Progressive Increase in Antimicrobial Resistance among Invasive Isolates of Haemophilus influenzae Obtained from Children Admitted to a Hospital in Kilifi, Kenya, from 1994 to 2002

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Etest susceptibilities to amoxicillin, chloramphenicol, and trimethoprim-sulfamethoxazole of 240 invasive isolates of Haemophilus influenzae cultured from children in rural Kenya were 66%, 66%, and 38%, respectively. Resistance increased markedly over 9 years and was concentrated among serotype b isolates. In Africa, the increasing cost of treating resistant infections supports economic arguments for prevention through conjugate H. influenzae type b immunization.

Among children in sub-Saharan Africa, Haemophilus influenzae type b (Hib) causes 20% of cases of radiologically confirmed pneumonia and 40% of cases of meningitis (10, 11). Meningitis and severe pneumonia are treated with chloramphenicol and benzylpenicillin, while mild pneumonia is treated with trimethoprim-sulfamethoxazole, ampicillin, or amoxicillin. Data on susceptibility to these antibiotics are scarce (7, 9, 13, 15), and alternative drugs are considerably more costly. The Global Alliance on Vaccines and Immunization (GAVI) has offered financial support for conjugate Hib vaccine introduction, but response has been slow, as health ministries are cautious about a long-term commitment to an expensive vaccine. Although the costs of the vaccine are easily calculated, the costs of managing H. influenzae disease in hospitals are much less apparent and, in the face of chloramphenicol resistance, may be considerable. Here we define the antimicrobial resistance patterns of H. influenzae for one district in Kenya in order to indicate effective alternative therapies that might be used in such calculations.

The study population comprised stored frozen isolates of Haemophilus influenzae cultured from blood or cerebrospinal fluid (CSF) from pediatric inpatients at the Kilifi District Hospital between January 1994 and December 2002. Before August 1998, cultures of CSF and blood were initiated on clinical suspicion of meningitis or sepsis at any point during admission. From August 1998 onwards, blood cultures were taken from all pediatric inpatients upon admission (1); lumbar puncture was undertaken systematically for patients with impaired consciousness, meningism, prostration, or seizures (other than febrile seizures) and for all children ≤60 days old with suspicion of sepsis. No case of epiglottitis was seen during the study. Hib conjugate vaccine was introduced to Kenya in November 2001, and Hib disease is now uncommon, but the vaccine has not yet been introduced into neighboring Ethiopia, Sudan, or Tanzania.

Before June 1998, blood was cultured in brain heart infusion broth (Oxoid, Basingstoke, United Kingdom) for 7 days at 37°C under 5% CO2 with obligatory subcultures at 2 and 7 days. Subsequently, BacTec Peds-Plus medium (Becton Dickinson, NJ) was incubated for 5 days and subcultured as indicated by the BacTec instrument. Broth medium was subcultured to 7% horse blood agar and chocolate agar. CSF samples were cultured on the same medium. Haemophilus species were identified by colony morphology, Gram staining, dependence on X and V factors, satellitism, and serotyping (16). Quality control was provided by the United Kingdom National External Quality Assessment Service (www.ukneqas.org.uk).

Antimicrobial susceptibilities were determined by Etest, a reliable and appropriate method for MIC determinations in developing countries (6, 14). A broth suspension with a 0.5 McFarland standard was evenly inoculated onto Haemophilus influenzae Test Medium agar (Oxoid, Basingstoke, United Kingdom). After drying, Etests for amoxicillin, cefotaxime, chloramphenicol, amoxicillin-clavulanate, and trimethoprim-sulfamethoxazole were placed on the medium, which was incubated at 35°C under 5% CO2 for 20 h. Haemophilus influenzae (ATCC 49247) was included in every batch as a control. MICs were read at the point of complete inhibition of growth. All Etests were done at the laboratories of the manufacturer, AB Biodisk, in Solna, Sweden. Although the Etest has not been approved by the NCCLS for Haemophilus influenzae testing, susceptibility breakpoints were taken from the 2003 NCCLS guidelines (12). Raw Etest readings that fell between 1-in-2 dilutions were rounded up for analysis. Beta-lactamase activity was detected by a chromogenic cephalosporin test (BR66A; Oxoid).
Isolates were serotyped by latex agglutination with poly- 
clonal rabbit antisera (Difco Laboratories, Michigan). Serotyping 
results were confirmed by PCR-based capsular genotyping 
using primers designed to amplify the capsule type-specific 
regions of the cap loci in each of the six capsular types of H. influenzae, a through f (5).

Hypotheses of association were tested by the chi-square test 
and chi-square test for trend. Logistic models were developed 
using backward stepwise regression to examine risk factors 
(age, sex, human immunodeficiency virus status, year of admis-
sion (in three strata), diagnosis of lower respiratory tract in-
fection or meningitis, source of isolate, serotype b, and resis-
tance to other antibiotics) for resistance to each antibiotic 
separately.

There were 245 inpatient episodes in which H. influenzae 
was isolated from blood or CSF. Two frozen isolates were 
irretrievable, two were not carried to Sweden, and two origi-
nated from the same patient in separate admissions 10 days 
apart. Of 240 episodes studied, 123 (51%) occurred in males 
and 124 (52%) among children aged 2 to 11 months; 116 (48%) 
strains were isolated from CSF; and 203 strains (85%) be-
tween age 2 to 11 months; 116 (48%) 
Among children aged 2 (5 of 19, 26%), or nontypeable (22). 
Human immunodeficiency virus testing among 128 patients 
presenting after July 1998 revealed a seroprevalence of 15%.

Antibiotic susceptibility patterns are shown in Table 1. Mul-
tiple resistance was common; 40% (94/236) of isolates were 
resistant or intermediate to at least two antibiotics and 28% 
(65/236) to three antibiotics. Of 79 isolates that were not sus-
cetable to chloramphenicol, 65 (82%) also were not suscepti-
able to amoxicillin. The clinical coverage of amoxicillin com-
bined with chloramphenicol was 73% (172/237), but this 
decreased to 50% (23/46) in 2001 and 32% (10/31) in 2002. All 
of the amoxicillin-resistant isolates and 19 of 20 intermediate 
isolates were beta-lactamase positive.

Susceptibility to trimethoprim-sulfamethoxazole decreased 
progressively from 53% among children aged <2 months to 
13% for children aged ≥60 months (P = 0.02). For amoxicillin 
and chloramphenicol, the prevalence of resistance peaked in 
the third year of life. There was no association between resis-
tance and sex. Resistance to amoxicillin, chloramphenicol, and 
trimethoprim-sulfamethoxazole increased markedly through-
out the 9 years of the study (Fig. 1).

The prevalence of resistance to amoxicillin was 39% (78/ 
201) for serotype b isolates and 6% (2/35) for others; for 
chloramphenicol the proportions were 38% (78/203) and 8% 
(3/36), respectively; for trimethoprim-sulfamethoxazole they 
were 66% (134/203) and 42% (15/36), respectively (P = 0.005 
for each comparison). In univariate analyses no other factor 
was significantly associated with resistance to any of the anti-
biotics. In multivariable analyses resistance was strongly asso-
ciated with time and with serotype b for each antibiotic (Table 
2). Resistance to amoxicillin was strongly associated with re-
sistance to chloramphenicol. All isolates that were resistant to 
either amoxicillin or chloramphenicol were also resistant to 
trimethoprim-sulfamethoxazole.

Comments. Chloramphenicol is the mainstay of inpatient 
therapy for H. influenzae infections in children >2 months old; 
amoxicillin is the most commonly used drug in outpatient ther-
apy of mild pneumonia. This study shows a dramatic and pro-
gressive increase in chloramphenicol and amoxicillin resistance 
over 4 years that now greatly exceeds previous estimates. In 
Nairobi in 1998 to 1999, chloramphenicol resistance was esti-
ated at 5% and amoxicillin resistance at 9% (7). In neigh-
boring Ethiopia, only 1 of 28 H. influenzae isolates from chil-
dren with meningitis in 1990 was resistant to chloramphenicol 
and 5 were resistant to ampicillin (15); in 1999, resistance to 
chloramphenicol and ampicillin was found in only 2 of 74 
isolates from children with meningitis (9).

Because of the paucity of culture facilities in the region, the 
selection of antibiotics for sick children in East Africa must be 
based on clinical rather than microbiological criteria (2). Po-
tential therapeutic alternatives must also be selected within the 
constraints of the local health care budget; for example, 
throughout the period of this study, the annual Kenya govern-
ment expenditure on health was only $9.50 per person (17).

TABLE 1. Resistance patterns of 240 invasive isolates of H. influenzae to five antibiotics determined by Etest

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Total no. of strains</th>
<th>Susceptible strains</th>
<th>Intermediate strains</th>
<th>Resistant strains</th>
<th>Minimum MIC</th>
<th>Maximum MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MIC a % of total</td>
<td>MIC b % of total</td>
<td>MIC c % of total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>237</td>
<td>≤1 66</td>
<td>2 8.4</td>
<td>8 ≥4</td>
<td>0.125</td>
<td>&gt;256</td>
</tr>
<tr>
<td>Amoxicillin-clavulanate</td>
<td>239</td>
<td>≤4/2 100</td>
<td>0 0</td>
<td>0 ≥8/4</td>
<td>0.094</td>
<td>0.75</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>240</td>
<td>≤2 100</td>
<td>0 2</td>
<td>0 ≥2</td>
<td>0.004</td>
<td>0.023</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>240</td>
<td>≤2 66</td>
<td>4 12</td>
<td>8 ≥8</td>
<td>0.19</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>240</td>
<td>0.5/9 38</td>
<td>1/19–2/38</td>
<td>5.4 ≥4/76</td>
<td>0.047</td>
<td>&gt;32 &gt;32</td>
</tr>
</tbody>
</table>

a All MIC values are in μg/ml.

b MIC50 and MIC90, MICs at which 50 and 90% of isolates, respectively, are inhibited.

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For meningitis, potential alternative parenteral drugs are ce-
foxime, ceftriaxone, or amoxicillin-clavulanate. For a 15-kg 
child, the costs of 7-day courses of these antibiotics in Kenya 
are $108, $40, and $67, respectively, compared to $5.50 for 
the combination of chloramphenicol and benzylpenicillin. The 
costs could be minimized by strengthening laboratory services 
and targeting prescriptions after CSF Gram stain or suscepti-
bility results are obtained. Chloramphenicol resistance rates of 
~20% led clinicians in two other developing countries to 
switch to first-line therapy with ceftriaxone. In Malawi, only 
patients with gram-negative bacilli on a CSF Gram stain were 
given ceftriaxone (8). In Papua New Guinea, all patients re-
cived ceftriaxone, but those with susceptible isolates were 
switched back to chloramphenicol to save costs; cure rates 
improved from 29% to 91% (3). These appealing approaches 
are clearly valid only in hospitals that routinely perform lumbar 
puctures for meningitis, which are still rare in Kenya (4).

The World Health Organization Regional Office for Africa
FIG. 1. Percentages of 240 *H. influenzae* isolates resistant to three commonly used antibiotics by year. (A) Amoxicillin; (B) chloramphenicol; (C) trimethoprim-sulfamethoxazole. Black shading, resistant isolates; grey, intermediate isolates; white, susceptible isolates. In each bar absolute numbers are shown for each category except where the frequency count was zero. Results of a χ² test for trend, merging intermediate with resistant cells, are as follows: (A) 61.7 ($P < 0.00005$); (B) 66.0 ($P < 0.00005$); (C) 31.06 ($P < 0.00005$).
TABLE 2. Logistic regression odds ratios for risk factors for resistance to amoxicillin, chloramphenicol, and trimethoprim-sulfamethoxazole

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Risk factor</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td></td>
<td>Isolated 1994–96</td>
<td>1.00</td>
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<td></td>
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<tr>
<td>Amoxicillin</td>
<td>Isolated 1997–99</td>
<td>10.2</td>
<td>1.13 91.0</td>
<td>0.0004</td>
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<td>Isolated 2000–02</td>
<td>24.8</td>
<td>2.82 219</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td></td>
<td>Serotype b</td>
<td>4.56</td>
<td>0.86 24.2</td>
<td>0.046</td>
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<tr>
<td></td>
<td>Chloramphenicol</td>
<td>24.3</td>
<td>9.51 62.2</td>
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<tr>
<td></td>
<td>resistance</td>
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<tr>
<td>Chloramphenicol</td>
<td>Isolated 1994–96</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isolated 1997–99</td>
<td>4.89</td>
<td>0.55 43.5</td>
<td>0.0005</td>
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<tr>
<td></td>
<td>Isolated 2000–02</td>
<td>17.5</td>
<td>2.09 146</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Serotype b</td>
<td>5.66</td>
<td>0.91 35.1</td>
<td>0.029</td>
</tr>
<tr>
<td></td>
<td>Amoxicillin</td>
<td>23.0</td>
<td>9.04 58.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>resistance</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isolated 1994–96</td>
<td>1.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>Isolated 1997–99</td>
<td>1.59</td>
<td>0.76 3.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Isolated 2000–02</td>
<td>5.27</td>
<td>2.53 11.0</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td></td>
<td>Serotype b</td>
<td>3.03</td>
<td>1.35 6.82</td>
<td>0.0067</td>
</tr>
</tbody>
</table>

a OR, odds ratio.
b CI, confidence interval.
c P values are derived from likelihood ratio tests comparing fitted to reduced models. Intermediate resistance is classified in this analysis as resistance. Hosmer-Lemeshow goodness-of-fit χ² tests for each model yielded P values of 0.43 for amoxicillin, 0.104 for chloramphenicol, and 0.97 for trimethoprim-sulfamethoxazole.

has initiated bacteriological surveillance of meningitis in 22 centers throughout Africa (www.afro.who.int/hib/index.html). Through its Accelerated Development and Introduction Plan for pneumococcal vaccine, GAVI has also funded sentinel site surveillance for *H. influenzae* and *Streptococcus pneumoniae* in East Africa (www.netspier.org). Knowledge of resistance to commonly used antibiotics can determine the prescribing practice of individual hospitals, but it may also, as is the case here, strengthen the case for prevention through the Hib conjugate vaccine, a standard long since achieved throughout most of the developed world.

This study is published with the permission of the director, Kenya Medical Research Institute, Nairobi.

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