Antimicrobial resistance of nasopharyngeal isolates of Streptococcus pneumoniae and Haemophilus influenzae from children in the Central African Republic

[Original Studies]

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Abstract
Background. To assist the Central African Republic (CAR) develop national guidelines for treating children with pneumonia, a survey was conducted to determine antimicrobial resistance rates of nasopharyngeal isolates of Streptococcus pneumoniae (SP) and Haemophilus influenzae (HI). Secondary purposes of the survey were to identify risk factors associated with carriage of a resistant isolate and to compare the survey methods of including only children with pneumonia vs. including all ill children.

Methods. A cross-sectional survey of 371 ill children was conducted at 2 outpatient clinics in Bangui, CAR.

Results. In all 272 SP isolates and 73 HI isolates were cultured. SP resistance rates to penicillin, trimethoprim-sulfamethoxazole (TMP-SMX), tetracycline and chloramphenicol were 8.8, 6.3, 42.3 and 9.2%, respectively. All penicillin-resistant SP isolates were intermediately resistant. HI resistance rates to ampicillin, TMP-SMX and chloramphenicol were 1.4, 12.3 and 0%, respectively. The most common SP serotypes/groups were 19, 14, 6 and 1; 49% of HI isolates were type b. History of antimicrobial use in the previous 7 days was the only factor associated with carriage of a resistant isolate. Resistance rates were similar among ill children regardless of whether they had pneumonia.

Conclusions. Resistance rates were low for antimicrobials recommended by the World Health Organization for children with pneumonia. We recommended TMP-SMX as the first line treatment for pneumonia in CAR because of its low cost, ease of dosing and activity against malaria.
INTRODUCTION

To reduce childhood deaths from pneumonia in the Central African Republic (CAR), the government is implementing an acute respiratory infection (ARI) control program developed by WHO (unpublished CDC foreign trip report by Stephen Redd, May 1992). The key intervention is to treat children with pneumonia, as defined by a simple clinical algorithm, with an antimicrobial active against Streptococcus pneumoniae (SP) and Haemophilus influenzae (HI). WHO currently recommends trimethoprim-sulfamethoxazole (TMP-SMX), amoxicillin or procaine penicillin as first line agents for outpatient therapy of pneumonia. We conducted a survey of antimicrobial resistance to help develop national treatment recommendations and to understand better the epidemiology of resistant SP and HI in central Africa.

In designing the survey we reviewed a draft set of WHO guidelines for conducting SP and HI resistance surveys in developing countries. These guidelines recommended studying nasopharyngeal (NP) isolates from children with WHO-defined pneumonia. This method is supported by studies showing that resistance rates among blood and NP isolates were similar in children with pneumonia. Because studies have also shown similar resistance rates among NP isolates from children with and without pneumonia, we simplified the WHO recommendation and collected NP specimens from all ill children, many of whom did not have WHO-defined pneumonia. Our goal was to compare the survey methods of including only children with WHO-defined pneumonia vs. including all ill children.

METHODS

Study design and population. We conducted a cross-sectional survey from January to February, 1995, during the season of peak pneumonia incidence. Children 2 to 59 months of age brought to a study site for any illness were eligible for enrollment. The two study sites were high volume outpatient clinics in Bangui, CAR's largest city. To determine resistance rates in a high risk population, all inpatients 2 to 59 months of age in the Complexe Pédiatrique hospital (the inpatient facility of one study site) were evaluated and cultured on a single day. The study protocol was approved by the Ministry of Public Health and Population (Bangui) and the Centers for Disease Control and Prevention (Atlanta) Human Subjects Review Board.

Data collection procedures. Oral consent was obtained from parents of eligible children. Parents were interviewed using a standardized questionnaire concerning the child's respiratory symptoms, "danger" signs (lethargy, inability to drink and history of a seizure in the prior 3 days) and prior hospitalizations. History of previous antimicrobial use was based on responses from parents and a review of children's health booklets for prescriptions. Children were examined by a trained nurse, and an NP specimen was obtained by gently passing a sterile calcium alginate-tipped swab (Calgiswab Type 1; Spectrum Laboratories, Dallas, TX) through the nares into the nasopharynx. Two agar plates were immediately inoculated (blood agar with 5 µg/ml of gentamicin for SP and bacitracin heated blood agar for HI). At the end of each morning, all plates were taken to CAR's National Laboratory (Bangui) for processing.
Laboratory methods. All plates were incubated at 35-37°C; HI plates were kept in a candle jar. SP and HI were identified by routine methods. SP isolates were screened for antibiotic resistance by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar with 5% sheep blood. SP isolates were screened for TMP-SMX resistance on Mueller-Hinton agar with lysed horse blood. Screening of SP isolates for penicillin resistance was done with a 1-µg/ml oxacillin disk. HI isolates were screened on Haemophilus sensitivity test medium. E-tests were performed on all resistant isolates (results published elsewhere).14 Resistant isolates were confirmed at the South African Institute for Medical Research (Johannesburg) by MIC determination using the broth microdilution method. Typing was performed with antisera from Statens Serum Institut (Copenhagen, Denmark). HI isolates were typed for the identification of serotype b and tested for beta-lactamase production (using beta-lactamase agar containing 5 million units of penicillin and a 0.5% phenol red indicator).

Interpretation of inhibitory zone sizes and MICs were those recommended by the National Committee on Clinical Laboratory Standards.15 A resistance rate was defined as the proportion of isolates that were either intermediately or highly resistant.

Definitions of variables. ARIs were classified according to WHO recommendations, except that "vomiting everything," a danger sign added to WHO recommendations after this survey was completed, was not included and thus not used for ARI classification.4 ARI was defined by a history of cough or difficult breathing. Severe pneumonia was defined as an ARI with a danger sign, stridor or lower chest wall indrawing. Nonsevere pneumonia was defined as an ARI with fast breathing (>=50 breaths/min for children <12 months and >= 40 breaths/min for children 12 to 59 months) that did not meet the criteria for severe pneumonia. "No pneumonia: cough or cold" was defined as an ARI that did not meet the criteria for either pneumonia category.

Data analysis. Weight-for-age Z scores and exact binomial 95% confidence intervals (CIs) for resistance rates were calculated.16 A two-tailed Fisher's exact test was used for comparing isolation rates, resistance rates of inpatients and outpatients and resistance rates among HI serotypes.17

To examine the appropriateness of surveying all ill children rather than surveying only children with pneumonia, we compared the resistance rates, P1 and P2, of two mutually exclusive groups: (1) children with pneumonia; and (2) children without pneumonia. For each bacterial/antimicrobial combination we computed an exact 95% CI for the difference P2 - P1.18 We used this interval to test whether the resistance rates for the two groups were equivalent. Two proportions may be considered statistically equivalent if one can reject a null hypothesis that assumes that the two proportions differ by more than some threshold considered large enough to be important. We defined equivalence as the "clinically relevant" difference of 10 percentage points or less between P1 and P2, and thus the resistance rates of children with and without pneumonia were considered to be statistically equivalent when the entire 95% CI for P2 - P1 fell within the range -10% to +10%. This approach to equivalence testing allows one to control the probability of incorrectly rejecting the null hypothesis and concluding that two populations are equivalent when they are not (type 1 error) and is appropriate when an incorrect determination of equivalence has serious negative consequences.19-23 In this study such an incorrect determination might result in adoption of a survey method that provided biased estimates of
antibiotic resistance for the population of children with pneumonia. The choice of a clinically relevant difference is somewhat arbitrary; the use of a CI allows readers to evaluate the evidence for equivalence at different thresholds.

Analyses for predictors of carriage with a resistant isolate included all children enrolled from the outpatient study sites, even if their cultures were negative. Eleven factors were studied: number of "main" symptoms (cough or difficult breathing, diarrhea and fever); whether the child had been previously seen by a health care provider for the same illness; and the nine factors shown in Table 1. Odds ratios (ORs) and 95% CIs were estimated from unconditional logistic regression models. For factors found to be significant in a univariable analysis, confounding was assessed by comparing unadjusted ORs from univariable models to ORs adjusted for other factors. Because adjusted ORs were always similar to unadjusted ORs, only unadjusted ORs are presented.

TABLE 1. Clinical characteristics, ARI classifications, previous antibiotic use and previous hospitalizations of children seen as outpatients, Bangui, Central African Republic, January through February, 1995

RESULTS

Description of participants. Of 374 eligible children from the 2 outpatient study sites, 371 children were enrolled (median age, 14 months; range, 2 to 58 months). Descriptive statistics are shown in Table 1. In addition 35 hospitalized children were enrolled.

Nearly one in four children had reportedly received an antimicrobial in the previous 7 days. Among outpatients 84 (22.6%) received 1 antimicrobial, and 4 (1.1%) received 2 antimicrobials. Fifty (13.5%) children received a sulfa drug (primarily TMP-SMX), 20 (5.4%) children received a beta-lactam (primarily amoxicillin or penicillin) and 21 (5.7%) received other antimicrobials (primarily metronidazole or a macrolide).

Culture results and antimicrobial resistance testing. Culture results from the outpatients were 211 SP only, 12 HI only, 61 both organisms and 87 neither organism: thus isolation rates were 73.3% for SP and 19.7% for HI. Isolation rates were higher among children without a history of antimicrobial use in the previous 7 days compared with children with such a history: 76.7% vs. 62.5% (P = 0.013) for SP; and 21.8% vs. 13.8% (P = 0.12) for HI. Isolation rates did not vary significantly by age, sex, nutritional status, history of hospitalizations or the presence of pneumonia or a danger sign.

Among outpatients SP resistance rates were relatively low for penicillin (8.8%, all intermediately resistant) and TMP-SMX (6.3%) (Table 2); only one isolate was resistant to both agents. HI resistance rates were also relatively low for ampicillin (1.4%) and TMP-SMX (12.3%); beta-lactamase was not detected in the single ampicillin-resistant isolate. There was a high rate of SP
resistance to tetracycline (42.3%), although no recent tetracycline use was documented in our study population. SP resistance rates were significantly higher among inpatients than outpatients for penicillin and TMP-SMX.

Table 2. Antimicrobial resistance among nasopharyngeal isolates of Streptococcus pneumoniae and Haemophilus influenzae from children 2 to 59 months of age, Bangui, Central African Republic, January through February, 1995

Table 3 shows the serotype/group distribution of outpatient SP isolates found to be resistant to >=1 antimicrobial tested by the disk diffusion method. The 4 most common serotypes/groups (19, 14, 6 and 1) accounted for 83.4% of resistant isolates, all 24 of the penicillin-resistant isolates and all 42 isolates resistant to >1 antimicrobial. Overall 11.3% of resistant isolates belonged to serogroups not included in the nonavalent conjugate pneumococcal vaccine.

Table 4. Serotype/group distribution of nasopharyngeal isolates of Streptococcus pneumoniae found to be resistant by the disk diffusion method that were cultured from children seen as outpatients in Bangui, Central African Republic, January through February, 1995

Of 73 outpatient HI isolates 36 (49.3%) were serotype b. Compared with non-type b isolates resistance rates were significantly higher among serotype b isolates for TMP-SMX (22.2% vs. 2.7%, P = 0.01) but not ampicillin (2.8% vs. 0%, P = 0.49).

Comparison of resistance rates among children with and without pneumonia. Resistance rates among isolates cultured from outpatients were similar for those with and without WHO-defined pneumonia, although there was insufficient evidence to conclude the resistance rates from these two groups were equivalent, according to our definition (i.e. a difference of <=10 percentage points) (Table 4).

Table 4. Antimicrobial resistance rates of nasopharyngeal isolates of Streptococcus pneumoniae and Haemophilus influenzae cultured from ill children seen as outpatients, stratified by the presence of WHO-defined pneumonia, Bangui, Central African Republic, January through February, 1995
Because fewer than one-third of the children from the outpatient sites had pneumonia, the time required to complete this survey would have increased ~3-fold if only children with pneumonia had been included. Assuming the isolation rates and the proportion of outpatients with pneumonia from this study (which required 11 day-long clinic visits), the time required to accumulate the same number of SP and HI isolates as this study would have been 36 and 29 days, respectively, if only children with pneumonia had been included.

Risk factors for nasopharyngeal carriage of resistant bacteria. Among outpatients, including children with negative cultures, 6.5% carried penicillin-resistant SP, 4.6% carried TMP-SMX-resistant SP, 0.3% carried ampicillin-resistant HI and 2.4% carried TMP-SMX-resistant HI. Of 11 factors studied only a history of antimicrobial use in the previous 7 days was significantly associated with carriage of resistant SP. In contrast no factor was significantly related to carriage of resistant HI; however, small numbers of resistant isolates limited our ability to identify significant associations.

Four of 20 children with a history of beta-lactam use in the previous 7 days carried penicillin-resistant SP, compared with 20 (5.7%) of 351 children who lacked such a history (OR 4.1; 95% CI 1.3 to 13.5). Similarly 6 (12.0%) of 50 children with a history of sulfa use in the previous 7 days carried a TMP-SMX-resistant SP, compared with 11 (3.4%) of 321 children who lacked such a history (OR 3.8; 95% CI 1.4 to 10.9).

**DISCUSSION**

Among children seen as outpatients in Bangui, CAR, resistance rates for most antimicrobials were relatively low (6 to 12%). These results resemble patterns of SP resistance from Rwanda, Nigeria, Ethiopia and Zambia, whereas higher rates have been reported from Kenya, Ghana and Egypt.5, 11, 24-33 For HI resistance studies from Egypt, Ethiopia and Kenya reported the same relatively low rates found in CAR, whereas South Africa, Nigeria and The Gambia had higher rates.7, 8, 11, 24, 26, 34-37

Pneumococcal resistance rates among hospitalized children were quite high. This finding has been noted in other settings and may be explained by increased exposure to antimicrobials that select out resistant strains or colonization by resistant strains acquired in the hospital.38-40

The distribution of pneumococcal serotypes/groups found in CAR was similar to results from other studies of children in Africa.5, 27, 30, 39 The carriage rate of HI type b in CAR (9.7%) was similar to rates found in other studies of noninvasive isolates from Africa.8, 26, 34

This study examined a simplified method for conducting resistance surveys in which eligibility is broadened to include all ill children, with or without pneumonia. We found resistance rates were similar among ill children with and without WHO-defined pneumonia; however, the finding did not reach statistical significance. Surveying all ill children is an attractive option because, compared with surveying only children with pneumonia, including all ill children is simpler, does not require a clinician to evaluate children for pneumonia and can be completed more rapidly (approximately one-third of the time, in CAR).
An analysis of predictors for NP carriage of a resistant SP isolate revealed only a single significant factor, history of recent exposure to the antimicrobial to which the isolate was resistant. This finding, which agrees with studies of both NP carriage and invasive isolates, supports current recommendations for the judicious use of antimicrobials.41-47

There are several limitations of this study. First, participants were chosen from a nonrandom sample of clinics located in CAR's largest city. Thus our results may not be generalizable to the rest of CAR. However, because antimicrobial availability is probably greatest in urban settings, resistance rates in other parts of CAR are probably equal to or lower than those found in this survey.10, 48, 49 Second, relatively small numbers of isolates might have limited our ability to identify significant factors related to carriage of resistant HI and to demonstrate equivalence of the two survey methods examined. Finally no information was available on human immunodeficiency virus infection, a potential confounder of the relationship between antimicrobial use and carriage of resistant SP.41, 50-52

Methodologic simplifications allow most developing countries to measure antimicrobial resistance rates. However, because the impact of resistance on the treatment of pneumonia is not well-understood, it is not clear when a national ARI program should change its antibiotic recommendations as resistance increases.47, 53-56 In CAR resistance rates were generally low and similar for TMP-SMX and penicillin/amoxicillin; therefore TMP-SMX was recommended as the first line treatment for children with pneumonia because of its lower cost, twice daily dosage and antimalarial effect. In countries with severely limited budgets for health care where resistance to the recommended antimicrobial is found to be high, it is important to weigh the cost of replacing a less expensive first line antimicrobial with a more expensive agent to which respiratory pathogens have less resistance. A cost effectiveness analysis may be useful in making these decisions.

Antimicrobial resistance of respiratory pathogens in Africa is a growing problem. The global public health community should focus efforts on improving surveillance, developing guidelines for the practical application of surveillance data, advocating policies for the rational use of antimicrobials and ensuring that children in need of antimicrobial treatment for pneumonia are treated promptly and correctly.

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Key words: Streptococcus pneumoniae; Haemophilus influenzae; pneumonia; acute respiratory tract infection; antimicrobial resistance; Central African Republic; developing countries; equivalence testing; epidemiologic methods