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Antimicrobial Resistance in *Haemophilus influenzae* Isolated during Population-Based Surveillance for Meningitis in Salvador, Brazil

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Antimicrobial susceptibility was determined for 150 *Haemophilus influenzae* isolates obtained during population-based surveillance for meningitis in Salvador, Brazil. Ten (6.7%) isolates were resistant to ampicillin and chloramphenicol. Of these, two isolates, a β-lactamase and non-β-lactamase producer, were resistant to amoxacillin-clavulinic acid. These findings indicate that present antibiotic regimens in Brazil may not be appropriate for the treatment of *H. influenzae* meningitis.

Haemophilus influenzae remains a major public health problem in the post-*H. influenzae* type b (Hib) conjugate vaccine era, responsible each year for more than 3 million cases of invasive disease and 400,000 deaths worldwide (11). In developing countries, where the *H. influenzae* disease burden is greatest, treatment regimens for bacterial meningitis are based on low-cost antibiotics because of the economic constraints associated with purchasing third-generation cephalosporins; in Brazil, the combination of ampicillin and chloramphenicol is standard initial therapy for suspected bacterial meningitis in the pediatric population (9). However, antimicrobial resistance has emerged in the developing world in bacterial pathogens commonly associated with meningitis (5). In South America, more than 20% of *Streptococcus pneumoniae* clinical isolates are nonsusceptible to penicillin (4). Furthermore, findings from reference laboratory collection surveys (2) and laboratory-based surveillance (14) indicate that *H. influenzae* is resistant to commonly used antibiotics, albeit at levels lower than those observed in developed countries (6). Yet little, if any, information on antimicrobial resistance in *H. influenzae* disease is available from population-based surveillance in South America. Data from surveillance become ever more critical in guiding therapeutic decisions since few hospitals in South America have the laboratory infrastructure to monitor antimicrobial resistance in local catchment populations.

Active population-based surveillance for bacterial meningitis was established in Salvador, a city of more than 2 million inhabitants in northeastern Brazil. According to the state health secretary's regulations, suspected cases of meningitis from the metropolitan region of Salvador are referred to the state infectious disease reference hospital. More than 95% of the case notifications for meningitis from the metropolitan region are identified at this site (case notification records, Secretary of Health for the State of Bahia). Between March 1996 and October 2000, the study team prospectively identified 524 consecutives cases of meningitis with *H. influenzae* isolated from the cerebrospinal fluid. *H. influenzae* was identified based on Gram stain morphology and growth requirement for hemin and β-NAD. Patients were enrolled into the study according to protocols approved by the institutional review board committees of the New York-Presbyterian Hospital and Oswaldo Cruz Foundation/Brazilian Ministry of Health. A sample of 150 (29%) isolates was randomly selected from the 524 identified during surveillance in order to achieve 4% precision for an observed prevalence value of 10% in the sample. The slide agglutination method was performed with type-specific antisera (Difco Laboratories, Detroit, Mich.) and identified 145 (95% of 150), 2 (1.3%), 2 (1.3%), and 1 (0.6%) isolates as serotypes b, a, nonencapsulated, and f, respectively.

The broth microdilution method was used to determine MICs (10). We evaluated susceptibility to antimicrobial agents used in the treatment of bacterial meningitis and acute respiratory infections for which *H. influenzae* is an etiologic agent: ampicillin, amoxicillin-clavulanic acid, cefotaxime, cefaclor, cefuroxime, chloramphenicol, ofloxacin, rifampin, tetracycline, co-trimoxazole (Sigma Chemical Co., St. Louis, Mo.), and azithromycin (Pfizer Ltd., Sao Paulo, Brazil). Antibiotics were added to the *Haemophilus* test medium (Difco Laboratories) in doubling dilution to give final concentrations ranging from 0.008 to 64 μg/ml. Microdilution trays were inoculated with a saline (0.9%) suspension of isolate strains to produce a final inoculum density of approximately 5×10^5 CFU/ml (100-μl final volume) and were examined for growth after 20 to 24 h of incubation at 35°C in ambient air. The MIC was defined as the lowest concentration of the antibiotic that inhibited growth. *H. influenzae* ATCC 49247 and 49766 were included as quality control standards in each assay (10). MIC determination showed that 10 (6.7% of 150) of the isolates were resistant to ampicillin, 16 (10.7%) to tetracycline, 12 (8.0%) to chloramphenicol, and 8 (5.3%) to co-trimoxazole (Table 1). All of the isolates tested were susceptible to cefuroxime, cefotaxime, ofloxacin, and azithromycin.

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TABLE 1. Distribution of MICs for 150 *H. influenzae* isolates from meningitis cases in Salvador, Brazil[a]

MIC (μg/ml)	No. of strains										
	AMP	CEC	CXM	AZM[b]	CEF	CHL	TET	RIF	SXT	OFL	AMC
0.008					78		1	1		3	
0.016	4				63					10	
0.031	2				4	1			2	60	2
0.062		1		1				1	9	77	
0.125	11		1	1					30		1
0.25	71	5	1	1	2		1	13	63		4
0.5	46	16	101	50	1	10	30	131	28		103
1	5	44	42	48	2	106	91	3	6†	35	
2	1†	45	5	2		18	9		4†		3
4		34				3†	2†		4*		
8	2*	4					1*		2*		2*
16	4*	1†				9*	10*	1*	2*		
32	3*					3*	5*				
64	1*										

[a] Data are presented as the number of strains. Breakpoints are those defined by the National Committee for Clinical Laboratory Standards. †, intermediate susceptible; *, resistant. AMP, ampicillin; CEC, cefaclor; CXM, cefuroxime; AZM, azithromycin; CEF, cefotaxime, CHL, chloramphenicol; TET, tetracycline; RIF, rifampin; SXT, co-trimoxazole; OFL, ofloxacin; and AMC, amoxicillin-clavulanic acid.

[b] Only 103 isolates were tested.

Multidrug resistance, defined as being intermediate or resistant to two or more classes of antibiotics, was found in 9.3% (14 of 150) of the isolates tested. The most frequently identified pattern was resistance to ampicillin, chloramphenicol, and tetracycline (10 isolates, 6.7%) and was followed by resistance to chloramphenicol and tetracycline (3, 2.0%). All 14 multidrug-resistant isolates were nonsusceptible to chloramphenicol, and 10 (6.7% of 150) isolates were resistant to ampicillin and chloramphenicol.

Nine of 10 (90%) ampicillin-resistant isolates were identified as β-lactamase producers, while one was β-lactamase negative and ampicillin resistant (BLNAR), according to the nitrocefin disk method (Cefinase; BBL Microbiology Systems, Cockeysville, Md.). All 139 ampicillin-susceptible isolates were β-lactamase nonproducers, including the five isolates for which cefotaxime MICs were relatively elevated (0.25 to 1.0 μg/ml). Two isolates were resistant to amoxicillin-clavulanic acid; one was BLNAR while the other was β-lactamase positive and amoxicillin-clavulanic acid resistant (BLPACR). The amoxicillin-clavulanic acid MICs for the BLNAR and BLPACR isolates were 8.0/4.0 μg/ml, whereas the cefotaxime MIC for only the BLNAR isolate was relatively elevated (1.0 μg/ml).

Our study found that a significant proportion (6.7%) of patients in Salvador, Brazil, developed meningitis due to strains resistant to ampicillin and chloramphenicol, antibiotics used routinely for the empirical treatment of bacterial meningitis in Brazil. These population-based findings corroborate those obtained from reference laboratory surveys in Brazil (2, 14) and South America (3), albeit in certain countries, such as Argentina, Chile, and Mexico (7), ampicillin resistance was identified in more than 20% of the clinical isolates. Together, these findings indicate that the choice of antibiotic regimen for the empirical treatment of bacterial meningitis may need to be reevaluated in Brazil and parts of South America where ampicillin and chloramphenicol are used. During surveillance in Salvador, all isolates were susceptible in vitro to third-generation cephalosporins, indicating that they would be an appropriate alternative. However, the cost of treating a patient with these alternative regimens can be up to 10 times greater than that for ampicillin and chloramphenicol (12) and represents a significant burden in regions such as Salvador, where the per capita public sector health expenditure is less than U.S. $20 each year.

Identification of *H. influenzae* BLNAR and BLPACR strains during surveillance in Salvador appears to be the first reported from Brazil and South America. The mechanism for resistance in BLNAR strains is believed to be associated with modifications in the penicillin binding proteins (13) and/or decrease in cell wall permeability (8). BLNAR strains may be resistant to β-lactams in combination with a β-lactamase inhibitor, as observed for the amoxicillin-clavulanic acid-resistant BLNAR isolate identified in this study and to first- and second-generation cephalosporins (1). Recent large surveys have not identified BLNAR and BLPACR among *H. influenzae* clinical isolates in South America (3, 6, 7). The isolation of these resistant strains in Salvador indicates that a new mechanism for drug resistance in *H. influenzae* may have recently emerged in this region.

Immunization with Hib conjugate vaccines is cost effective and is the priority for reducing the burden of *H. influenzae* disease and addressing antimicrobial-resistant Hib in South America. All ampicillin-resistant strains identified during surveillance for meningitis in Salvador were Hib. Hib conjugate vaccine campaigns in South America, such as that which was recently initiated in Brazil, will therefore provide the benefit of reducing future costs associated with treating antimicrobial-resistant Hib disease.

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REFERENCES

1. **Barry, A. L., P. C. Fuchs, and M. A. Pfaller.** 1993. Susceptibilities of β-lactamase-producing and -nonproducing ampicillin-resistant strains of *Haemophilus influenzae* to ceftibuten, cefaclor, cefuroxime, cefixime, cefotaxime, and amoxicillin-clavulanic acid. Antimicrob. Agents Chemother. **37:**14–18.
2. **Critchley, I. A., C. Thornsberry, G. Piazza, M. Jones, M. L. Hickey, A. L. Barth, C. Mendes, F. F. Rossi, H. S. Sader, L. M. Teixeira, and D. F. Sahm.** 2000. Antimicrobial susceptibility of *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* collected from five centers in Brazil, 1997–98. Clin. Microbiol. Infect. **6:**178–184.
3. **De Andrade, A. L., M. C. Brandileone, J. L. Di Fabio, R. M. Oliveira, S. A. Silva, S. S. Baiocchi, and C. M. Martelli.** 2001. *Haemophilus influenzae* resistance in Latin America: systematic review of surveillance data. Microb. Drug Resist. **7:**403–411.
4. **Di Fabio, J. L., E. Castaneda, C. I. Agudelo, F. De La Hoz, M. Hortal, T. Camou, G. Echaniz-Aviles, M. Noemi, C. Barajas, I. Heitmann, J. C. Hormazabal, M. C. Brandileone, V. S. Dias Vieira, M. Regueira, R. Ruvinski, A. Corso, M. Lovgren, J. A. Talbot, C. De Quadros, et al.** 2001. Evolution of *Streptococcus pneumoniae* serotypes and penicillin susceptibility in Latin America, Sireva-Vigia Group, 1993 to 1999. Pediatr. Infect. Dis. J. **20:**959–967.
5. **Hart, C. A., and S. Kariuki.** 1998. Antimicrobial resistance in developing countries. BMJ **317:**647–650.

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6. **Hoban, D. J., G. V. Doern, A. C. Fluit, M. Roussel-Delvallez, and R. N. Jones.** 2001. Worldwide prevalence of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella catarrhalis* in the SENTRY Antimicrobial Surveillance Program, 1997–1999. Clin. Infect. Dis. **32** (Suppl. 2)**:**S81–S93.
7. **Lopez-Vidal, Y., E. Palavecino, and F. Rossi.** 2002. Prevalence of beta-lactamase production in *H. influenzae* isolated in Latin America in 1998–1999: results of the LASER study. Int. J. Antimicrob. Agents **19:**201–205.
8. **Mendelman, P. M., D. O. Chaffin, T. L. Stull, C. E. Rubens, K. D. Mack, and A. L. Smith.** 1984. Characterization of non-β-lactamase-mediated ampicillin resistance in *Haemophilus influenzae*. Antimicrob. Agents Chemother. **26:** 235–244.
9. **Ministério da Saúde.** 1998. Guia de Vigilância Epidemiológica—Meningites em geral. Ministério da Saúde, Fundação Nacional de Saúde, Brasilia, Brazil.
10. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 5th ed. Approved standard M7-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
11. **Peltola, H.** 2000. Worldwide *Haemophilus influenzae* type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. Clin. Microbiol. Rev. **13:**302–317.
12. **Rojo, P.** 2001. Access to essential drugs in developing countries. Gac. Sanit. **15:**540–545. (In Spanish.)
13. **Ubukata, K., N. Chiba, K. Hasegawa, Y. Shibasaki, K. Sunakawa, M. Nonoyama, S. Iwata, and M. Konno.** 2002. Differentiation of beta-lactamase-negative ampicillin-resistant *Haemophilus influenzae* from other *H. influenzae* strains by a disc method. J. Infect. Chemother. **8:**50–58.
14. **Zanella, R. C., S. T. Casagrande, S. Bokermann, S. C. Almeida, and M. C. Brandileone.** 2002. Characterization of *Haemophilus influenzae* isolated from invasive disease in Brazil from 1990 to 1999. Microb. Drug Resist. **8:**67–72.

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