

# Antibiotic resistance of bacteria responsible of acute respiratory tract infections in children

Makhtar Camara,<sup>1</sup> Assane Dieng,<sup>1</sup> Abdoulaye Diop,<sup>1</sup> Amadou Diop,<sup>1</sup> Amadou Diop,<sup>2</sup> Djibril Boiro,<sup>3</sup> Jean Baptiste Niokhor Diouf,<sup>4</sup> Amary Fall,<sup>5</sup> Ousmane Ndiaye,<sup>3</sup> Mbayame Niang,<sup>5</sup> Cheikh Saad Bouh Boye<sup>1</sup>

<sup>1</sup>Bacteriology and Virology Laboratory, Le Dantec teaching Hospital, Dakar; <sup>2</sup>Bacteriology and Virology Laboratory, Albert Royer teaching Hospital, Dakar; <sup>3</sup>Paediatric Unit, Abass NDAO Teaching Hospital, Dakar; <sup>4</sup>Paediatric Unit, Roi Baudouin Hospital, Dakar; <sup>5</sup>Medical Virology Unit, Institute Pasteur, Dakar, Senegal

## Summary

**Background and aims.** *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* are the most common causative agents of acute respiratory tract infections (RTIs). The objective of this study was to assess their susceptibility to several antibiotics.

**Materials and methods.** A total of 58 strains (16 *S. pneumoniae*, 19 *H. influenzae* and 23 *M. catarrhalis*) were isolated from samples collected in two paediatric centres, and their

susceptibility to commonly used antibiotics tested by E-test.

**Results.** Among *H. influenzae* isolates, 10.5% were resistant to ampicillin (all  $\beta$ -lactamase-positive), and 88.9% were susceptible to cefaclor. High  $\beta$ -lactam resistance rates (penicillin: 31.3% and cephalosporins: 18.7 to 31.3%) had been observed among *S. pneumoniae* strains. Only 50% of isolates were susceptible to azithromycine. 91.3% of *M. catarrhalis* isolates  $\beta$ -lactamases producers were resistant to ampicillin while susceptible to the most tested antibiotics.

**Conclusions.** Except *M. catarrhalis*  $\beta$ -lactamases producing strains, frequency of antibiotic resistance was mainly observed among *S. pneumoniae*, and to a lesser extent among *H. influenzae* clinical isolates, suggesting the need for continuous surveillance of antimicrobial resistance patterns in the management of RTIs.

Correspondence: Makhtar Camara, CHNU Aristide Le Dantec, Cheikh Anta Diop University, Laboratory of Bacteriology-Virology, 30 Avenue Pasteur, BP 7325, Dakar, Senegal.  
Tel.: +221.33.822.5919 - Fax: +221.33.823.5368.  
E-mail: camaramakhtar@yahoo.fr

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## Introduction

Respiratory tract infections (RTIs) are the main cause of morbidity and mortality worldwide (7). Such diseases mainly involve bacteria such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. In Africa, acute respiratory infections induce 16% of death in children under 5 years old (15). Despite this high burden, there are few studies reporting updates of antimicrobial susceptibility of respiratory pathogens in Africa (8). There are limited antimicrobial surveillance data in Africa, and results from one country may not be applicable to another because of epidemiological discrepancies.

This makes surveillance of antibiotic resistance very important for guiding empirical therapy, especially in Africa, where health structures and patients must often rely on affordable first-line antibiotics that may have lost their clinical effectiveness (8).

Effective empirical therapy of bacterial diseases requires knowledge on local antimicrobial resistance (AMR) patterns since respiratory tract infection are usually treated without identification of the causal agent or its antibiotics susceptibility profile.

The objectives of this study were to identify these clin-

ical isolates, and to assess their susceptibility to several antibiotics.

## Materials and methods

### Sample collection

We analysed data from paediatric samples collected in patient aged between 1 to 60 months visiting two medical centers (Abass NDAO teaching hospital and Roi BAU-DOUIN hospital) in Dakar, Senegal.

From October 2014 to October 2015, sputum (21), bronchoalveolar lavage (19), acute otitis media effusions (01), blood (01), sinus fluids (11), and throat swab (05) samples were collected and referred to the biotechnology unit of the laboratory of bacteriology and virology of Aristide Le Dantec university teaching hospital for routine bacterial tests.

The samples were immediately cultured and the strains isolated were then identified according to the standard methods of microbiology. *H. influenzae*, *S. pneumoniae*, and *M. catarrhalis* strains were identified if the bacterial load was at least  $10^5$  CFU/mL.

### Identification of bacterial isolates

*H. influenzae* was identified by its macroscopic aspects in culture (such as the growth of tiny, moist, and smooth grey colonies) as well as metabolic characteristics: absence of haemolysis, positive catalase and oxidase tests, growth in simultaneous presence of X and V factors, satellite growth around streaks of *Staphylococcus aureus*, and other biochemical features using API NH<sup>®</sup> galleria (BioMerieux, Marcy-l'Étoile, France).

*M. catarrhalis* was identified by the presence of tiny, round, and smooth colonies; absence of haemolysis; positive catalase and oxidase tests; and others biochemical characters using API<sup>®</sup> NH galleria (BioMerieux, France).

*S. pneumoniae* was identified by the presence of tiny, round, flat, and transparent colonies with central depression (checker piece and nail head colonies); haemolysis of  $\alpha$ -viridans; negative catalase and oxidase tests; absence of bile-sculin hydrolysis; lysis by bile-salts; susceptibility to optochin; and other biochemical characters using API<sup>®</sup> Strep (BioMerieux, France).

### Antibiotic susceptibility testing

E-test method was used to study the antibiotic susceptibility of the three pathogens. Bacterial suspensions of each of the three pathogens were diluted to obtain a final concentration of  $10^5$  CFU/mL (an optical density of 0.5 on the McFarland scale) and inoculated on Haemophilus Test Medium for *H. influenzae*, on chocolate agar supplemented with Polyvitex<sup>®</sup> for *M. catarrhalis*, and on Mueller-Hinton supplemented with 5% sheep blood for *S. pneumoniae*.

Strips (E-test, bioMerieux SA, LYON) containing selected antibiotics were then placed on the inoculated plates. Plates were then incubated at 37°C under CO<sub>2</sub> atmosphere for 18-24 hours. Quality control for antimicrobial suscepti-

bility testing was performed using the ATCC 49247 strain of *H. influenzae*, ATCC 49619 strains of *S. pneumoniae*. All antibiotics displayed acceptable minimal inhibitory concentrations (MICs) values compared to the control strains.

MICs were calculated as MIC<sub>50</sub> (MIC inducing inhibition of 50% of isolates) and MIC<sub>90</sub> (MIC inducing inhibition of 90% of isolates). Percentages of susceptibilities were determined based on Clinical Laboratory Standards Institute (CLSI) break points (4).

### Beta-lactamase tests

The production of beta-lactamase has been investigated for *H. influenzae* and *M. catarrhalis* isolates using a nitro-cefin-based test (Cefinase, Becton Dickinson Microbiology Systems, and Cockeysville, MD, USA).

### Analysis of results

WHONET software (version 6) was used to analyse results of the antibacterial susceptibility test. MIC<sub>50</sub>, and MIC<sub>90</sub> were calculated.

## Results

In this study, 58 isolates (19 of *H. influenzae*, 16 *S. pneumoniae* and 23 of *M. catarrhalis*) have been identified and tested for antibiotic susceptibility.

### Antibiotic susceptibility testing of *H. influenzae*

The results of susceptibility testing for *H. influenzae* isolates are summarized in Table 1.

All *H. influenzae* isolates were susceptible to amoxicillin/clavulanic acid (MIC<sub>90</sub>=1 mg/L), cephalosporins (cefuroxim MIC<sub>90</sub>=3 mg/L, cefixim MIC<sub>90</sub>=0.094 mg/L), fluoroquinolones (ciprofloxacin: MIC<sub>90</sub>=0.094 mg/L; levofloxacin: MIC<sub>90</sub>=0.023 mg/L; ofloxacin: MIC<sub>90</sub>=0.016 mg/L), and azithromycin (MIC<sub>90</sub>=2 mg/L). However, resistance patterns to ampicillin (10.5%), cefaclor (11.2%) and /sulfamethoxazole/trimethoprim (100%) were observed. All ampicillin-resistant isolates produced  $\beta$ -lactamase.

### Antibiotic susceptibility testing of *S. pneumoniae*

Table 2 shows an overview of antibiotic susceptibility rates of *S. pneumoniae*. High  $\beta$ -lactams resistance rates had been observed among *S. pneumoniae* strains, including penicillin G (31.3%), cephalosporins (18.7 to 31.3%). All isolates were resistant to sulfamethoxazole/trimethoprim (100%). Only 50% of *S. pneumoniae* isolates were susceptible to azithromycin while all isolates were susceptible to fluoroquinolone antibiotics (levofloxacin MIC<sub>90</sub>=1 mg/L, ofloxacin MIC<sub>90</sub>=2 mg/L) and clindamycin (MIC<sub>90</sub>=0.19 mg/L).

### Antibiotic susceptibility testing of *M. catarrhalis*

All *M. catarrhalis* isolates were susceptible to amoxicillin/clavulanic acid (MIC<sub>90</sub>=0.5 mg/L) and macrolides (azithromycin MIC<sub>90</sub>=0.75 mg/L, clarithromycin MIC<sub>90</sub>=1.5 mg/L) (Table 3). Fluoroquinolones tested

showed very good activity (100% and 95.2% for levofloxacin and ciprofloxacin, respectively), with low MIC<sub>90</sub> (0.094 mg/L). Among isolates tested, 95.7% were susceptible to cefuroxim and cefaclor. However, 91.3% of *M. catarrhalis* isolates positive for  $\beta$ -lactamase were resistant to ampicillin (data not shown).

## Discussion

The results from this study indicate a low frequency (10.5%) of *H. influenzae* beta-lactamase producing strains, all resistant to ampicillin. Our results are in agreement with

**Table 1. Susceptibility of *Haemophilus influenzae* isolates.**

Antibiotics	R, %	S, %	MIC <sub>50</sub> , mg/L	MIC <sub>90</sub> , mg/L	Range, mg/L
Ampicillin	10.5	89.5	0.25	1	0.04-1.5
Amoxicillin/clavulanic acid	0	100	0.5	1.5	0.125-2
Cefuroxim	0	100	1	3	0.19-4
Cefaclor	11.2	88.9	3	16	1.5-48
Cefixim	0	100	0.047	0.094	0.016-0.38
Ciprofloxacin	0	100	0.016	0.023	0.003-0.023
Levofloxacin	0	100	0.012	0.016	0.006-0.016
Ofloxacin	0	100	0.032	0.047	0.016-0.047
Sulfamethoxazole/trimethoprim	100	0	32	32	0.75-32
Azithromycin	0	100	1	2	0.38-2
Clarithromycin	5.3	94.7	4	8	1.5-12

R, Resistant; S, Susceptible; MIC, Minimum Inhibitory Concentration.

**Table 2. Susceptibility of *Streptococcus pneumoniae* isolates.**

Antibiotics	R, %	S, %	MIC <sub>50</sub> , mg/L	MIC <sub>90</sub> , mg/L	Range, mg/L
Penicillin G	31.3	68.7	0.032	0.125	0.006-0.5
Amoxicillin/clavulanic acid	12.5	87.5	0.032	0.032	0.016-1
Cefuroxim	18.7	81.3	0.047	0.38	0.016-1.5
Cefaclor	31.3	68.7	0.75	1.5	0.094-2
Cefixim	24.5	75.5	0.38	1.5	0.125-1.5
Levofloxacin	0	100	0.5	1	0.38-1
Ofloxacin	0	100	1.5	2	1-2
Sulfamethoxazole/trimethoprim	100	0	3	32	0.064-32
Clindamycin	0	100	0.094	0.19	0.016-0.25
Azithromycin	50	50	0.5	1.5	0.016-1.5
Clarithromycin	19.7	81.3	0.064	0.125	0.016-0.125

R, Resistant; S, Susceptible; MIC, Minimum Inhibitory Concentration.

**Table 3. Susceptibility of *Moraxella catarrhalis* isolates.**

Antibiotics	R, %	S, %	MIC <sub>50</sub> , mg/L	MIC <sub>90</sub> , mg/L	Range, mg/L
Amoxicillin/clavulanic acid	0	100	0.064	0.5	0.016-3
Cefuroxim	4.3	95.7	0.75	2	0.064-12
Cefaclor	4.3	95.7	1.5	2	0.38-12
Cefixim	0	100	0.125	0.5	0.047-0.5
Ciprofloxacin	4.8	95.2	0.032	0.094	0.012-0.25
Levofloxacin	0	100	0.032	0.094	0.012-0.25
Sulfamethoxazole/trimethoprim	0	100	0.25	0.25	0.25-0.25
Azithromycin	0	100	0.064	0.75	0.023-0.75
Clarithromycin	0	100	0.047	1.5	0.023-1.5

R, Resistant; S, Susceptible; MIC, Minimum Inhibitory Concentration.

findings from a previous study conducted in Dakar (10) and in other countries such as Italia (2), Northern Taiwan (14), Cuba (13), and South Africa (5). This suggests that beta-lactamase production could be the major mechanism of antibiotic resistance for these organisms.

A high rate (100%) of *H. influenzae* resistance to cotrimoxazole was observed, contrasting therefore with results reported by Gueye et al., in Dakar in 2009 (10).

The cephalosporins, fluoroquinolones, and macrolides antimicrobial families were active and could therefore be used as alternative options in RTI treatment.

Penicillin G resistance in *S. pneumoniae* infection was observed in Africa as well as in other countries (1,6). In our study, high resistance rate (31.3%) of *S. pneumoniae* to penicillin G was observed, which is similar to data previously reported in a study conducted by Gueye and al. in Dakar (10). However, our data are not consistent with results reported in Sub-Saharan Africa in which resistance level remained below 6% overall (12). This is similar to findings from West Africa, especially in Gambia where surveillance studies have been carried out extensively and showed that only 6.6% of the isolates had intermediate resistance and none had full resistance to penicillin (3). Resistance to sulfamethoxazole/trimethoprim were detected in all isolates tested. This is in disagreement with results from a study conducted in Dakar in 2009 (10). However, increasing resistance rates to sulfamethoxazole/trimethoprim have been reported between 2003 and 2006 in the East Africa region (9).

*M. catarrhalis* isolates showed high susceptibilities towards most of the antibiotics tested, except for  $\beta$ -lactamase positive strains for which higher rate of resistance (91.3%) was observed. In our study, we observed that beta-lactamase production was the primary mechanism of ampicillin resistance for *M. catarrhalis*; all isolates that were resistant to ampicillin were beta-lactamase producer. Similar results were observed in a previous surveillance study conducted in other parts of the world (11).

## Conclusions

In summary, the results from this study indicate that none of the isolates exhibited multiple resistances patterns. Except *M. catarrhalis* ampicillin-resistant  $\beta$ -lactamases producing strains, frequency of antibiotic resistance was mainly observed among *S. pneumoniae*, and to a lesser extent among *H. influenzae* clinical isolates. Overall, cephalosporins, fluoroquinolones, and macrolides remain active on these three pathogens and could be used as alternative treatment for RTIs in association with continuous surveillance of antimicrobial resistance patterns.

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