Increasing Chloramphenicol Resistance in *Streptococcus pneumoniae* Isolates from Papua New Guinean Children with Acute Bacterial Meningitis[▽]

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In Papua New Guinean (PNG) children with acute bacterial meningitis (ABM), all *Haemophilus influenzae* isolates were resistant to chloramphenicol. Although *Streptococcus pneumoniae* isolates had a median chloramphenicol MIC of 3 μg/ml, it was ≥4 μg/ml in 42.8%, and the likelihood of an area under the 24-hour concentration-time curve/MIC ratio of >100 h at a MIC of ≥4 μg/ml was approximately 50%. All isolates were ceftriaxone sensitive. These data support ceftriaxone rather than conventional chloramphenicol for all PNG children with suspected ABM.

In Papua New Guinea (PNG), *Haemophilus influenzae* type b (Hib) and *Streptococcus pneumoniae* are the major causes of acute bacterial meningitis (ABM) in children (6). Chloramphenicol has been used widely in PNG since the 1980s as empirical therapy for severe bacterial infections, including ABM, but increasing resistance of Hib isolates has prompted progressive changes to national treatment guidelines. The latest version recommends ceftriaxone for all children with suspected ABM (8). Hib vaccine was implemented as part of the PNG vaccine schedule in 2008. Its increasing coverage may see *S. pneumoniae* emerge as the dominant local pathogen in pediatric ABM, especially as pneumococcal vaccination is not currently part of the schedule.

The antibiotic susceptibility of *S. pneumoniae* in PNG is unknown, but if most strains are sensitive, the expected reduction in invasive Hib disease might allow chloramphenicol to be reconsidered as an inexpensive component of the ABM treatment algorithm. To assess this, we characterized the antimicrobial susceptibility of Hib and *S. pneumoniae* isolated from children age 2 months to 10 years who were admitted to Modilon Hospital, the sole referral hospital in Madang Province, between October 2006 and December 2009 with proven ABM (≥20 white cells/mm^3 in cerebrospinal fluid [CSF] and positive culture from CSF and/or blood) (5).

Preliminary *S. pneumoniae* identification was through the presence of Gram-positive cocci forming flat, alpha-hemolytic colonies with a >14-mm inhibition zone around optochin discs. Small Gram-negative coccobacilli growing preferentially on chocolate agar were assumed to be *H. influenzae*. Invasive bacterial isolates were stored frozen in skim-milk broth until confirmatory identification, serotyping, and susceptibility testing were performed. Susceptibilities to antibiotics, including

chloramphenicol and ceftriaxone, were determined using the Kirby-Bauer disc diffusion method (1). The MICs of Hib and *S. pneumoniae* to penicillin antibiotics, chloramphenicol, and tetracycline were determined using Etest strips (AB Biodisk, Solna, Sweden).

We estimated the likelihood of attaining a target chloramphenicol pharmacokinetic/pharmacodynamic (PK/PD) ratio at each *S. pneumoniae* MIC. After an initial intramuscular or intravenous dose of 25 mg/kg of body weight in Melanesian children, the mean ± standard deviation (SD) area under the concentration-time curve from 0 to 6 h (AUC_{0-6}) was 101 ± 26 or 108 ± 51 μg · h/ml, respectively, and 134 ± 63 or 110 ± 41 μg · h/ml, respectively, after subsequent doses (11). The AUC_{0-24} was estimated assuming that children received four doses, one every six hours, of chloramphenicol. We considered an AUC_{0-24}/MIC ratio of >100 h to be the target PK/PD parameter. In the absence of definitive data for chloramphenicol from animal and human studies, this cut point was derived from published time-kill curves, a rabbit model of pneumococcal meningitis, and clinical observational data for linezolid (2, 10), an antibiotic with a structure, pharmacodynamic properties, and a toxicity similar to those of chloramphenicol.

Hib and *S. pneumoniae* were isolated from 21 and 22 children, respectively. Difficulties keeping cultures viable until processing in a reference bacteriology laboratory limited susceptibility testing to 17 *S. pneumoniae* and 15 *H. influenzae* isolates. Thirteen of 15 *H. influenzae* isolates were Hib, and the remaining two were serotype A. The antibiotic susceptibilities of Hib and *S. pneumoniae* using disc diffusion and Etest methods are summarized in Table 1. All 14 *H. influenzae* isolates with available antimicrobial susceptibility data were resistant to chloramphenicol. Of the 14 *S. pneumoniae* isolates tested, the chloramphenicol MIC ranged from 0.75 to 24 μg/ml. The median chloramphenicol MIC was 3 μg/ml, and six (42.8%) had a MIC of ≥4 μg/ml. All *S. pneumoniae* isolates were fully susceptible to ceftriaxone. The likelihood of attaining an AUC_{0-24}/MIC ratio of >100 h for *S. pneumoniae* isolates at different chloramphenicol MICs is shown in Fig. 1. This is close

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TABLE 1. Antibiotic susceptibility testing for *Haemophilus influenzae* and *Streptococcus pneumoniae* isolates[a]

Antibiotic	*Haemophilus influenzae* (n = 15 isolates)		*Streptococcus pneumoniae* (n = 17 isolates)	
	No. of isolates S/I/R by disc diffusion	Median (IQR) Etest MIC (µg/ml)	No. of isolates S/I/R by disc diffusion	Median (IQR) Etest MIC (µg/ml)
Penicillin	1/0/14	29 (12–≥256)	11/2/2	0.032 (0.025–0.166)
Chloramphenicol	0/0/14	16 (12–32)	12/2/1	3 (2–4)
Sulfamethoxazole-trimethoprim	0/0/15		6/7/3	
Tetracycline	1/0/14	>32 (>32–>32)	11/4/1	0.38 (0.20–1.00)
Ceftriaxone	10/0/0		15/0/0	

[a] Ampicillin disc diffusion and Etest were performed on *H. influenzae* isolates, while oxacillin and penicillin were used for disc diffusion and Etest, respectively, in *S. pneumoniae* isolates. S, sensitive; I, intermediate; R, resistant; IQR, interquartile range.

to 100% at low MICs (0.5, 1.0, and 2.0 µg/ml) but falls to approximately 50% and <20% at 4 µg/ml and ≥8 µg/ml, respectively.

Applying currently recommended breakpoints, *S. pneumoniae* isolates with chloramphenicol MICs of ≤4, 8, and ≥16 µg/ml are considered susceptible, intermediate, and resistant, respectively (1). There is, however, evidence that for an isolate with a MIC of 4 µg/ml there is a low likelihood of therapeutic success with standard-dose parenteral chloramphenicol. First, the present PK/PD model indicates that the chance of attaining the target AUC_{0-24}/MIC ratio at this chloramphenicol MIC is 51 to 70%, depending on route and timing of chloramphenicol dosing. As nearly half our *S. pneumoniae* isolates had a MIC of ≥4 µg/ml, a substantial proportion of children with *S. pneumoniae* ABM treated with chloramphenicol may not attain the PK/PD target. Target concentrations could be further compromised by variability in protein binding and CSF penetration (7). Second, in a South African study of *S. pneumoniae* ABM (4), there was a substantially higher mortality rate among children with penicillin-resistant *S. pneumoniae* who were treated with chloramphenicol than among those with penicillin-sensitive organisms. All isolates were chloramphenicol susceptible on initial disc diffusion testing, but subsequent analysis showed that two-thirds had minimum bactericidal concentrations of ≥4 µg/ml. The authors hypothesized that excess mortality was due to diminished chloramphenicol susceptibility of penicillin-resistant *S. pneumoniae* isolates.

The median *S. pneumoniae* MIC of 3 µg/ml is 2-fold higher than that reported in studies of invasive *S. pneumoniae* isolates conducted in the PNG highlands from 1996 to 2000 (D. Lehmann, personal communication). This rise in MICs and the complete resistance of Hib to chloramphenicol in the present study suggest that all ABM infections should be treated with an expanded-spectrum cephalosporin, consistent with PNG national recommendations (8). It is likely that other countries in Oceania and beyond where chloramphenicol has been used widely and vaccination programs do not cover *S. pneumoniae* will be in a similar epidemiologic situation. The strategy of empirical ceftriaxone therapy for children with ABM due to Hib that is resistant to chloramphenicol and reversion to chloramphenicol if the results of *in vitro* testing demonstrate susceptibility has been assessed in PNG and reduces mortality as well as costs associated with the use of expanded-spectrum cephalosporins (3). However, this policy will be applicable only to the few PNG centers with bacteriology laboratories.

In addition to cost, widespread use of expanded-spectrum cephalosporins promotes emergence of vancomycin-resistant enterococci and extended-spectrum beta-lactamase-producing organisms (9). Because of this, simple algorithms guiding antibiotic therapy could prove valuable in the absence of even basic bacteriology. These algorithms could be based partly on exclusion of ABM through initial observation following a single febrile seizure, absence of neck stiffness or a bulging fontanel in a child with normal consciousness, and/or a positive thick blood film for malaria in a child without signs of meningism (5).
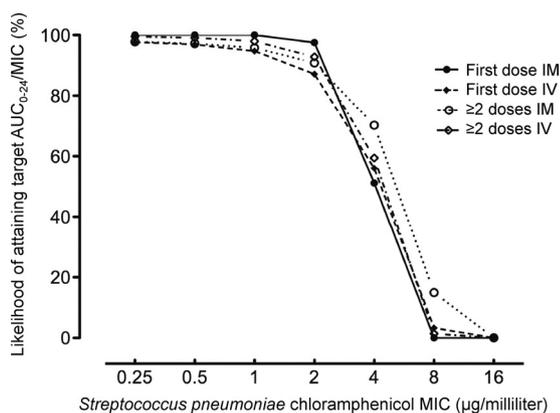
FIG. 1. The likelihood of attaining an AUC/MIC ratio of >100 h by *Streptococcus pneumoniae* MIC. Individual plots by route and timing of chloramphenicol administration are shown.

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