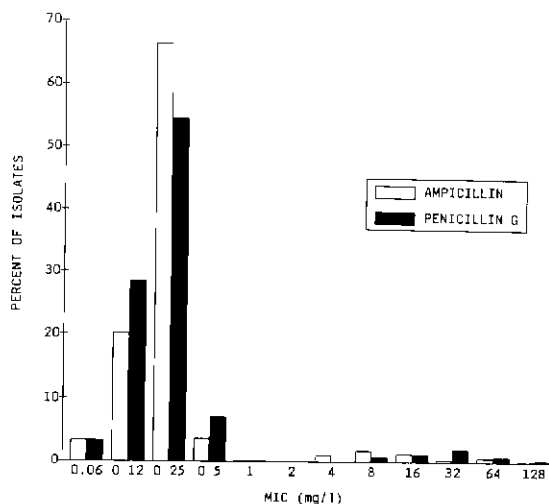
The prevalence of antibiotic resistance among invasive and noninvasive *Haemophilus* isolates was low compared to recent surveys in the USA and Europe (6, 13). For example, only 6% of isolates in the current study were resistant to ampicillin, compared to 20% in a recent collaborative study performed in the USA (6). With the exception of isolates from Thailand, resistance to chloramphenicol was rare in the current study. The low prevalence of resistance to the different antibiotics was unexpected in view of the availability of antibiotics, without the

need for a prescription, in many developing nations. There are two possible explanations for this finding. First, although antibiotics may be available without prescription, data are lacking on the actual per capita consumption of antibiotics in many areas of the developing world. Thus, the true amount of antibiotics consumed by the populations studied may be less than that expected based upon their availability. Alternatively, transmissible resistance elements may not yet have entered the bacterial population of most of the countries studied. If the latter hypothesis is true, once such elements enter the bacteria in question, resistance may develop in the future over a relatively brief period of time, as has been observed in the USA and Europe (3, 4).

In contrast to reports from the USA, resistance or decreased susceptibility to TMP/SMX was relatively common in some of the developing nations, especially Pakistan. Overall, approximately 4% of the *Haemophilus* isolates tested in this study were resistant to TMP/SMX, and 6% had intermediate susceptibility to TMP/SMX, compared to <0.7% in either category among isolates from the USA (6). Whenever TMP/SMX resistance is reported in an area, modifications in the choice of oral therapy for case management of acute respiratory infections may be necessary.<sup>b</sup>

Penicillin G is no longer routinely used for the treatment of *Haemophilus* infections in the USA or Europe; as a result, modern guidelines for the interpretation of MICs of penicillin G were never firmly established (4). None the less, parenteral penicillin G is recommended as part of the case management of

Fig. 1. Distribution of minimum inhibitory concentrations (MIC) of ampicillin and penicillin G against 426 isolates of *Haemophilus* recovered from children in 11 developing countries. The MICs were determined by microbroth dilution using *Haemophilus* Test Medium.



<sup>b</sup> World Health Organization. Case management of acute respiratory infections in children in developing countries. Report of a Working Group Meeting, Geneva, 3–6 April 1984. Unpublished document WHO/RSD/85.15 Rev. 1

pneumonia in children in the developing world.<sup>b</sup> The strong correlation between the MICs of penicillin G and ampicillin, together with the confirmatory  $\beta$ -lactamase assay results, suggest that the interpretive guidelines for ampicillin could be applied to penicillin. Based upon our data and using these guidelines, we anticipate that penicillin would be active against 94% of the isolates tested. These data, along with pharmacokinetic studies of penicillin in children with pneumonia in developing nations (14), provide support for the current WHO recommendations.<sup>b</sup> However, monitoring of antibiotic susceptibility patterns of *Haemophilus* isolates should be continued and prospective studies designed to monitor the impact of therapy on the outcome of acute respiratory infection morbidity and mortality are needed.

### Acknowledgments

A. Niles, R. Heeren, S. Cole, J. Granoff, and K. Lottenbach provided expert technical assistance. We also thank Dr J. Bale and Dr M. Greene (National Academy of Sciences/National Research Council, USA) for their support and advice. Work performed at Washington University was supported in part by contract AID-2429-8-312 from the Board on Science and Technology for International Development (BOSTID), National Academy of Sciences/National Research Council, by means of a grant from the US Agency for International Development; and by grant RO1 AI 24332 from the National Institute of Allergy and Infectious Diseases, United States Public Health Service. Dr Weinberg is a recipient of the Burroughs Wellcome Fellowship in Infectious Diseases from the Infectious Diseases Society of America.

### Résumé

#### Sensibilité aux antimicrobiens des isolements d'*Haemophilus* provenant d'enfants de 11 pays en développement

Les infections respiratoires aiguës constituent une cause importante de morbidité et de mortalité chez les enfants des pays en développement. *Haemophilus influenzae* est à l'origine de bon nombre des infections respiratoires bactériennes de l'enfant. Toutefois, on sait peu de chose du profil de la sensibilité d'*H. influenzae* aux antibiotiques dans les pays en développement.

On a étudié la sensibilité à 11 antibiotiques de 426 isolements d'*Haemophilus* recueillis dans le cadre d'une étude collective multicentrique sur l'étiologie et l'épidémiologie des infections respiratoires aiguës, menée dans 11 pays en développement. On a déterminé les concentrations mini-

males inhibitrices (CMI) par des microdilutions en bouillon, en prenant un milieu de culture récemment mis au point (*Haemophilus* Test Medium, contenant un bouillon de Mueller-Hinton enrichi en cations, un extrait de levure, de l'hématine bovine, du  $\beta$ -NAD et de la thymidine-phosphorylase). On a procédé à l'identification bactériologique en associant diverses méthodes, dont: la morphologie des colonies, les besoins en facteurs de croissance, la production de porphyrine, la formation de colonies translucides entourées de halos sur gélose de Levinthal contenant de l'immunsérum d'âne ("burro") anti-type b, l'agglutination au latex, et l'immunoélectrosynérèse.

La résistance à chaque antibiotique a été définie pour les concentrations minimales inhibitrices (mg/l) suivantes: amoxicilline/acide clavulanique,  $\geq 8/4$ ; ampicilline,  $\geq 4$ ; céfador,  $\geq 32$ ; céfamandole,  $\geq 16$ ; ceftriaxone,  $\geq 4$ ; chloramphénicol,  $\geq 8$ ; érythromycine,  $\geq 1$ ; rifampicine,  $\geq 4$ ; tétracycline,  $\geq 8$ ; et triméthoprime/sulfaméthoxazole (TMP/SMX),  $\geq 4/76$ .

On a obtenu 252 isolements d'*Haemophilus* après prélèvement par des techniques invasives (hémocultures, cultures de liquide céphalorachidien, de liquide pleural, ou de liquide d'aspiration pulmonaire) chez 240 enfants provenant de sept pays; 161 d'entre eux (64%) étaient des isolements d'*H. influenzae* de type b, 14 (6%) des isolements d'*H. influenzae* encapsulés n'appartenant pas au type b (représentant des sérotypes a, c, d, e et f), 65 (26%) des isolements d'*H. influenzae* non typables et 12 (5%) des isolements d'*H. parainfluenzae*.

La résistance aux antibiotiques était rare dans la plupart des pays. Sur les 252 isolements précités, 15 étaient résistants à l'ampicilline (CMI de 4-128 mg/l); ils ont tous produit de la  $\beta$ -lactamase (méthode acidimétrique). Sur ces 15 isolements, 6 appartenaient au type b et 9 n'étaient pas typables. L'un d'entre eux était également résistants à l'association amoxicilline/acide clavulanique; toutefois, aucun n'était résistants au céfador, au céfamandole, ni à la ceftriaxone. Quatre isolements d'*Haemophilus* (provenant tous de Thaïlande) étaient résistants au chloramphénicol et à la tétracycline. trois d'entre eux étaient également résistants à l'ampicilline. Dans ces isolements, la résistance au TMP/SMX a été surtout retrouvée au Pakistan: sur les 13 isolements résistants, 12 provenaient du Pakistan; les 25 isolements ayant présenté une sensibilité intermédiaire (CMI de 1-2 mg/l) provenaient également de ce pays. Aucune résistance à la rifampicine n'a été observée, quel que soit le pays. En revanche, on a trouvé une résistance à l'érythromycine dans les isolements de

tous les pays étudiés (94% d'isolements résistants).

On a obtenu 174 isolements d'*Haemophilus* spp. après prélèvement par des méthodes non invasives (cultures de prélèvements de gorge ou de rhinopharynx) chez 172 enfants provenant de huit pays; 22 d'entre eux (13%) étaient des isolements d'*H. influenzae* de type b (on n'a pas recherché le sérotype des isolements restants). En général, le profil de la sensibilité des isolements obtenus après prélèvement par des méthodes non invasives a été le même que celui des isolements obtenus après prélèvement par des méthodes invasives.

Dans 97% des isolements, les concentrations minimales inhibitrices pour la pénicilline et l'ampicilline concordent pour chaque dilution au double, et la correspondance était totale entre les concentrations minimales inhibitrices de la pénicilline et la production de  $\beta$ -lactamase.

Nous en concluons donc que: 1) la prévalence de la résistance à l'ampicilline dans les isolements d'*Haemophilus* provenant d'enfants atteints d'infections respiratoires aiguës, dans les pays en développement sur lesquels a porté l'enquête, est faible par rapport à celle rapportée dans des enquêtes récentes menées aux Etats-Unis d'Amérique et en Europe, 2) la résistance au TMP/SMX existe dans certains pays en développement (Pakistan), et pourrait nécessiter des modifications du traitement à administrer par voie orale lors de la prise en charge des cas d'infections respiratoires aiguës; 3) compte tenu des méthodes utilisées dans cette étude, les directives relatives à l'interprétation des concentrations minimales inhibitrices observées pour l'ampicilline pourraient être directement appliquées à la pénicilline G; 4) le rôle de la pénicilline G comme médicament de première intention pour le traitement des infections respiratoires aiguës est confirmé; et 5) il faudrait poursuivre au niveau mondial la surveillance de la sensibilité des isolements d'*Haemophilus* aux antibiotiques.

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