# Percentage, Bacterial Etiology and Antibiotic Susceptibility of Acute Respiratory Infection and Pneumonia among Children in Rural Senegal

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**Summary**

**Acute respiratory infections (ARI) are still a major health problem in most developing countries. So far no study has evaluated the importance of childhood ARI in rural Senegal. We prospectively studied ARI, the percentage of pneumonia and related mortality, as well as the bacterial composition of nasopharyngeal flora using nasopharyngeal aspirates in 114 children, aged 2–59 months, presenting at Ndioum's pediatric ward. Excluded from the trial were those children that had had antimicrobial therapy in the previous 2 weeks. The Kirby–Bauer method was used to determine antibiotic resistance throughout the study. The percentage of ARI and pneumonia among the population tested was 24 per cent and 11 per cent respectively. *Streptococcus pneumonia* was often resistant to cotrimoxazole (31 per cent) but only 9 per cent were resistant to chloramphenicol and 14 per cent to penicillin. *Haemophilus influenzae* (HI) was uniformly sensitive to ampicillin, and only 4 per cent were resistant to chloramphenicol and 11 per cent to cotrimoxazole. We conclude that SP and HI resistance to cotrimoxazole is important and warrants larger clinical trials using chloramphenicol. Information campaigns and intense management of comorbidities are desirable in this type of population. Comorbidities (tuberculosis, malaria, HIV-AIDS, severe malnutrition) are determinant variables in many ARI cases and carry a high negative prognosis value.**

## Introduction

More than 98 per cent of the 10.630 million children under 5 years of age who die each year are in developing countries and acute respiratory infections (ARI) are accountable for 4 million of those deaths. Pneumonia is thought to arise from inhalation of *Streptococcus pneumoniae* (SP) or *Haemophilus influenzae* (HI) present in nasopharyngeal secretions. One of the World Health Organization's (WHO) main objectives in the Integrated Management of Childhood Illness (IMCI) is the prevention of death due to pneumonia. It also emphasizes that case management of pneumonia is the major area where improvement is still needed. It recommends that any child under 5 years of age presenting at a first-level health center with a cough or respiratory distress should have a thorough examination to exclude possible pneumonia. Assessment should verify rate of respiration, chest indrawing, cyanosis and feeding ability,[1] and thus enable sequential and rational administration of antimicrobials. Incidence of SP and HI in nasopharyngeal aspirates has been established in many different countries and settings;[1] it has also been established that SP and HI are the predominant pathogens involved in childhood pneumonia in developing countries.[2,3] WHO has recommended data collection on antimicrobial resistance for each country, but few trials have provided data concerning ARI in rural communities,[4,5] and none in Senegal. Lung biopsy or needle aspirations are the only methods that assertively demonstrate the etiology of bacterial pneumonia. However, it has been shown that micro-organisms detected in lung aspirates are present 80 per cent of the time in children's nasopharynx. The converse is often true and, based on WHO methodology, nasopharyngeal sampling has been shown to give valid results.[6,7] Another assumption is that organisms found in the nose of children who do not have pneumonia are similar to those found in the lungs of children who do have pneumonia.[6,8,9] Nasopharyngeal aspirates are thus a suitable substitute for lung aspirate for the determination of the type of pathogens involved in ARI and their susceptibility to antibiotics. In this regard antibiotic susceptibility tests occupy a central

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position because it has been established that clinical efficacy is directly related to antibiotic susceptibility.[10] The goals of this study, which was conducted at Ndioum's rural hospital in Senegal and involved children aged 2–59 months old, were to evaluate the percentage of ARI and pneumonia in the population tested, to establish the bacterial composition of nasopharyngeal flora and its *in vitro* resistance, and to correlate *in vitro* susceptibility or resistance with clinical evolution in children with WHO-defined pneumonia.

## Patients and Methods

From March to May 2000, we prospectively enrolled all children aged between 2 and 59 months presenting at Ndioum's hospital pediatric service. Excluded from the trial were those children that had had antimicrobial therapy in the previous 2 weeks. Verbal parental consent as well as approval from the hospital director was obtained. The hospital is the main care provider of the Saint-Louis district, apart from the Saint-Louis hospital which is 5 h away, and the pediatric service covers a population of 100 000 children under 16 years of age. Five thousand consultations and 1000 hospitalizations take place every year at the ward. Parents sometimes report that they have been travelling for 10 days when they arrive at the ward. Infectious diseases are the primary cause of hospitalization. The national vaccination programme is well accepted by the population and coverage is good, but it does not include *Haemophilus influenzae* type b vaccine. For each children, details of the clinical examination made upon arrival were recorded on a standardized form.

*Specimen collection and processing*

Nasopharyngeal aspirate was taken at admission from every child, using a suction set or a child's feeding tube connected to suction. Sampling and child identification were done at the first visit to the clinic and no subsequent sampling was done even if the child presented at a later time during the study. A tube was passed along the inferior nasal wall until it reached the posterior nasopharyngeal wall. Suction was then activated and left on for about 3 s. It was stopped before withdrawal of the tube. Specimens were immediately brought to the laboratory and processed. All laboratory procedures and methods were carried out in accordance with WHO guidelines.[11] A standardized laboratory form was used for each child. For each nasopharyngeal sample, a Gram stain was done. In accordance with WHO recommendation, samples that had more than 25 epithelial cells at 100× were rejected.[12] All others were immediately inoculated onto three solid media: a trypticase soy agar plate containing 5 per cent sheep blood with an optochin disk on the site of inoculation; a chocolate blood agar plate containing bacitracin (10 UI/ml); and a MacConkey agar plate. The sheep blood and chocolate agar plates were incubated at 37 ºC in a candle extinction jar while the MacConkey agar plate was incubated at 37 ºC without special atmosphere. Bacterial growth was recorded after 24 h of incubation and semi-quantitative evaluation was done using the four-quadrant method.

*Bacterial identification and susceptibility testing*

The Kirby–Bauer method, accepted by the WHO as a standard testing method,[12] was used to determine antibiotic resistance throughout the study. SP was identified by typical colony morphology, alpha-hemolysis on sheep blood agar, susceptibility to optochin (zone of inhibition > 14 mm) and bile solubility. HI was identified by typical colony morphology on chocolate blood agar with bacitracin and by growth requirements for nicotinamid adenine dinucleotide (factor V) and haemin (factor X). Using the disc diffusion method, SP and HI were tested against penicillin, cotrimoxazole, chloramphenicol and ampicillin, cotrimoxazole, and chloramphenicol, respectively. *In vitro* susceptibility of SP isolates to oxacillin (for penicillin) (1 µg), cotrimoxazole (1.25/23.75 µg) and chloramphenicol (30 µg) was tested on a Mueller–Hinton agar plate containing 5 per cent sheep blood and incubated at 37 ºC in a candle extinction jar, in accordance with National Committee for Clinical Laboratory Standards recommendations.[13] SP isolates with an inhibition zone diameter ≤ 19 mm for oxacillin (no equivalent MIC breakpoint), ≤ 15 mm for cotrimoxazole (equivalent MIC breakpoint ≥ 4/76 µg/l) and ≤ 20 mm for chloramphenicol (equivalent MIC breakpoint ≥ 8 µg/l) were defined as resistant. Technical limitations prevented us from performing MIC testing on SP isolates that had a zone of inhibition > 13 mm but < 20 mm for oxacillin, as recommended by the NCCLS. This limits the interpretation of the results for the strains falling in this category to 'probably moderately resistant' to penicillin instead of a clear-cut result of resistant or insensitive. SP isolates with an inhibition zone diameter ≥ 20 mm for oxacillin (equivalent MIC breakpoint ≤ 0.6 µg/l), ≥ 19 mm for cotrimoxazole (equivalent MIC breakpoint ≤ 0.5/9.5 µg/l) and ≥ 21 mm for chloramphenicol (equivalent MIC breakpoint ≤ 4 µg/l) were defined as being susceptible. Zones for inhibition found to be between resistant and susceptible categories were declared intermediate to those antibiotics, except for oxacillin and chloramphenicol, which do not have an intermediate zone of inhibition.

*In vitro* susceptibility testing of HI isolates to ampicillin (10 µg), cotrimoxazole (1.25/23.75 µg) and chloramphenicol (30 µg) was performed on a HTM plate and incubated at 37 ºC in a candle extinction jar.[13] HI isolates with an inhibition zone diameter

≤ 18 mm for ampicillin (equivalent MIC breakpoint ≥ 4 μg/l), ≤ 10 mm for cotrimoxazole (equivalent MIC breakpoint ≥ 4/76 μg/l) and ≤ 25 mm for chloramphenicol (equivalent MIC breakpoint ≥ 8 μg/l) were defined as resistant. HI isolates with an inhibition zone diameter ≥ 22 mm for ampicillin (equivalent MIC breakpoint ≤ 1 μg/l), ≥ 16 mm for cotrimoxazole (equivalent MIC breakpoint ≤ 0.5/9.5 μg/l) and ≥ 29 mm for chloramphenicol (equivalent MIC breakpoint ≤ 2 μg/l) were defined as susceptible. Zones of inhibition found to be between resistant and susceptible categories were declared intermediate to those antibiotics, except for chloramphenicol, which does not have an intermediate zone of inhibition.

## Results

One hundred and forty children aged 2–59 months were examined; 12 had received antibiotics in the previous 2 weeks and were excluded. Nasopharyngeal aspirates were taken from 128 children and 14 of them did not meet minimal quality criteria. Thus 114 children (52 girls, 62 boys; mean age 17.8 months, 64 per cent under 2 years) were included in the study. The principal indication for consultation were diarrhea ($n$ = 37, 32 per cent), ARI ($n$ = 27, 24 per cent), fever ($n$ = 7, 6 per cent), and measles ($n$ = 6, 5 per cent). Twenty-six children (23 per cent) were malnourished with a weight/height ratio < 60 ($n$ = 17, 15 per cent) or between 60 and 80 ($n$ = 9, 8 per cent). Twenty-nine children were hospitalized and eight died during the hospital stay, three within 24 h and five later.

Table 1 shows the isolation percentages of pathogenic bacteria and their susceptibility profiles. Isolation percentages from nasopharyngeal aspirates were 56 per cent for SP (64/114) and 46 per cent for HI (53/114). Seventy-three per cent (83/114) of children had a flora containing SP, HI or both. Nasopharyngeal aspirate revealed SP alone in 30 cases (26 per cent), HI alone in 19 cases (17 per cent), both in 34 cases (30 per cent), and neither of them in 31 cases (27 per cent). Among 114 children, 27 presented signs of ARI according to WHO. Table 1 also compares nasopharyngeal carriage percentages and resistance in patients with and without ARI based on WHO definition. SP and HI carriage percentages are not statistically different between these two groups ($\alpha \leq 0.05$).

Overall resistance to cotrimoxazole was shown in 31 and 11 per cent of SP and HI isolates, respectively. In contrast, ampicillin was uniformly active against HI isolates. Multidrug resistance concerned 11 isolates (nine SP: three Peni-Cotri, six Chlor-Cotri, and two HI both Chlor-Cotri). All pathogens but one from children presenting with ARI were susceptible to chloramphenicol. Age (less than or greater than 2 years as in the literature) was not found to be a determining factor for carriage and resistance percentages.

TABLE 1
*Carriage and resistance percentages[a] in children, based on the presence or absence of WHO-defined ARI*

| Children | Overall resistance $n$ (%) | |
|---|---|---|
| | *S. pneumoniae* | *H. influenzae* |
| With ARI ($n$ = 27) | | |
| Pen or Amp[b] | 3/17 (18) | 0/10 (0) |
| Cotrimoxazole | 7/17 (41) | 2/10 (20) |
| Chloramphenicol | 0/17 (0) | 1/10 (10) |
| Without ARI ($n$ = 87) | | |
| Pen or Amp | 6/47 (13) | 0/43 (0) |
| Cotrimoxazole | 13/47 (28) | 4/43 (9) |
| Chloramphenicol | 6/47 (13) | 1/43 (2) |
| All children ($n$ = 114) | | |
| Pen or Amp | 9/64 (14) | 0/53 (0) |
| Cotrimoxazole | 20/64 (31) | 6/53 (11) |
| Chloramphenicol | 6/64 (9) | 2/53 (4) |

[a] For the definition of penicillin resistance in SP, see Patients and Methods section.
[b] Penicillin for SP, Ampicillin for HI.

Twelve cases of pneumonia (seven of class 2, five of class 3) were diagnosed, representing 11 per cent; all were seen in children < 2 years (six girls, six boys; mean age 14 months), with a mortality rate of 33 per cent (4/12). Fifty per cent (4/8) of all children dying during this study had pneumonia. All 12 children with pneumonia were hospitalized and antimicrobial therapy was instituted. Of major concern was the delay in arrival at the hospital, which put children at risk and is reflected in the rapid fatal evolution (within 48 h) for four of the eight children who died during this study. Antimicrobial susceptibility correlated well with clinical outcome in three children. One child's outcome was favorable in spite of *in vitro* resistance to the antibiotic prescribed. He was free of any major comorbidity. Two children died, even though their flora was susceptible to the antimicrobial given, probably due to severe comorbidities (tuberculosis and malaria) as well as malnutrition. Five of the 12 children with pneumonia had a flora containing neither SP nor HI; two of these five children died within 48 h of admission.

## Discussion

An estimated 5000 consultations take place at Ndioum's pediatric service each year. Its remote location, extreme climate, and lack of communication render healthcare delivery very difficult while favoring delay in child hospitalization. We surveyed 114 children aged 2–59 months (64 per cent under 2

years of age) from March to May 2000. Pneumonia as defined by WHO concerned children < 2 years exclusively. Tambe, *et al.*,[5] reported the highest pneumonia prevalence in the less-than-2-year age group. The percentage of ARI was 24 per cent (27/114) while that of pneumonia was 11 per cent and mortality among this latter group was 33 per cent (4/12), representing 50 per cent (4/8) of all deaths occurring during this study. The percentage of ARI was recorded as being between 7.6 and 26.8 per cent in Tanzania, India and Egypt[5,14–16] and a higher percentage in children between 6 and 24 months was noted in India.[5] Our collective results are a good indicator of the pediatric population encountered in rural north-east Senegal in particular and provide a valid estimate of a rural Third World population in general.

This study, realized in the field, has the following limitations. First, cultures were obtained from nasopharyngeal aspirates and not lung aspirates or blood. Second, aspirates were taken from all children even though most of them did not have ARI. Third, we did not test urine for antibiotic activity. This suggests that we underestimated carriage percentages and overestimated resistance percentages.[17] Lastly, isolates were not serotypes. The limits of nasopharyngeal aspirations as a means of detecting SP or HI were revealed by the 50 per cent SP and/or HI yield; 27 per cent of the specimens were free of either SP or HI and 42 per cent of specimens from children with pneumonia revealed neither SP nor HI. It must be noted, however, that for the limited number of cases of pneumonia we encountered, no systematic correlation between *in vitro* susceptibility patterns and clinical evolution could be established. No particular correlation could be established either between age and type and intensity of colonization. Carriage percentages revealed a clear cleavage between developing and developed countries, the former disclosing consistently higher carriage percentages than the latter. Regarding nasopharyngeal carriage of SP or HI, our data are similar to reports from other Third World countries, particularly from Central Africa Republic (CAR)[18] and Egypt.[8] Higher SP carriage percentages were reported from Gambia (98 per cent), Papua New-Guinea (98 per cent), Kenya (89 per cent), Dakar-Senegal (72 per cent), Zambia (72 per cent), Pakistan (61 per cent), and South Africa (56 per cent) and a lower rate from Virginia, USA (39 per cent).[1,6,19,20] HI carriage was reported from Pakistan (43 per cent) and South Africa (42 per cent) and HI type b from Gambia (13 per cent), Kenya (9 per cent), Papua New-Guinea (6 per cent), and Wales, UK (1 per cent).[1,10,19,20] Because few studies have tested antibiotic activity, differences in carriage percentages of HI and SP may have been due to variations in the proportion of children who recently had antibiotics.

Regarding antibiotic resistance, a significant SP *in vitro* resistance percentage to cotrimoxazole (31 per cent) was found, as well as complete susceptibility of HI isolates to ampicillin. Resistance of SP and HI was in the high range when compared with data from other developing countries. Resistance was highest against cotrimoxazole (31 per cent), an antimicrobial agent broadly used in that part of Senegal as a first-line agent, particularly in feverish children and in areas where malaria is endemic. This has been shown to increase percentages of resistant SP.[21] Most SP and HI isolates were susceptible to chloramphenicol and ampicillin, respectively. SP resistance to penicillin (7–14 per cent), cotrimoxazole (7–33 per cent) and chloramphenicol (1–37 per cent) has been reported from South Africa, Pakistan, CAR, and Zambia.[8,21] SP resistance to penicillin (43 per cent), cotrimoxazole (46 per cent) and chloramphenicol (9 per cent) was reported from the USA.[22] HI resistance to cotrimoxazole (0–26 per cent), the higher figure occurring in tuberculosis-infected children in South Africa, and to chloramphenicol (0–36 per cent) has been reported from the Philippines, Papua New-Guinea, Thailand, and Pakistan.[20,23,24] We found no HI strains resistant to ampicillin (universal or near universal susceptibility of HI isolates to ampicillin has been reported in most developing countries). These countries reported a low percentage of β-lactamase positive HI strains when compared with developed countries. It has been reported that HI infections in children with tuberculosis showed a high degree of *in vitro* resistance to conventional ARI treatment.[20] Our unique case of tuberculosis showed SP and HI isolates fully susceptible. Rowe, *et al.*[18] reported no significant difference with regard to antimicrobial resistance between sick children with and without WHO-defined pneumonia.

We observed similar carriage and resistance percentages among children with and without ARI as defined by WHO (Table 1). Age was not a predictive factor for SP or HI colonization among our population. As in CAR,[18] perhaps another risk factor for colonization is previous exposure to antibiotics; however, our study did not include such patients.

Comorbidities associated with pneumonia (tuberculosis, malaria, HIV-AIDs, severe malnutrition) carry a high negative prognostic value in terms of outcome. Twelve WHO-defined pneumonia cases were diagnosed during the study, a number that did not allow us to test fully the correlation between *in vitro* results and *in vivo* outcome. However, it is obvious that ARI is one of the most prevalent illnesses in north-east Senegal and its related mortality is elevated.

## Conclusions

WHO guidelines have now been used for a long time and have proved their efficacy in most situations.

However ARI, and particularly pneumonia, still carry a high mortality rate in most developing countries, as was the case in this study. Cotrimoxazole is recommended as a first-line agent against pneumonia in rural Senegal because of its few side-effects, ease of administration, and low cost. However, *in vitro* resistance to cotrimoxazole, particularly in SP and (to a lesser degree) in HI, the two most prevalent pathogens recovered in half of the patients, is of concern. To establish the correlation between *in vitro* susceptibility to antibiotics and clinical outcome, using the few antimicrobials available in the Third World, would necessitate large clinical studies. Information on general health issues aimed at targeting populations, as well as intense management of ARI comorbidities, notably malnutrition, are desirable if ARI's death toll is to be reduced. Further studies using chloramphenicol, found to be highly active in this study and presently recommended only for severe ARI, must be carried out in spite of its rare, but feared, side-effects.

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