Antibiotic resistance of nasopharyngeal isolates of *Streptococcus pneumoniae* from children in Lesotho

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Villages associated with the Lesotho Highlands Development Agency were randomized with a bias in favour of larger villages, and children <5 years of age from cluster-randomized households in these villages were chosen for the assessment of antibiotic resistance in pneumococci. Children of the same age group attending clinics in the capital, Maseru, were selected for comparison. Nasopharyngeal cultures of Streptococcus pneumoniae *from both groups of children were examined for antibiotic resistance and a questionnaire was used to assess risk factors for the acquisition of resistant strains.*

Carriage of penicillin- and tetracycline-resistant pneumococci was significantly higher among 196 Maseru children compared with 324 rural children ($P < 0.05$ and $P = 0.01$, respectively). Maseru children tended to visit clinics at an earlier age compared with their rural counterparts. The rural children were less exposed to antibiotics ($P < 0.01$), were less frequently hospitalized ($P < 0.001$), and rarely attended day-care centres ($P < 0.001$). The very low incidence of antibiotic resistance in rural Lesotho and the higher incidence in Maseru are in stark contrast with the much higher frequencies found in the Republic of South Africa, many European countries, and the USA.

Introduction

Streptococcus pneumoniae, the most important cause of pneumonia worldwide, has been implicated in 70–80% of severe pneumonia episodes in Africa (*1*). Although reliable statistics on the etiology of pneumonia in African children are lacking, one recent Gambian study reported pneumococcus as the causative organism in 69% of childhood cases of pneumonia (*2*). Respiratory infections and diarrhoeal diseases are also the most common causes of childhood mortality, especially in developing countries (*3*). As the problem of antibiotic resistance in pneumococci is increasing in both developed and developing countries (*4*), the periodic monitoring of resistance to recommended antibiotics is important.

Penicillin resistance is not usually a problem in the treatment of pneumococcal pneumonia, because the antimicrobial levels obtained in blood and lung tissues exceed the minimal inhibitory concentrations (MICs) of penicillin and most other β-lactam agents (*4*, *5*). However, β-lactam resistance is extremely important in the treatment of pneumococcal meningitis, where both intermediate resistance to penicillin (with MICs of 0.1–1.0 μg/ml) and full resistance (with MICs of >1.0 μg/ml) have a profound effect on the outcome of meningitis (*5*). Furthermore, southern African isolates with MICs >1 μg/ml are very likely to exhibit intermediate or high-level resistance to the third-generation cephalosporins, ceftriaxone and cefotaxime (*6*, *7*). As these cephalosporins are commonly used for the initial (empiric) treatment of meningitis, a high prevalence of penicillin resistance may compromise their usefulness, making it necessary to have treatment with vancomycin, ideally in synergic combination with ceftriaxone or cefotaxime (*8*).

Pneumococci, together with *Haemophilus influenzae*, are frequent causes of otitis media and sinusitis. Treatment of acute otitis media may also be compromised by resistance to β-lactam antibiotics (*5*, *9*), while treatment with co-trimoxazole in combination with trimethoprim/sulfamethoxazole (TMP/SMX) against *in vitro*-resistant strains has not yet been adequately evaluated. Several surveys in South Africa, Australia, Pakistan, Spain and other European countries have shown that the incidence of antimicrobial resistance in pneumococci carried asymptomatically in the nasopharynx of children, although not correlating exactly with pneumococcal

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strains that cause disease, is a good indication of the prevalence of antimicrobial-resistant strains in these communities (*4*, *10–12*). We therefore studied the resistance of nasopharyngeal pneumococci to commonly used antimicrobial agents in <5-year-olds in Lesotho. The children investigated lived in rural households and villages associated with the Lesotho Highlands Development Agency (LHDA) project, as well as in the capital city of Maseru and surrounding villages. The latter group was studied separately and the findings contrasted with those from the rural areas. The studies were approved by the Committee on Research and Human Subjects of the University of the Witwatersrand and comply with the Ethical and Biosafety Guidelines of the South African Medical Research Council. Free and informed consent was obtained from the parents or guardians of the children in the studies.

Materials and methods

Children. Both rural children from villages associated with the LHDA and children from Maseru were selected for the study. The rural (LHDA) study was conducted during the summer months from late August 1995 through February 1996, while the Maseru study was performed in November and December 1995.

Rural children <5 years of age were selected from cluster randomized households recruited for the LHDA project. Eight villages in remote mountainous areas at different tiers above the actual water levels of dams, which formed part of a multiphase construction of dams and tunnels in the Lesotho Highlands, were randomly chosen for the present study. Randomization of the villages included a built-in bias in favour of larger villages. This study was one of several relating to the possible impact of the LHDA project on health in the region. A specially trained field worker and one of us (*M.M.*) took nasopharyngeal swabs from children <5 years of age from households in the chosen villages. A questionnaire was also used to obtain answers relating to possible risk factors for the selection and acquisition of antibiotic-resistant pneumococci. Children <5 years of age attending clinics in Maseru during the study period were investigated in a similar way by collecting nasopharyngeal swabs and using a slightly modified questionnaire.

A total of 324 rural children and 196 children from Maseru were investigated for nasopharyngeal carriage of *S. pneumoniae*, the isolates being tested for antimicrobial susceptibility, with antimicrobial-resistant strains being serotyped.

Questionnaire. A simple questionnaire to elicit information on possible risk factors relating to antibiotic resistance in nasopharyngeal pneumococci included questions on past hospitalization of the child and the reason for this, antibiotics received during the past month, attendance at a day-care centre, any other <5-year-olds in the families and, in the case of the Maseru children, the reason for the current visit to the clinic.

Microbiological methods. Nasopharyngeal swabs using calcium alginate swabs (Calgiswab, Puritan, Hardwood Products Co., Guilford, ME, USA) were gently introduced nasally into the nasopharynx of children, withdrawn and then inoculated directly onto blood agar plates (Oxoid Columbia base with 5% horse blood (Oxoid, Basingstoke, Hampshire, England)) containing 5 μg/ml of gentamicin as a selective agent for isolation of pneumococci (*13*). Nasopharyngeal swabs from children in rural villages were taken in the morning to ensure that these plates, as well as those collected at the Maseru clinics, were delivered within 3 hours to the Biology Department of the National University of Lesotho (NUL) in Maseru where they were incubated at 37 °C overnight. Identification of pneumococcal isolates was based on typical colonial morphology, α-haemolysis on blood agar plates, and susceptibility to optochin.

Procedures for drug susceptibility testing in Maseru. Initial isolation was performed in Maseru as described above. Screening for resistance was carried out by disc diffusion testing according to National Committee for Clinical Laboratory Standards (NCCLS) guidelines (*14*). In order to assess susceptibility to benzyl penicillin, 1-g oxacillin discs were used and zones of inhibition of 19 mm were considered to indicate resistance. Inocula were prepared by making suspensions of colonies from overnight cultures on blood agar to the required turbidity standard. Disc-diffusion tests were also performed on the following antibiotics (disc concentrations are given in parentheses): chloramphenicol (30 g), erythromycin (15 g), clindamycin (2 g), tetracycline (30 g), TMP/SMX (1.25/23.75 g) and rifampicin (5 g) according to NCCLS guidelines (*14*). Mast-rings (Mast Diagnostics, Merseyside, England) with discs containing the specified antibiotic concentrations were used.

Transportation. Isolates were sent to the South African Institute for Medical Research (SAIMR), Johannesburg, initially in cooked meat medium. When it became clear that an unacceptable number of cultures failed to survive transportation, Dorset egg medium slopes in Bijou bottles were used in addition. Both media were prepared by the SAIMR media preparation facility. A sweep of characteristic colo-

nies from primary isolation plates was transferred to the transport media, which were incubated overnight and then sent to Johannesburg in batches by courier service.

Procedures performed at SAIMR, Johannesburg. These are described below.

Antibiotic susceptibility testing
All pneumococcal cultures received from Maseru were subcultured and, after confirming their identity, were subjected to disc-diffusion testing according to NCCLS guidelines (*14*). The same procedures employed in Maseru, including the use of Mast-rings, were followed in the Johannesburg laboratory.

The Etest procedure
The Etest procedure was performed on selected isolates, including all those that showed antibiotic resistance on disc-diffusion testing. Isolates from Maseru and rural children were tested against benzyl penicillin and TMP/SMX in Maseru according to the manufacturer's instructions (AB Biodisc, Solna, Sweden) by one of the authors (*M.M.*). An excellent correlation exists between the size of growth inhibition around an antibiotic-impregnated strip forming an exponential antibiotic gradient in an agar-based medium and the MIC of the antibiotic against which a specific bacterial isolate is tested (*15*). This technique has also been shown to give good results with pneumococcal isolates (*16*).

Confirmation of antimicrobial resistance
All isolates that showed resistance or "borderline resistance", i.e. zones up to 2 mm greater than critical concentrations denoting resistance (break-point diameters) as well as all isolates which, on Etest evaluation, showed discrepancies with the SAIMR disc-diffusion results were tested by the NCCLS dilution method in microdilution trays for MIC determination (*17*). Critical MIC concentrations denoting resistance (breakpoints) for benzyl penicillin were 0.1–1 μg/ml for intermediate resistance and ≥2 μg/ml for full resistance, while the TMP/SMX breakpoints were 1/19–2/38 μg/ml for intermediate resistance and ≥4/76 μg/ml for full resistance. The breakpoints for the other antibiotics are listed in the NCCLS guidelines (*16*).

Antigenic typing of isolates
Capsular typing of all antibiotic-resistant isolates was performed using the "capsular swelling" technique. The Statens Seruminstitut, Copenhagen, supplied the group- and type-specific antisera and the Danish nomenclature was used for the designation of antigenic types.

Results

Prevalence of pneumococci in the nasopharynx. Nasopharyngeal carriage of *S. pneumoniae* was found in 60% (310 out of 520) of the children (Table 1). Carriage among the rural children aged ≤36 months (131 out of 216; 61%) was significantly higher ($P = 0.02$) than in those aged >36 months (51 out of 97; 53%). This was not the case for the Maseru children among whom the carrier rate in the <18 months age group was lower than for the same age group of rural children or in the 19–36-months age group from Maseru (Table 1). These latter differences were, however, not statistically significant.

Transportation of cultures. Of the 201 and 109 cultures from rural and Maseru children, respectively, 39 and 4 were lost for further testing in Johannesburg, some having been overgrown by contaminants and the majority having failed to grow on subculture. As the Maseru study started approximately 2 months after that of the rural study, the Dorset egg medium was already in use, resulting in few cultures being lost. Those isolates which showed typical *S. pneumoniae* features and were susceptible to optochin in the NUL laboratory in Maseru were included in the carriage rates shown in Table 1.

Antibiotic susceptibility findings. The antimicrobial susceptibility results obtained in the Maseru and Johannesburg laboratories are summarized in Table 2.

Table 1: **Nasopharyngeal carriage of *S. pneumoniae* in rural and urban (Maseru) children in Lesotho, by age**

Age group (months)	Rural group		Maseru group		Total	
	No. of children	No. of carriers	No. of children	No. of carriers	No. of children	No. of carriers
<18	104	68 (65)[a]	109	57 (52)[a]	213	125 (59)[a]
19–36	119	80 (67)	50	32 (64)	169	112 (66)
≥37	97	51 (53)	37	20 (54)	134	71 (53)
All ages	324[b]	201[b] (62)	196	109 (56)	520[b]	310[b] (60)

[a] Figures in parentheses are percentages.
[b] There were two carriers and another two children who were not carriers whose ages were not recorded.

Table 2: **Antibiotic resistance patterns of nasopharyngeal pneumococci in Lesotho children**

	No. of pneumococcal isolates susceptible/resistant to:															
	Penicillin			Chloramphenicol		Tetracycline			Erythromycin/ clindamycin[a]			Rifampicin			TMP/SMX[a]		
	S[b] (0.06)[c]	I[b] (0.101)	R[b] (>1)	S (≤4)	R (≥8)	S (≤2)	I (4)	R (28)	S (<0.25)	I (0.5)	R (≥1)	S (≤1)	I (2)	R (≥24)	S (0.5)[d]	I (27)	R (≥4)
Rural group:																	
Total[e]	195	4	0	200	0	199	0	1	201	0	0	197	0	0	190	5	4
JHB subgroup[f]	158	4	0	162	0	161	0	1	162	0	0	162	0	0	156	4	2
Maseru group:																	
Total[e]	102	5	2	107	2	104	0	5	109	0	0	109	0	0	101	3	5
JHB subgroup[f]	98	5	2	103	2	100	0	5	105	0	0	105	0	0	97	3	5

[a] Erythromycin and clindamycin showed similar susceptibility patterns and the breakpoint concentrations for S, I and R were the same. TMP = trimethoprim; SMX = sulfamethoxazole.
[b] S = susceptible; I = intermediate resistance; R = full resistance.
[c] Breakpoint concentrations in μg/ml denoting the three susceptibility/resistance patterns.
[d] Expressed in terms of trimethoprim (TMP) concentrations. Ratios of TMP to sulfamethoxazole (SMX) are 1 to 19 for all breakpoint concentrations.
[e] All evaluable cultures, including susceptible strains lost during transport to Johannesburg (see text for details).
[f] All isolates available for retesting in Johannesburg (JHB).

Disc-diffusion screening

The initial disc-diffusion screening of the isolates performed in Maseru considerably overestimated the antibiotic resistance rates compared with the MIC findings. Invariably discrepancies were due to resistance that could not be confirmed by MIC testing in Johannesburg. Since none of the 267 cultures that were evaluated in Johannesburg gave false-susceptible results on disc-diffusion testing in Maseru, the finding on susceptibility in the 43 cultures that were tested only in Maseru were accepted as correct. Resistance in this group of isolates was only accepted when, in the case of penicillin and TMP/SMX, they were confirmed by Etest. In a few instances, susceptibility findings from the rural children were, as a result of recording anomalies, regarded as not evaluable (total isolates evaluated varied from 197 to 201; see Table 2). The antibiotic susceptibility results are given in Table 2, where the combined findings of the two laboratories, after correction of the resistance findings which could not be confirmed by either MIC testing in Johannesburg or the Etest in Maseru, are recorded separately as the "Total" group. Those results which were available for confirmation testing by MIC determination in Johannesburg only are labelled the "JHB subgroup" in the Table. The MIC findings following disc-diffusion screening were regarded as final proof of the resistance status of the isolates, except in the case of the few Etest-confirmed resistant isolates among the 39 rural and 4 Maseru isolates which were lost during transportation to Johannesburg.

Comparison of antibiotic resistance in pneumococci between the two groups

Resistance patterns of pneumococcal isolates in the Maseru and rural Lesotho groups of children are given in Table 3. Penicillin and tetracycline resistance among pneumococcal isolates from Maseru children (6.4% and 4.6% respectively) was significantly commoner than those from rural children (2.0% and 0.5% respectively). The *P*-value for the penicillin comparison suggests marginal significance at 0.046, while tetracycline resistance was much commoner among Maseru children (odds ratio (OR) = 9.87 (95% confidence intervals (CIs) = 1.04–454.7); *P*-value = 0.01) (Table 3).

Resistance to TMP/SMX was somewhat commoner than to penicillin or tetracycline in both groups but did not differ significantly between the Maseru and rural groups (7.3% and 4.5% respectively). Overall resistance to any of the antibiotics tested, although higher in the Maseru group (11.9% vs. 6.2% in rural children) did not reach statistical significance ($P = 0.08$; Table 3).

When antibiotic resistance among the smaller subgroups of isolates, which could be retested in Johannesburg, was analysed (see Table 2), similar trends were evident but only tetracycline resistance proved to be significantly commoner in the Maseru children (OR = 8.05 (95% CI = 0.90–184.77); *P*-value = 0.03. For penicillin resistance between the two groups of Lesotho children the corresponding values were OR = 2.82 (95% CI = 0.69–13.44); *P*-value = 0.09). The overall resistance to any antibiotic was 13 out of 105 (12.4%) in Maseru children

Table 3: **Comparison of antibiotic resistance among pneumococci in the nasopharynx of children in Maseru vs. rural Lesotho children**

	Proportions of resistant isolates[a]		
Antibiotic	Maseru	Rural	Significance
Penicillin	7/109 (6.4)[b]	4/199 (2.0)[b]	OR = 3.35 (95% CI 0.82–15.89)[c] $P = 0.046$
Chloramphenicol	2/109 (1.8)	0/200 (0)	NS[d]
Tetracycline	5/109 (4.6)	1/200 (0.5)	OR = 9.57 (95% CI 1.04–454.71) $P = 0.01$
Erythromycin/ clindamycin	0/109 (0)	0/201 (0)	NS
Rifampicin	0/109 (0)	0/197 (0)	NS
TMP/SEX[e]	8/109 (7.3)	9/199 (4.5)	OR = 1.67 (95% CI 0.57–4.90) $P > 0.05$
Any antibiotic tested	13/109 (11.9)	12/194 (6.2)	OR = 2.05 (95% CI 0.84–5.03) $P = 0.08$

[a] Based on critically evaluated findings from both Lesotho and SAIMR (Johannesburg) laboratories.
[b] Figures in parentheses are percentages.
[c] Odds ratio (OR) with 95% confidence intervals.
[d] NS = not significant.
[e] TMP/SMX = trimethoprim/sulfamethoxazole.

and 9 out of 162 (5.5%) in the rural setting. The difference between the two Lesotho studies appears to be marginally significant (OR = 2.40 (95% CI = 0.92–6.38); *P*-value = 0.047).

Multiple drug resistance among pneumococcal carriers. There were three isolates in the Maseru children that were resistant to three or more antimicrobial agents, compared with none in the rural study.

The respective patterns were resistant to penicillin-tetracycline-TMP/SMX, and penicillin-tetracycline-chloramphenicol while the third strain was resistant to the latter three antibiotics as well as TMP/SMX.

Levels of antibiotic resistance. High levels of resistance were more frequently encountered in Maseru children compared with those in the rural study, while high-level resistance to penicillin occurred only in the Maseru children (Table 4).

Age-related carriage of resistant pneumococci. The carriage of pneumococcal isolates showing resistance to any of the antibiotics tested was significantly more common in the >18 months age group (10 out of 52, 19.2%), compared with younger children (3 out of 57, 53%) in the Maseru study (OR = 4.29 (95% CI = 1.01–25.42); *P* = 0.03). An opposite (5 out of 131 (3.8%) vs. 7 out of 68 (10.3%)) but not statistically significant trend was seen in the Lesotho study (OR = 2.87 (95% CI = 0.74–11.91); *P* = 0.07).

Serotypes associated with antibiotic resistance. A total of 14 antibiotic-resistant isolates from Maseru children and 9 from rural Lesotho children were available for serotyping. Twenty isolates (14 from Maseru and 6 from rural children) belonged to serotype 19F, 7 to serotype 6A (4 from Maseru and 3 from rural children), and there was one isolate each of serotype 1 and serotype 14 (both from Maseru).

Potential risk factors associated with antibiotic resistance. The frequency of risk factors that may have played a role in the emergence of antibiotic resistance in pneumococci affecting children in the two study populations is presented in Table 5. The frequency of exposure to antibiotic treatment, hospitalization, and attendance of day-care centres was significantly more common in the Maseru children. In contrast, there were more <5-year-olds sharing the same household in the rural area, compared with the Maseru children. However, when the same risk factors were evaluated directly in association with carriage of antibiotic-resistant pneumococci, only one was found to be statistically significant. Among the Maseru families, the odds of finding an antibiotic-resistant carrier with siblings <5 years of age in a household were 0.45 times that of finding carriers with no siblings in this age group (odds ratio = 0.00–1.12; *P* = 0.045), i.e., children with siblings in a family were less likely to be a carrier of resistant pneumococci.

Discussion

The object of the present study was to determine the prevalence of antibiotic resistance in nasopharyngeal isolates of *S. pneumoniae* among two groups of children in Lesotho based on the premise that the frequency of such resistance reflects the prevalence of resistance in pneumococci causing invasive disease (*7, 10–12, 18*). The findings of this study could, therefore, form a rational basis for future treatment of community-acquired pneumonia, otitis media and acute bacterial meningitis (*5, 18*). Children <5 years

Table 4: **Levels of antibiotic resistance in pneumococcal isolates in children in Maseru and rural Lesotho**

	No. of isolates with			
	Intermediate resistance[a]		High resistance[a]	
Antibiotic	Maseru (*n* = 109)	Rural (*n* = 199)[b]	Maseru (*n* = 109)	Rural (*n* = 199)[b]
Penicillin	5 (4.6)[c]	4 (2.0)[c]	2 (1.8)	0 (0)
TMP/SMX[d]	3 (2.7)	5 (2.5)	5 (4.6)	4 (2.0)
Chloramphenicol	—[e]	—[e]	2 (1.8)	0 (0)
Tetracycline	0 (0)	0 (0)	5 (4.6)	1 (0.5)

[a] Based on MIC breakpoints denoting full (high-level) resistance (see text).
[b] The number tested for chloramphenicol and tetracycline was 200.
[c] Figures in parentheses are percentages.
[d] TMP/SMX = trimethoprim/sulfamethoxazole.
[e] Intermediate resistance to chloramphenicol is not recognized as a separate category.

Table 5: **Frequency of risk factors in Maseru and rural Lesotho children, which may relate to selection of antibiotic resistance in pneumococci**

Risk factor	Maseru (n = 196)[a]	Rural (n = 323)[a]	Significance
Antibiotic use	43 (21.9)[b]	10 (3.1)[b]	OR = 8.80 (95% CI 4.13–1.22)[c] $P < 0.001$[d]
Hospitalization	14 (7.14)	1 (0.31)	OR = 24.77 (95% CI 3.69–1050.48) $P < 0.001$[e]
Attendance in day-care centre	39 (19.9)	6 (1.8)	OR = 13.12 (95% CI 5.34–38.56) $P < 0.001$[e]
Presence of <5-year-olds in same household[f]	65 (33.2)	178 (55.1)	OR = 0.40 (95% CI 0.27–0.59) $P < 0.001$[d]

[a] Number of children evaluated for each of the risk factors.
[b] Figures in parentheses are percentages.
[c] Odds ratio (OR) with 95% confidence intervals.
[d] P-value based on Yates' corrected χ^2 analysis.
[e] P-value based on Fisher's exact test.
[f] Based on the assumption that overall carriage and carriage of resistant strains may be more common in young children and the potential for spread may be high in households with young children (*4, 11, 20, 22*).

of age were chosen because the nasopharyngeal carrier rate of pneumococci is highest in this age group (*4, 11, 18*). The administration of antibiotics to children is likely to facilitate the development of resistance in pneumococcal strains to the antimicrobial agent used, especially in the hospital setting (*11*).

The finding of relatively low frequencies of penicillin resistance among children in rural Lesotho (2.5%) and Maseru (6.4%), even lower than most recent reports in the literature (*4, 11, 12, 19, 20*), is reassuring. The reason for this must include the remoteness of most households from hospitals, clinics and other medical facilities resulting in a low antibiotic selection pressure, as well as limited exposure to overcrowded conditions outside households, thus reducing the opportunity for transmission of novel strains. The high incidence of penicillin resistance in neighbouring South Africa (*4, 7, 11, 21*), however, poses a real threat to rural Lesotho children, especially as the major Lesotho Highlands Water Scheme is likely to promote increased population movement between the two countries and also provide additional risks favouring the selection of resistant pneumococci and their acquisition by rural children.

As penicillin resistance could jeopardize the treatment of acute bacterial meningitis, penicillin or amoxycillin should be reserved for the treatment of pneumococcal infections other than pneumonia and otitis media in Lesotho. However, for the initial (empiric) treatment of meningitis, amoxycillin would be more appropriate in order to cover *H. influenzae* type b as well, assuming that the incidence of β-lactamase-producing strains remains low in Lesotho and that immunization against the latter pathogen is not yet widespread in Lesotho.

As the penicillin-resistant isolates in rural Lesotho are still in the intermediate category of resistance, the third-generation cephalosporins (ceftriaxone and cefotaxime) and possibly high doses of intravenous amoxycillin could be appropriate for the empiric treatment of acute meningitis in rural Lesotho. However, ceftriaxone or cefotaxime should already at this time be considered for patients from Maseru with pneumococcal meningitis. The prevalence of high-level penicillin resistance in this area is still low (2%) and only these latter strains are likely to exhibit sufficient cross-resistance with ceftriaxone or cefotaxime to warrant the addition of vancomycin (*5, 8, 18*). The use of ceftriaxone, because of its long half-life, necessitating ideally only two daily intravenous or intramuscular doses for the treatment of meningitis, is particularly attractive for developing countries, provided its use is reserved for only well-defined serious infections including meningitis, gonorrhoea, chancroid and typhoid fever. It should be noted that the use of penicillin plus chloramphenicol for the empiric treatment of acute meningitis may not be effective if caused by intermediately penicillin-resistant pneumococci (*21*).

The relatively low incidence of penicillin resistance in pneumococci in Lesotho should not lead to complacency. The rapid increase in penicillin resistance in recent years in the USA (*22*) and elsewhere (*20*) may also occur in Lesotho as a result of rapid socioeconomic changes relating to the Lesotho Highland Water Scheme.

It is reassuring that antibiotic-resistant serotype 23F strains were not encountered in the present study because this serotype and serotypes 19A, 6A and 14 are particularly likely to be associated with antibiotic resistance (*4, 20*). Serotypes 6A and 14 were found among resistant isolates in Lesotho chil-

dren, but serotype 19F was by far the commonest type in the present study. However, without more details about the invasive types in the population it is difficult to draw any useful conclusions from our limited data.

The relatively high prevalence of resistance to TMP/SMX (7.3% and 4.5% in Maseru and rural Lesotho, respectively), although still low compared with some other countries (*4*, *12*, *18*, *20*), is a cause for concern because the situation may change as the incidence of resistance to this combination is already appreciably higher in neighbouring South Africa (*11*). However, at present the use of TMP/SMX in Lesotho is still indicated as first-line candidate for the treatment of pneumonia but continued surveillance, possibly in the form of periodic surveys, is highly recommended. The need for surveillance also applies to penicillin resistance, since early detection of escalation of resistance in Lesotho could lead to an expeditious review of treatment options for meningitis.

Differences between resistance findings in Maseru and rural Lesotho. The prevalence of penicillin and tetracycline resistance was significantly more common in urban Maseru children than in rural Lesotho children. Although, probably because of small numbers, statistically significant differences could not be established, there was a consistent trend of more multiple-resistant isolates, as well as high levels of resistance based on MIC determinations, to penicillin, TMP/SMX, chloramphenicol and tetracycline in the children in Maseru (Table 4). These findings may well relate to the greater accessibility of clinic and hospital facilities and availability of antibiotics to the urban children.

Risk factors associated with antibiotic resistance. Although no direct association could be established between the carriage of resistant pneumococci and prior exposure to antibiotic treatment, hospitalization and clinic attendance, all three of these risk factors were significantly more common in the Maseru children as a group, compared to the rural children (Table 5). These findings strongly suggest that antibiotic use, hospitalization and clinic attendance may well predispose to acquisition of resistant pneumococci, which accord with findings reported in other studies (*4*, *7*, *11*, *18–20*). The higher prevalence of tetracycline resistance in the Maseru children suggests that such strains may initially have arisen in adults exposed to tetracyclines and were subsequently transmitted to children. Tetracycline treatment is contraindicated in children and it is unlikely that this group of antibiotics was widely used in children in Lesotho.

More children in the <5-years age group living in the same dwelling as a pneumococcal carrier were encountered in the rural setting compared with Maseru ($P = 0.001$) (Table 5). There was also a higher pneumococcal carriage rate in rural children <36 months of age than in older children ($P = 0.02$). Despite these findings which theoretically should promote the development of antibiotic resistance, this did not occur because the selection pressure of antibiotic usage that favours the emergence of resistant mutants was lacking. It can, however, be expected that as access to antibiotics increases in rural communities in developing countries, the incidence of antibiotic resistance would rise. Interestingly, there was a trend for younger children in Maseru (mean age, 21.2 ± 17 months) compared with rural children (mean age, 29.9 ± 18 months) to attend clinics in their respective regions ($P < 0.01$). The earlier exposure of Maseru children to clinics and access to antibiotics, with the likelihood of the same children attending clinics at a later stage when they are ill, are in keeping with greater antibiotic pressure selecting for antibiotic resistance in the Maseru children. The fact that the homes of antibiotic-resistant carriers in Maseru were less likely to house several other young children than homes with carriers of susceptible strains suggests that the smaller families in Maseru are more likely to be exposed to antibiotics. This finding may be related to the likelihood that parents with smaller families may enjoy a higher educational and/or economic status and as a result have greater access to clinics and antibiotics.

Performance of the microbiology laboratory in Maseru. This laboratory failed to screen antibiotic resistance by the relatively simple disc-diffusion test. However, the performance in the same laboratory of the technically similar, but more exacting, Etest was excellent for the prediction of penicillin resistance. The poor performance with the disc-diffusion testing procedure may have been due to poor storage or a defect in the batch of discs, or failure to press the discs down firmly onto the agar surface before incubation. Also, inexperience in determining accurately the size of zones of inhibition and failure to detect contaminants may have contributed to the discrepant findings. Discrepancies between the routine disc-diffusion and Etest findings were not recognized early. Regular and correct use of both susceptible and resistant, control strains, could have resulted in the early detection and correction of errors.

The reasons for the failure of the Etest in Lesotho to accurately predict TMP/SMX resistance are not clear but should also be addressed. Although the use of the Etest for TMP/SMX susceptibility testing is recommended by the manufacturing company,

specific scientific evaluation of this drug combination is not listed in their comprehensive bibliography list. The excellent performance of the Etest in detecting penicillin resistance in pneumococci accords with experience elsewhere (*16*).

Problems with the transportation of cultures from Lesotho to Johannesburg also require elucidation. It is possible that cultures were incubated for longer than the prescribed 18 hours in cooked meat medium, leading to autolysis of the pneumococci. Dorset egg medium appears to be superior for transportation of pneumococcal cultures, and its suitability for transport purposes has been confirmed in a separate study conducted by us (*23*). Because of the need for a laboratory or laboratories in Lesotho with the capability of monitoring antibiotic resistance on an ongoing basis, the problems identified in this study ought to be corrected.

The incidence of antibiotic resistance in the pneumococcus is still low in Lesotho, one of the poorest developing countries, and the use of antimicrobial agents based on the WHO Essential Drugs List, with the possible exception of the requirement of ceftriaxone for the treatment of meningitis, is still appropriate. Carefully planned surveillance of antibiotic resistance in Lesotho and other developing countries will assist in formulating relevant policies for the use and control of antimicrobial drugs in countries burdened with high morbidity and mortality rates due to respiratory infections, an important cause of which is the pneumococcus.

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Résumé

Résistance aux antibiotiques d'isolements rhinopharyngés de *Streptococcus pneumoniae* provenant d'enfants du Lésotho

Dans nombre de pays en développement, et notamment au Lésotho, on connaît mal la fréquence de la résistance à la pénicilline et aux autres antibiotiques chez le pneumocoque, cause la plus importante de pneumonie dans l'ensemble du monde. Comme le taux de portage rhinopharyngé de *Streptococcus pneumoniae* antibiorésistants par les enfants en bas âge permet de se faire une idée de la prévalence des pneumocoques résistants dans une communauté, nous avons étudié les taux d'antibiorésistance des pneumocoques nasopharyngés chez des enfants du Lésotho.

Au cours de l'été (fin août 1995 à février 1996) nous avons étudié les taux de portage nasopharyngé de *S. pneumoniae*, de même que leur résistance à la pénicilline et autres antibiotiques, chez 324 enfants de moins de 5 ans vivant dans des familles rurales dont s'occupait la Lesotho Highlands Development Agency ainsi que chez un groupe analogue de 196 autres enfants fréquentant des dispensaires de la capitale, Maseru. Pour l'étude en milieu rural, on a tiré au hasard des villages situés à différents niveaux au-dessus du plan d'eau prévu de barrages en construction, cette randomisation comportant un biais en faveur des villages les plus importants. Tous les enfants de moins de 5 ans qui résidaient dans les ménages constituant les grappes randomisées ont été examinés. On a ensuite inclus dans l'étude les enfants de moins de 5 ans qui fréquentaient les dispensaires de Maseru. Un questionnaire a été utilisé pour déterminer les facteurs de risque éventuels pouvant être associés à l'acquisition de souches résistantes.

On a constaté qu'en milieu rural, le taux de portage rhinopharyngé de *S. pneumoniae* était sensiblement plus élevé chez les enfants d'âge inférieur ou égal à 36 mois que chez les enfants plus âgés (148/223, soit 66,4% contre 51/97, soit 52,6%; $P = 0,02$). Chez les enfants de Maseru, le taux de portage de pneumocoques résistants aux antibiotiques était plus élevé que chez les enfants de milieu rural (pénicilline, 6,4% contre 2,0%, $P = 0,046$; tétracycline, 4,6% contre 0,5%; $P = 0,01$; cotrimoxazole, 7,3 5 contre 4,5%; pas de signification statistique). A Maseru, la résistance aux antibiotiques était plus répandue chez les enfants âgés de plus de 18 mois que chez les enfants plus jeunes ($P = 0,03$). Cette observation a été mise en rapport avec le fait que les enfants de la capitale sont amenés plus tôt au dispensaire que les enfants de milieu rural. On a également constaté que les enfants de Maseru recevaient sensiblement plus souvent des antibiotiques ($P < 0,01$), étaient plus fréquemment hospitalisés ($P < 0,001$) et étaient plus souvent amenés dans des centres de soins ambulatoires ($P < 0,001$). En ce qui

concerne la détermination de la résistance aux antibiotiques, on a eu des problèmes avec la technique de diffusion sur disque, mais l'épreuve Etest a donné de bons résultats dans le cas de la pénicilline.

Cette étude montre que dans les régions rurales du Lésotho, la résistance aux antibiotiques est peu répandue chez les enfants, et qu'elle est plus élevée — bien qu'encore relativement faible— chez ceux de Maseru qui ont plus facilement accès aux antibiotiques, aux hôpitaux, aux dispensaires et aux centres de soins ambulatoires. Les résultats font en outre nettement ressortir que l'accessibilité des antibiotiques constitue un risque important de sélection et d'acquisition de souches résistantes de *S. pneumoniae*. Bien que les souches résistantes de pneumocoques soient peu répandues au Lésotho comparativement à l'Afrique du Sud voisine, à certains pays d'Europe et aux Etats-Unis, il est recommandé de poursuivre la surveillance de l'antibiorésistance dans ce pays, où des familles nombreuses comptant beaucoup d'enfants en bas âge sont exposées au risque d'acquérir des souches résistantes.

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