High prevalence of antibiotic resistance in nasopharyngeal bacterial isolates from healthy children in rural Uganda: A cross-sectional study

ELIZEUS RUTEBEMBERWA[1], BETTY MPEKA[2], GEORGE PARIYO[3], STEFAN PETERSON[1,4,5], EDISON MWOROZI[6], FREDDIE BWANGA[7] & KARIN KÄLLANDER[1,4,8]

[1]*Makerere University School of Public Health, Kampala, Uganda,* [2]*Abt Associates, Kampala, Uganda,* [3]*Johns Hopkins Bloomberg School of Public Health, Baltimore, MD, United States,* [4]*Division of Global Health, Department of Public Health Sciences, Karolinska Institutet, Stockholm, Sweden,* [5]*International Maternal and Child Health Unit, Department of Woman and Child Health, Uppsala University, Sweden,* [6]*Department of Paediatrics and Child Health, Makerere University Medical School, Kampala, Uganda,* [7]*Department of Microbiology, Makerere University Faculty of Medicine, Kampala, Uganda, and* [8]*Malaria Consortium, London, United Kingdom*

Abstract

Background: In Uganda, the main causes of death in children under 5 years of age are malaria and pneumonia—often due to delayed diagnosis and treatment. In preparation for a community case management intervention for pneumonia and malaria, the bacterial composition of the nasopharyngeal flora and its *in vitro* resistance were determined in children aged five or under to establish baseline resistance to commonly used antibiotics.

Methods: In a population-based survey in April 2008, nasopharyngeal specimens were collected from 152 randomly selected healthy children under 5 years of age in the Iganga/Mayuge Health and Demographic Surveillance Site (HDSS). Medical history and prior treatment were recorded. Demographic characteristics and risk factors for carriage of resistant strains were obtained from the HDSS census. Bacteria were isolated and analysed for antibiotic susceptibility using disk diffusion and E test.

Results: *Streptococcus pneumoniae* (S. pneumoniae) carriage was 58.6%, and, while most (80.9%) isolates had intermediate resistance to penicillin, none was highly resistant. Whereas no isolate was resistant to erythromycin, 98.9% were resistant to trimethoprim-sulphamethoxazole (co-trimoxazole).

Conclusions: *In vitro* resistance in *S. pneumoniae* to co-trimoxazole treatment was high, and the majority of isolates had intermediate resistance to penicillin. To inform treatment policies on the clinical efficacy of current treatment protocols for pneumonia in health facilities and at the community level, routine surveillance of resistance in pneumonia pathogens is needed as well as research on treatment efficacy in cases with resistant strains. Improved clinical algorithms and diagnostics for pneumonia should be developed.

Key words: *Antibiotic resistance, children, community case management, pneumonia, Streptococcus pneumoniae*

Introduction

Worldwide in 2013, 935,000 children younger than 5 years of age die of pneumonia, and in sub-Saharan Africa an estimated 16% of child deaths are attributed to pneumonia (1). Bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (Hib), and *Staphylococcus aureus* are responsible for the majority of these infections, but also viruses, parasites, and fungi can cause pneumonia

(2,3). Reducing the pneumonia death toll requires preventive interventions (e.g. adequate nutrition and immunizations), but the main difference between life and death for a child sick with pneumonia hinges on prompt and appropriate treatment with an effective drug (4). Yet in 2012 UNICEF published *Pneumonia and diarrhoea—Tackling the deadliest diseases for the world's poorest children*, which revealed that less than 29% of the worlds' children with potential pneumonia receive antibiotics for their illness (5).

In an attempt to improve access to essential medicines in countries with weak health systems, strategies for managing pneumonia in the community are recommended (6). These strategies typically use trained community health workers (CHWs) to assess, classify, and treat children using antibiotic and anti-malarial drugs. While community case management (CCM) of malaria and pneumonia can reduce mortality (7,8) there is a concern that CHWs' performance is poorer when they are required to manage several illnesses and that over-treatment and antibiotic resistance in bacteria could be negative consequences of these programmes (9).

The worldwide incidence of infections caused by pneumococci resistant to penicillin and other antimicrobial agents has increased at an alarming rate during past decades (10). Resistance is believed to be driven by antibiotic use (both appropriate and inappropriate), dose, and duration of therapy (11). The use of broad-spectrum antibiotics, as a substitute for precise diagnosis or to enhance the likelihood of treatment success, and use of sub-standard medicines, which give sub-optimal blood drug concentrations, enhance the selection rate of resistant bacteria (12). While prophylactic use of trimethoprim-sulphamethoxazole (TMP-SMX, or co-trimoxazole) to prevent opportunistic infections in HIV+ patients is policy in several sub-Saharan countries, many of these countries are also using co-trimoxazole as first-line treatment for non-severe pneumonia.

A major factor in the pathogenesis of pneumococcal disease, its transmission, and spread of drug resistance is the nasopharyngeal reservoir of bacteria which is carried by up to 60%–70% of healthy children (13). The colonizing strains, which are carried for a mean duration of 6 weeks, can easily spread from one child to another (14), and their characteristics and susceptibility patterns have been shown to be similar to those involved in invasive illness (15). Hence, studies of the nasopharyngeal reservoir of bacteria can provide useful information about the prevalence and resistance patterns of respiratory pathogens in a population, and how these characteristics change over time.

High rates of *in vitro* resistance to antibiotics have been found in nasopharyngeal isolates in both Gram-positive (e.g. *Streptococcus pneumoniae*) and Gram-negative (e.g. *Haemophilus influenzae* and *Moraxella catarrhalis*) respiratory organisms (16,17). We were unable to find any population-based study of nasopharyngeal carriage and resistance in respiratory pathogens in Uganda. Hence, our objective was to establish the bacterial composition of nasopharyngeal flora and its *in vitro* resistance in healthy children at the community level. The study was carried out as a baseline before the introduction of integrated community case management of fever using amoxicillin for presumptive pneumonia and artemether–lumefantrine (Coartem) for presumptive malaria.

Materials and methods

A cross-sectional survey of nasopharyngeal carriage among healthy children under five was conducted in the Iganga/Mayuge Health and Demographic Surveillance Site (HDSS) in Eastern Uganda in April 2008. The HDSS follows standard INDEPTH network methodology (18) and keeps a census of 65,000 people living in a geographically defined area totalling 3931 km^2. A sample of 152 households with children younger than 5 years of age was selected from the HDSS population register using simple random sampling. Each of the selected households was visited, and one child aged between 2 and 59 months was selected using a lottery method. The primary caretaker was interviewed using a pretested questionnaire for information on socio-demographic characteristics, illness symptoms, and treatments in the last 2 weeks. The household identifier was linked to the HDSS database for retrieval of household characteristics such as crowding, water source, and number of children ≤5 years.

With caretaker consent a nasopharyngeal specimen was collected by a lab technician in each of the 152 children. Pre-packed sterile calcium alginate swabs on flexible aluminium shafts (BD, Franklin Lakes, New Jersey, USA) were used. The swabs were placed in Amies transport medium in a tube. Tubes were transported within 12 h to the Microbiology lab at Makerere University Medical School.

Each sample was registered in a book, where date of receipt, lab number, and household number were entered. Each swab was streaked within 3 h on blood agar plates supplemented with 7% sheep blood as well as on chocolate agar. An optochin disk was placed in the first streak area, and plates were incubated at 37°C in 5%–10% CO_2 for 24–48 h. *S. pneumoniae* was identified by an inhibition zone

Table I. Disk diffusion interpretation.

		Zone diameter interpretation (mm)		
Agent	Disk content/mL	Susceptible	Intermediate	Resistant
Oxacillin	1 μg oxacillin	≥20	[a]	[a]
Trimethoprim-sulphamethoxazole	1.25/23.75 μg	≥19	16–18	≤15
Erythromycin	15 μg	≥21	–	≤20

[a]If the zone diameter for oxacillin is <20 mm, an isolate cannot be reported as susceptible, intermediate, or resistant, and MIC testing must be conducted for an appropriate penicillin (or other β-lactam) drug (19).

around the optochin disk and the presence of α-haemolytic, flat, irregularly shaped colonies with central depression with maturity. *H. influenzae* was identified by morphology (convex, smooth, pale, grey, or transparent) and with oxidase test, Gram stain, satellitism, and growth dependence on factors X and V. *M. catarrhalis* isolates were identified using standard microbiological methods: colony morphology (grey-white hemispheric colonies about 1 mm in diameter), Gram stain, oxidase test, DNase production, and a nitrate reduction test.

S. pneumoniae colonies from a 24-h-old subculture were suspended in sterile normal saline to achieve a density equal to a 0.5 McFarland standard. The inoculum was swabbed onto three plates of Mueller–Hinton agar supplemented with 7% sheep blood. Antimicrobial susceptibility to penicillin (1 μg oxacillin disc was used), erythromycin (15 μg), and TMP-SMX (co-trimoxazole) (1.25/23.75 μg) was determined by disk diffusion (Biolab Inc., Budapest, Hungary). *Streptococcus pneumoniae* ATCC 6303 was used as control, and results were within acceptable ranges. Minimum inhibitory concentration (MIC) of penicillin for *S. pneumoniae* was determined using E test (bioMérieux, Marcy l'Etoile, France). Antibiotic discs or E test strips were placed on the swabbed plates, which were then incubated for 24 h at 37°C in 5% CO_2. Zone diameters and MIC of various antibiotics were read and interpreted using Clinical and Laboratory Standards Institute guidelines (19). The disk diffusion interpretation is shown in Table I. *H. influenzae* was only analysed for detection of β-lactamase activity, determined by the chromogenic cephalosporin nitrocefin (BR66A; Oxoid Limited, Basingstoke, United Kingdom) method using known β-lactamase positives as controls.

The study protocol was reviewed by the Institutional Review Board at Makerere University School of Public Health, and ethical approval was obtained from Uganda National Council for Science and Technology (Ref HS 72). Verbal consent was obtained from district and village leaders as well as from caretakers while the studied children gave assent.

Table II. Socio-demographic characteristics of the household heads, caretakers, and children.

Variable	Number of children (%)
Child age	
≤2 years	77 (50.7)
>2 years	75 (49.3)
All	152 (100)
Child sex	
Girl	79 (52.0)
Boy	73 (48.0)
All	152 (100)
Residence	
Rural	110 (82.1)
Urban	24 (17.9)
All	134 (100)
Water source	
Spring water	95 (73.6)
Well water	33 (25.6)
Piped water	1 (0.8)
All	126 (100)
Number of children under 5 in household	
1	70 (49.3)
2	54 (38.0)
3	11 (7.8)
4	6 (4.2)
6	1 (0.7)
All	142 (100)
Number of people per bedroom	
0–2	13 (10.3)
3–5	84 (66.1)
5+	30 (23.6)
All	127 (100)

Results

Descriptive statistics

The median age of the 152 children was 24 months (Interquartile Range (IQ) 12–33.5). Fifty-two per cent of the children were girls (Table II). Complete socio-demographic profile was available for 82.9% (126/152) of the included children, of whom 82.1% were from rural villages and 73.6% used spring water as their primary water source. The median number of children less than 5 years of age per household was 2 (IQ 1–2), and the median number of household members per bedroom (as a measure of crowding) was 4 (IQ 3–5).

Table III. Antimicrobial susceptibility of isolated respiratory pathogens, $n = 89$ (%).

Agent	Bacterium	Susceptible	Intermediate	Resistant
Oxacillin[a]	*S. pneumoniae*	17 (19.1)	c	c
Nitrocefin[b]	*Moraxella* sp.	2 (9.5)	–	19 (90.5)
	Haemophilus sp.	12 (75.0)		4 (25.0)
Trimethoprim-sulphamethoxazole[a]	*S. pneumoniae*	1 (1.1)	0	88 (98.9)
Erythromycin[a]	*S. pneumoniae*	89 (100)	–	0
Penicillin[b]	*S. pneumoniae*	17 (19.1)	72 (80.9)	0

[a]Determined by disk diffusion.
[b]Determined by E test.
[c]If the zone diameter for oxacillin is <20 mm, an isolate cannot be reported as susceptible, intermediate, or resistant, and MIC testing must be conducted for an appropriate penicillin (or other β-lactam) drug (19).

Illness and antibiotic use in the previous 2 weeks

According to reports of the mothers, 95% (145/152) of the children had been sick in the previous 2 weeks. Among these, the most common symptoms were fever (91.7%; 133/145), running nose (61.4%; 89/145), and cough (44.8%; 65/145). No child was reported to have had fast breathing. Of the children who had been sick, 27.6% (40/145) had reportedly been given an antibiotic, mostly co-trimoxazole (77.5%; 31/40) and ampicillin (12.5%; 5/40). Only one child had been given amoxicillin. Antibiotics were given to 29.2% (19/65) of children with cough, 27.3% (24/88; 1 observation missing) of those with running nose, and 26.3% (35/131; 2 observations missing) of those with fever.

Nasopharyngeal carriage

S. pneumoniae was recovered from 58.6% (89/152) of the children, *M. catarrhalis* from 15% (23/152), and *H. influenzae* from 11% (16/152). Of all children sampled 34% (52/152) had none of the three bacteria, 20% (30/152) had two of the three bacteria (16 had *M. catarrhalis* + *S. pneumonia*, and 14 had *H. influenzae* + *S. pneumonia*). No child had all three bacteria. There was no difference in pneumococcal carriage between boys and girls, between children ≤6 months versus older children, bedroom crowding, or number of under-5 children in the household.

Antimicrobial susceptibility

Of the pneumococcal isolates 98.9% (88/89) were resistant to co-trimoxazole, and 80.9% (72/89) had intermediate resistance to penicillin. None was highly resistant to penicillin (≥2 μg/mL) (Table III). Sex, previous illness symptoms, treatment taken, and socio-economic or household profile had no effect on carriage of non-susceptible or intermediately resistant *S. pneumoniae*. Of the 16 *H. influenzae* isolates, 4 (25%) were producing β-lactamase.

Discussion

This study, which is one of the first population-based studies from East Africa that determines both the bacterial composition of the nasopharyngeal flora and its *in vitro* resistance in healthy children at the community level, shows colonization rates of 59% for *S. pneumonia*, 15.1% for *M. catarrhalis*, and 10.5% for *H. influenzae*. The *S. pneumoniae* colonization rate has been shown to vary geographically. While the 59% carriage rate found in our study is consistent with the 51%–60% prevalence found in coastal Kenya (20), it is much lower than the 97% observed in Gambian infants (21). Other studies from children in hospital settings in southern Africa generally show higher carriage rates (>69%) (22,23). Apart from differences in sampling frames, other factors influencing nasopharyngeal carriage rates include age, season, number of siblings, and acute respiratory illness (24). Our study did not show any significant association between pneumococcal carriage and these known risk factors, probably due to the small sample size.

Almost all pneumococcal strains were resistant *in vitro* to the treatment that was first line for pneumonia at the time of the study, co-trimoxazole, and 81% had intermediate resistance to penicillin. No isolate had high resistance to penicillin (≥2.0 μg/mL). Nevertheless, the 99% co-trimoxazole resistance is very high, and similar levels have only been observed in one other study, also from Uganda but in an adult population (25). Previous studies on pneumococcal isolates showed that co-trimoxazole resistance varied depending on sampling frame: from 39% in healthy village children in the Gambia (21), 83.5% in healthy children in a Ugandan hospital (26), and 64% in patients with invasive disease in South Africa (27). Yet the extreme levels of pneumococci resistant to co-trimoxazole reported

from studies in Uganda have not been observed in any other country. While the result of the susceptibility testing indicates a very homogeneous susceptibility pattern, a clonal spread could be suspected. However, the study area is large, and it is unlikely that the children sampled lived very close to each other or would ever have met. The molecular characteristics of pneumococci in healthy carriers and cases with invasive disease need to be determined to explain further the transmission and the high prevalence of non-susceptible bacteria in the study area.

Penicillin resistance patterns also vary greatly between countries (0%–79%), and it is well documented that resistance in pneumococci is increasing globally (16). Most studies on carriage of resistant pneumococci are conducted on hospital-based samples, such as another Uganda study which found 83.5% intermediate penicillin resistance (26). Of the few population-based studies from sub-Saharan Africa that were identified, intermediate penicillin resistance ranged from 14% in village-based infants in the Gambia (21,28), 45% in children in urban Ghanaian kindergartens (29), to 67% in healthy Tanzanian children attending an urban clinic (30). Again, it appears that Uganda, where we found 80% intermediate penicillin resistance, is on the higher end of the global penicillin-non-susceptible *S. pneumoniae* spectrum.

Despite these remarkable *in vitro* resistance rates of *S. pneumoniae* in the nasopharyngeal flora, the clinical relevance of these findings has not been clearly established. While there is evidence of the inferiority of co-trimoxazole *in vivo* efficacy in treating pneumonia (31), most studies have not been able to show any consistent association between *in vitro* resistance to co-trimoxazole and failure of therapy in cases of non-severe pneumonia (32). Studies that documented impact of β-lactam resistance on pneumococcal pneumonia mortality (33,34) had not been adjusted for severity of disease or HIV infection (35). In addition, susceptibility is rarely determined in community settings, but data primarily come from hospitalized patients where aggressive intravenous and/or multi-drug therapy makes the impact of therapy difficult to assess (11).

In 2009 the World Health Organization (WHO) recommended that while co-trimoxazole may be an alternative in some settings, national treatment policies should change to amoxicillin as first-line treatment of children 2–59 months of age diagnosed with pneumonia (36,37). Yet, despite the widespread pneumococcal *in vitro* resistance to co-trimoxazole, the treatment is still used as a first choice for treatment of pneumonia in many countries, primarily due to its low cost, few side effects, and because it can be safely used by health workers at the peripheral health facilities and at home by mothers (31). While agencies like UNICEF and WHO recommend that 'the clinical efficacy of pneumonia treatment should be monitored regularly to revise national treatment policies based on antimicrobial resistance information, clinical outcomes and other data' (38), such surveillance has been sporadic in most of sub-Saharan Africa.

Hence, more studies on how *in vitro* resistance in pneumococci translates into clinical outcomes in patients with varying treatment regimens and disease severity are urgently needed to optimize the clinical management of pneumonia (11,35). Meanwhile, monitoring of the bacterial reservoir in the society over time can provide important information on the characteristics and spread of respiratory bacteria, especially in relation to interventions such as community case management and introduction of vaccines against respiratory pathogens.

The most important factor driving resistance is previous exposure to antibiotics (24,35). In our study one-third of children in the community had been given an antibiotic in the previous 2 weeks. Since a child in this context is expected to suffer from 0.3 episodes of pneumonia per year (39) it is apparent that over-treatment with antibiotics is common. The WHO case management guidelines (40,41), which are based on simple clinical signs to help health workers identify and appropriately manage pneumonia and other illnesses in the community and health facilities, have shown to increase rational prescribing of medicines (42). However, as WHO guidelines do not make a distinction between viral and bacterial pneumonia, these children continue to receive antibiotics because of the concern that it may not be safe to do otherwise.

Hence, studies on how to reduce unnecessary prescribing, not only in health facilities but also at the community level and in the private sector, are key to preserve the efficacy of existing antibiotics. There is a need for simple methodologies to help health workers collect relevant data that can inform decision-makers to change policy and guidelines from first- to second-line antibiotics (43). Scaling up the pneumococcal vaccine (PCV) for children is a potential strategy to reduce the incidence of pneumococcal disease and possibly also reduce the problem with resistant strains among PCV serotypes. However, the actual role of the PCV in relation to pneumococcal resistance development is not yet fully understood, and it is likely that pneumococcal vaccine pressure has led to the selection of resistance in non-vaccine pneumococcal serotypes (44); this

calls for research on a vaccine that targets the antibiotic resistance mechanism itself (45). Research is also warranted to identify inexpensive and efficacious alternative antibiotic regimens that are associated with good adherence such as through co-formulated combination therapy, less frequent dosing, and shorter courses. More specific diagnostic criteria for acute respiratory infection and improved diagnostic tests should be developed and evaluated in clinical practice.

There are some methodological limitations of this study to keep in mind. The very high prevalence of reported illness is likely a result of caretaker over-reporting, and as a consequence illness data should be interpreted with caution. It is possible that the study purpose was not explained sufficiently well, leading caretakers to give answers which she or he expected to be 'desired' by the interviewer (46). This study was also based on a small study sample, which did not allow for stratified analysis. While we were unable to type the pneumococcal isolates for sero-types and clones, data on serotypes and resistance patterns from the same study population have been collected at a later stage and will be published elsewhere (Lindstrand et al., unpublished). Because the intention of the study was to have a baseline estimate of nasopharyngeal carriage and resistance rates in the child population before rollout of blister-packed amoxicillin through community health work-ers, the funding did not allow for these molecular analyses.

We would like to conclude that among the pneumococci isolated from healthy children in the community, *in vitro* resistance to co-trimoxazole treatment was high, and the majority of strains had intermediate resistance to penicillin. To inform treatment policies on the clinical efficacy of current treatment protocols for pneumonia in facilities and at the community level, routine surveillance of resist-ance in pneumonia pathogens is needed as well as rigorous research on the association between pneumococcal *in vitro* resistance in population-based samples and *in vivo* efficacy of treatment of clinical pneumonia. The revised WHO treatment guidelines for pneumonia in children, which recom-mend the use of amoxicillin over co-trimoxazole, should be rolled out widely. Pneumococcal vaccin-ation should be scaled up, and improved clinical algorithms and diagnostics for pneumonia should be developed.

Acknowledgements

References

1. Liu L, Oza S, Hogan D, Perin J, Rudan I, Lawn JE, et al. Global, regional, and national causes of child mortality in 2000-13, with projections to inform post-2015 priorities: an updated systematic analysis. Lancet. 2015; 385:430–40.

2. WHO. Conclusions from the WHO multicenter study of serious infections in young infants. The WHO Young Infants Study Group. Pediatr Infect Dis J. 1999;18:S32–4.

3. Enright MC, McKenzie H. Moraxella (Branhamella) catar-rhalis–clinical and molecular aspects of a rediscovered pathogen. J Med Microbiol. 1997;46:360–71.

4. Reyes H, Perez-Cuevas R, Salmeron J, Tome P, Guiscafre H, Gutierrez G. Infant mortality due to acute respiratory infections: the influence of primary care processes. Health Policy Plan. 1997;12:214–23.

5. UNICEF. Pneumonia and diarrhoea - Tackling the deadliest diseases for the world's poorest children. New York, NY: UNICEF; 2012.

6. WHO/UNICEF. Joint statement: Management of pneumo-nia in community settings. WHO/FCH/CAH/04.06. Geneva/ New York: WHO/UNICEF; 2004.

7. Kidane G, Morrow RH. Teaching mothers to provide home treatment of malaria in Tigray, Ethiopia: a randomised trial. Lancet. 2000;356:550–5.

8. Sazawal S, Black RE. Effect of pneumonia case management on mortality in neonates, infants, and preschool children: a meta-analysis of community-based trials. Lancet Infect Dis. 2003;3:547–56.

9. Druetz T, Siekmans K, Goossens S, Ridde V, Haddad S. The community case management of pneumonia in Africa:

a review of the evidence. Health Policy Plan. 2015; 30:253–66.

10. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, et al. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect Dis. 2005;5:481–93.

11. Klugman KP. Clinical impact of antibiotic resistance in respiratory tract infections. Int J Antimicrob Agents. 2007;29(Suppl 1):S6–10.

12. World Health Organization. WHO global strategy for containment of antimicrobial resistance. WHO/CDS/CSR/DRS/2001.2a. Geneva: WHO; 2001.

13. Henriques Normark B, Christensson B, Sandgren A, Noreen B, Sylvan S, Burman LG, et al. Clonal analysis of *Streptococcus pneumoniae* nonsusceptible to penicillin at daycare centers with index cases, in a region with low incidence of resistance: emergence of an invasive type 35B clone among carriers. Microb Drug Resist. 2003;9:337–44.

14. Uzuner A, Ilki A, Akman M, Gundogdu E, Erbolukbas R, Kokacya O, et al. Nasopharyngeal carriage of penicillin-resistant *Streptococcus pneumoniae* in healthy children. Turk J Pediatr. 2007;49:370–8.

15. Friedland IR, Klugman KP. Antibiotic-resistant pneumococcal disease in South African children. Am J Dis Child. 1992;146:920–3.

16. Fuller JD, McGeer A, Low DE. Drug-resistant pneumococcal pneumonia: clinical relevance and approach to management. Eur J Clin Microbiol Infect Dis. 2005;24:780–8.

17. Garcia-Rodriguez JA, Fresnadillo Martinez MJ. Dynamics of nasopharyngeal colonization by potential respiratory pathogens. J Antimicrob Chemother. 2002;50(Suppl S2): S59–73.

18. Sankoh O, Byass P. The INDEPTH Network: filling vital gaps in global epidemiology. Int J Epidemiol. 2012;41: 579–88.

19. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk susceptibility tests; approved standard. 11th ed. Wayne, PA, USA: Clinical and Laboratory Standards Institute; 2012. p. M02–A11.

20. Abdullahi O, Nyiro J, Lewa P, Slack M, Scott JA. The descriptive epidemiology of *Streptococcus pneumoniae* and Haemophilus influenzae nasopharyngeal carriage in children and adults in Kilifi district, Kenya. Pediatr Infect Dis J. 2008;27:59–64.

21. Hill PC, Akisanya A, Sankareh K, Cheung YB, Saaka M, Lahai G, et al. Nasopharyngeal carriage of *Streptococcus pneumoniae* in Gambian villagers. Clin Infect Dis. 2006; 43:673–9.

22. Huebner RE, Wasas A, Mushi A, Mazhani L, Klugman K. Nasopharyngeal carriage and antimicrobial resistance in isolates of *Streptococcus pneumoniae* and Haemophilus influenzae type b in children under 5 years of age in Botswana. Int J Infect Dis. 1998;3:18–25.

23. Woolfson A, Huebner RE, Wasas A, Chola S, Godfrey-Fausett P, Klugman K. Nasopharyngeal carriage of community-acquired antibiotic resistant *Streptococcus pneumoniae* in a Zambian paediatric population. Bull World Health Organ. 1997;75:453–62.

24. Deeks SL, Palacio R, Ruvinsky R, Kertesz DA, Hortal M, Rossi A, et al. Risk factors and course of illness among children with invasive penicillin-resistant *Streptococcus pneumoniae*.The *Streptococcus pneumoniae* Working Group. Pediatrics. 1999;103:409–13.

25. Blossom DB, Namayanja-Kaye G, Nankya-Mutyoba J, Mukasa JB, Bakka H, Rwambuya S, et al. Oropharyngeal colonization by *Streptococcus pneumoniae* among HIV-infected adults in Uganda: assessing prevalence and antimicrobial susceptibility. Int J Infect Dis. 2006;10:458–64.

26. Joloba ML, Bajaksouzian S, Palavecino E, Whalen C, Jacobs MR. High prevalence of carriage of antibiotic-resistant *Streptococcus pneumoniae* in children in Kampala Uganda. Int J Antimicrob Agents. 2001;17:395–400.

27. Adrian PV, Klugman KP. Mutations in the dihydrofolate reductase gene of trimethoprim-resistant isolates of *Streptococcus pneumoniae*. Antimicrob Agents Chemother. 1997;41:2406–13.

28. Warda K, Oufdou K, Zahlane K, Bouskraoui M. Antibiotic resistance and serotype distribution of nasopharyngeal isolates of *Streptococcus pneumoniae* from children in Marrakech region (Morocco). J Infect Public Health. 2013;6:473–81.

29. Dayie NT, Arhin RE, Newman MJ, Dalsgaard A, Bisgaard M, Frimodt-Moller N, et al. Penicillin resistance and serotype distribution of *Streptococcus pneumoniae* in Ghanaian children less than six years of age. BMC Infect Dis. 2013;13:490.

30. Moyo SJ, Steinbakk M, Aboud S, Mkopi N, Kasubi M, Blomberg B, et al. Penicillin resistance and serotype distribution of *Streptococcus pneumoniae* in nasopharyngeal carrier children under 5 years of age in Dar es Salaam, Tanzania. J Med Microbiol. 2012;61:952–9.

31. Rajesh SM, Singhal V. Clinical effectiveness of co-trimoxazole vs. amoxicillin in the treatment of non-severe pneumonia in children in India: a randomized controlled trial. Int J Prev Med. 2013;4:1162–8.

32. Qazi SA. Antibiotic strategies for developing countries: experience with acute respiratory tract infections in Pakistan. Clin Infect Dis. 1999;28:214–18.

33. Turett GS, Blum S, Fazal BA, Justman JE, Telzak EE. Penicillin resistance and other predictors of mortality in pneumococcal bacteremia in a population with high human immunodeficiency virus seroprevalence. Clin Infect Dis. 1999;29:321–7.

34. Feikin DR, Schuchat A, Kolczak M, Barrett NL, Harrison LH, Lefkowitz L, et al. Mortality from invasive pneumococcal pneumonia in the era of antibiotic resistance, 1995-1997. Am J Public Health. 2000;90:223–9.

35. Peterson LR. Penicillins for treatment of pneumococcal pneumonia: does in vitro resistance really matter? Clin Infect Dis. 2006;42:224–33.

36. Grant GB, Campbell H, Dowell SF, Graham SM, Klugman KP, Mulholland EK, et al. Recommendations for treatment of childhood non-severe pneumonia. Lancet Infect Dis. 2009;9:185–96.

37. WHO. Revised WHO classification and treatment of pneumonia in children at health facilities: evidence summaries. Geneva: World Health Organization; 2014.

38. UNICEF/WHO. Pneumonia: the forgotten killer of children. New York/Geneva: UNICEF/WHO; 2006.

39. Rudan I, O'Brien KL, Nair H, Liu L, Theodoratou E, Qazi S, et al. Epidemiology and etiology of childhood pneumonia in 2010: estimates of incidence, severe morbidity, mortality, underlying risk factors and causative pathogens for 192 countries. J Glob Health. 2013;3:010401.

40. WHO. Integrated management of childhood illness: A WHO/UNICEF Initiative. Bull World Health Organ. 1997;75:5–128.

41. Young M, Wolfheim C, Marsh DR, Hammamy D. World Health Organization/United Nations Children's Fund joint statement on integrated community case management: an equity-focused strategy to improve access to essential

treatment services for children. Am J Trop Med Hyg. 2012; 87:6–10.

42. Chopra M, Patel S, Cloete K, Sanders D, Peterson S. Effect of an IMCI intervention on quality of care across four districts in Cape Town, South Africa. Arch Dis Child. 2005;90:397–401.

43. Heyman G, Cars O, Bejarano MT, Peterson S. Access, excess, and ethics–towards a sustainable distribution model for antibiotics. Ups J Med Sci. 2014;119:134–41.

44. Klugman KP. Contribution of vaccines to our understanding of pneumococcal disease. Philos Trans R Soc Lond B Biol Sci. 2011;366:2790–8.

45. Henriques-Normark B, Normark S. Bacterial vaccines and antibiotic resistance. Ups J Med Sci. 2014;119:205–8.

46. Hardon AP, Boonmongkon P, Streefland P, Tan ML, Hongvivatana T, van der Geest S, et al. Applied health research - anthropology of health and health care. Amsterdam: Het Spinhuis Publishers; 2001.