Original Article

Antimicrobial susceptibility of select respiratory tract pathogens in Dakar, Senegal

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Abstract

Background : *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pyogenes*, and *Streptococcus pneumoniae* are the most common causative agents of respiratory tract infections (RTIs). The increase in resistance to current antibacterial agents highlights the need to monitor the resistance pattern of these bacterial pathogens.

Methodology: In this study, we assessed the antibacterial susceptibility of these pathogens causing respiratory tract infections in Dakar, Senegal, during 2007–2008. A total of 290 bacterial isolates (75 *H. influenzae*, 10 *M. catarrhalis*, 105 *S. pneumoniae*, and 100 *S. pyogenes*) were collected.

Results and Conclusions: All *H. influenzae* isolates were susceptible to amoxicillin/clavulanic acid, ofloxacin, clarithromycin, cephalosporins, and macrolides. Overall, 26.7% of *H. influenzae* isolates were completely resistant to ampicillin. Among the *M. catarrhalis* isolates, 30% were resistant to ampicillin. All the isolates of *H. influenzae* and *M. catarrhalis* that were resistant to ampicillin were beta-lactamase producing strains. Among the *S. pneumoniae* isolates, 33.3% isolates exhibited intermediate susceptibility to penicillin G, and one isolate was completely resistant. All five isolates that were resistant to erythromycin expressed the M phenotype. *S. pyogenes* exhibited high susceptibility to all other antibiotics, except tetracycline. Our study suggests that except for *M. catarrhalis*, all other bacterial isolates are susceptible to cephalosporins, macrolides, and fluroquinolones.

Keywords : *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, susceptibility

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Introduction

Respiratory tract infections (RTIs) such as acute otitis media, sinusitis, bronchitis, tongillonpharyngitis, and community-acquired pneumonia are major causes of morbidity and mortality worldwide [1]. *Haemophilus influenzae*, *Moraxella catarrhalis*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* are the common causative pathogens for these RTIs. Oral penicillins are not active against beta-lactamase producing pathogens such as *H. influenzae* and *M. catarrhalis*. Although macrolides are useful alternatives for beta-lactamase producing bacteria, common respiratory pathogens have developed increased resistance to macrolides, [2,3,4]. Similarly, resistance to fluoroquinolones is also emerging rapidly [5].

The three major RTI surveillance studies—The Alexander Project, PROTEKT, and the RTI component of SENTRY—presented the impact of antimicrobial resistance in different parts of the world [6,7,8]. The data from these studies revealed that antibacterial resistance showed significant geographical heterogeneity. Although these studies provide significant data on global changes in the resistance pattern, none of these studies was conducted in West Africa. The PALM (Pan African Link through Microbiology) Project, which has been initiated by Smith Kline Beecham, conducted multi-centric surveillance of antibiotic resistance in nine African countries, namely Kenya (East Africa), Cameroon (Central Africa), Nigeria, Senegal and Cote d 'Ivoire (West Africa), Morocco, 1'Algérie, and Tunisia (North Africa), and Malta [9]. However, very little data has been generated through this network [10]. Therefore, in this study, we aim to determine the antibacterial susceptibility of *H. influenzae*, *M. catarrhalis*, *S. pneumoniae*, and *S. pyogenes* in Dakar, the capital city of Senegal, during 2007–2008.

Material and Methods

Sample collection

We analysed data from patients who refer to our laboratory from three medical centres in Dakar, Senegal (the Departments of Paediatrics and ORL of the University Hospital of Aristide Le Dantec, the Pneumology Department of the University Hospital of Fann, and a private medical setting). These data, collected between May 2007 and May 2008, include 290 isolates (75 of *H. influenzae*, 10 of *M. catarrhalis*, 105 *S. pneumoniae,* and 100 of *S. pyogenes*). The clinical samples examined were sputum, bronchoalveolar lavage, acute otitis media effusions, blood, pus swab, sinus fluids, and throat swab. These samples were collected from patients with upper respiratory tract infections (acute otitis media, sinusitis, and tongillopharyngitis) or lower respiratory tract infections (community-acquired pneumonia and acute bronchitis). These samples were sent to the biotechnology unit of the Bacteriology and Virology Laboratory of Dantec Teaching Hospital where they were immediately cultured. The strains isolated were then identified according to the standard methods of microbiology.

Identification of bacterial isolates

H. influenzae was identified by the presence of tiny, moist, and smooth gray colonies; absence of hemolysis; positive catalase and oxidase tests; presence of growth factors X and V; satellite growth around streaks of *Staphylococcus aureus*; and other biochemical characters (using API NH® galleria, BioMérieux, La Balme-les-Grottes, France).

M. catarrhalis was identified by the presence of tiny, round, and smooth colonies; absence of hemolysis; positive catalase and oxidase tests; and others biochemical characters (using API® NH galleria, BioMérieux, La Balme-les-Grottes, France).

S. pneumoniae was identified by the presence of tiny, round, flat, and transparent colonies with central depression (checker piece and nail head colonies); hemolysis of α-viridans; negative catalase and oxidase test; absence of bile-esculin hydrolysis; lysis by bile-salts; susceptibility to optochin; and other biochemical characters (using API® Strep BioMérieux, La Balme-les-Grottes, France).

S. pyogenes was identified by the presence of thin and smooth colonies appearing as Gram-positive cocci grouped into chains; negative catalase test; showing growth inhibition around a disc containing 0.04 units of Bacitracin; and other tests conducted using (using API® Strep BioMérieux, La Balme-les-Grottes, France).

Antibiotic susceptibility testing

Susceptibility of each isolate of all four pathogens to twelve antibiotics was analyzed using both standard agar disc diffusion method and E-test. Bacterial suspensions were diluted to obtain a final concentration of 10^5 CFU/ml (an optical density of 0.5 on the McFarland scale). *H. influenzae* suspension was inoculated on Haemophilus test medium; *M. catarrhalis* on chocolate agar supplemented with Polyvitex®; *S. pneumonia* on Mueller-Hinton supplemented with 5% sheep blood; and *S. pyogenes* on Mueller-Hinton supplemented with 5% horse blood. Discs (diffusion method) or strips (E-test) containing selected antibiotics were then placed on the inoculated plates. These plates were then incubated at 37°C in a CO_2 atmosphere for 18–24 hours. Quality control strains used for antimicrobial susceptibility testing were the ATCC 49247 strain of *H. influenzae*, ATCC 49619 strains of *S. pneumoniae*, and the ATCC 29213 strain of *S. pyogenes*. All antibiotics demonstrated acceptable MICs values toward the control strains.

Minimal inhibitory concentrations (MICs) was calculated as MIC_{50} (MIC causing inhibition of 50% of isolates) and MIC_{90} (MIC causing inhibition of 90% of isolates). Percentage susceptibilities were calculated based on Clinical Laboratory Standards Institute (CLSI) break points [11].

Beta-lactamase tests

The *H. influenzae* and *M. catarrhalis* isolates were examined for production of beta-lactamase using a nitrocefin-based test (Cefinase, Becton Dickinson Microbiology Systems, Cockeysville, Md).

Erythromycin and clindamycin double-disc diffusion test

The test was performed to identify the erythromycin resistant phenotype. On a blood agar plate, an erythromycin disc (15 µg) was placed 20 mm from the centre of a disc containing 10 µg of clindamycin. Blunting of the clindamycin inhibition zone proximal to the erythromycin disc indicated an inducible resistant phenotype. Susceptibility only to clindamycin with no blunting indicated the M-phenotype. Resistance to both erythromycin and clindamycin indicated constitutive resistance.

Table 1. Numbers of isolates of *H. influenzae*, *M. catarrhalis*, *S. pneumoniae* and *S. pyogenes*, grouped according to specimen type, gender and age during the period 2007–2008.

	H. influenzae **(n = 75)**	***M catarrhalis*** **(n = 10)**	***S. pneumoniae*** **(n = 105)**	***S. pyogenes*** **(n = 100)**
Specimen				
Aom	4	-	20	-
Bal	39	-	48	-
Blood	-	-	10	-
Pus swab	-	-	2	-
Sputum	30	10	25	-
Sinus fluids	2	-	-	-
Throat swab	-	-	-	100
Gender				
Female	38	5	37	29
Male	37	5	68	71
Age groups (years)				
<1	-	7	3	-
4 -	-	-	4	-
6-9	3	-	4	-
10-14	5	-	5	11
15-29	49	3	48	53
≥30	18	-	41	36

Aom = Acute otitis media effusions, Bal = Bronchoalveolar lavage

Analysis of results

WHONET software was used to analyze the antibacterial susceptibility test results [12].

Results

Patient demographics

Except for *M. catarrhalis* isolates, most of the respiratory bacteria isolates included in this study were obtained from patients between 15–29 years of age (Table 1). The most frequent sources of bacterial isolates were bronchoalveolar lavage and sputum for *H. influenzae* and bronchoalveolar lavage for *S. pneumoniae*. All *M. catarrhalis* isolates were obtained from sputum, and all *S. pyogenes* isolates were collected from throat swabs.

Antibiotic susceptibility testing

H. influenzae

The results of susceptibility testing for *H. influenzae isolates* are summarised in Table 2. All *H. influenzae* isolates were susceptible to amoxicillin/clavulanic acid (MIC_{90} = 0.5 mg/L), cephalosporins (MIC_{90} = 2 mg/L), macrolides (azithromycin:MIC_{90} = 2 mg/L; clarithromycin: MIC_{90} = 8 mg/L), and ofloxacin (MIC_{90} =2 mg/L). Susceptibility to cotrimoxazole (MIC_{90} = 16 mg/L) and chloramphenicol (MIC_{90} = 6 mg/L) was observed for 68% and 82.7% of isolates, respectively. Most of the isolates (98.7%) were resistant to tetracycline. Overall, 26.7% of isolates were fully resistant to ampicillin (MIC_{90} = 16 mg/L), and 1.3% isolates exhibited intermediate susceptibility. Beta-lactamase was produced by all the isolates that were resistant to ampicillin.

M. catarrhalis

Among all isolates tested, 70% (7 isolates out of 10) were susceptible to ampicillin (MIC_{90} = 48 mg/L). These three strains that were resistant to ampiciilin were beta-lactamase producing strains. Although 87.5% of isolates were susceptible to cefaclor, the drug exhibited high MIC_{90} of 256 mg/L. Among the macrolides tested, 87.5% isolates were susceptible to azithromycin and 62.5% were susceptible to clarithromycin. However, azithromycin demonstrated higher efficacy (MIC_{90} = 8 mg/L) than clarithromycin (MIC_{90} = 24 mg/L). Only 50% isolates were susceptible to cotrimoxazole (Table 2).

S. pneumoniae

Table 3 shows antibiotic susceptibility rates of *S. pneumoniae*. Of all isolates, only one isolate was completely resistant to penicillin G and 35 isolates (33.3%) exhibited intermediate susceptibility. Cephalosporins had MIC_{90} of 0.38 mg/L. Furthermore, most of the isolates (99%) demonstrated high susceptibility towards cephalosporins. Overall, 49.5% and 41.9% of isolates were susceptible to

Table 2. *In-vitro* activities of antibiotics against *H. influenzae* and *M. catarrhalis* isolates.

***H. influenzae* (n = 75)**							
	Percentages of isolates			**MICs (mg/L)**		**Breakpoints**	
Antibiotic name	**R**	**I**	**S**	**MIC_{50}**	**MIC_{90}**	**S**	**R**
			E-test				
Ampicillin (E)	26.7	1.3	72	0.5	16	≤1	≥4
AmoxClavulanic acid(E)	0	0	100	0.125	.5	≤4/2	≥8/4
Cefuroxime(E)	0	0	100	0.25	2	≤4	≥16
Cefaclor(E)	0	0	100	0.094	2	≤8	≥32
Ofloxacin(E)	0	0	100	0.5	2	≤2	-
Azithromycin (E)	0	0	100	1	2	≤4	-
Clarithromycin (E)	0	0	100	4	8	≤8	≥32
			Disc-diffusion test				
Cotrimoxazole(D)	8	24	68	-	-	≥16	≤10
Chloramphenicol (D)	5.3	12	82.7	-	-	≥29	≤25
Tetracycline (D)	98.7	1.3	0	-	-	≥29	≤25
Beta-lactamase + (%)	23.7					-	-
***M. catarrhalis* (n = 10)**							
	Percentages of isolates			**MICs (mg/L)**		**Breakpoints**	
Antibiotic name	**R**	**I**	**S**	**MIC_{50}**	**MIC_{90}**	**S**	**R**
			E-test				
Ampicillin (E)	30	0	70	0.75	48	≤8	≥32
AmoxClavulanic acid (E)	0	0	100	0.25	1.5	≤8/4	≥32/16
Cefuroxime (E)	0	14.3	85.7	1.5	8	≤8	≥64
Cefaclor (E)	12.5	0	87.5	0.75	256	≤8	≥32
Cefotaxime (E)	0	0	100	0.125	4	≤8	≥64
Ofloxacin (E)	0	12.5	87.5	0.094	3	≤2	≥8
Azithromycin (E)	12.5	0	87.5	0.75	8	≤8	≥32
Clarithromycin (E)	37.5	0	62.5	2	24	≤2	≥8
			Disc-diffusion test				
Cotrimoxazole (D)	30	20	50	-	-	≤16	≥10
Chloramphenicol (D)	0	0	100	-	-	≤8	≥32
Tetracycline (D)	0	30	70	-	-	≤19	≥14
Beta-lactamase + (%)	30						

R: Resistant; S: Susceptible; I: Intermediate susceptible

cotrimoxazole and tetracycline, respectively. Only 3.8% of isolates were completely resistant to erythromycin and all were of the M phenotype (erythromycin-resistant, clindamycin susceptible). Among the macrolides tested, azithromycin was more active (MIC_{90} = 0.23 mg/L) with only 1% of isolates exhibiting intermediate susceptibility to it. Rate of resistance to clarithromycin, erythromycin, and tetracycline was slightly higher among isolates with intermediate susceptibility to penicillin G (8.6%, 5.7%, 5.7%, and 31.4% respectively) as compared to those that were completely susceptible (2.9%, 2.9%, and 26.1%, respectively; data not shown) However, none of these differences was statistically significant (p value > 0.05).

S. pyogenes

Only one percent of the isolates showed intermediate susceptibility to erythromycin, clindamycin, and azithromycin. Most of the isolates were completely susceptible to all antibiotics tested, except tetracycline. All isolates were fully resistant to tetracycline. Among the cephalosporins tested, cefpodoxime was the most active with an MIC_{90} of 0.16 mg/L (Table 3).

Table 3: *In-vitro* activities of antibiotics against *S. pneumoniae* and *S. pyogenes* isolates.

***S. pneumoniae* (n = 105)**							
	Percentages of isolates			**MICs (mg/L)**		**Breakpoints**	
Antibiotic name	**R**	**I**	**S**	**MIC_{50}**	**MIC_{90}**	**S**	**R**
E-test							
Penicillin G (E)	1	33.3	65.7	0.032	0.5	≤0.064	≥2
AmoxClavulanic acid (E)	0	0	100	0.016	0.032	≤2/1	≥8/4
Cefuroxime (E)	0	1	99	0.064	0.38	≤0.5	≥ 2
Cefaclor (E)	0	1	99	0.19	0.38	≤1	≥4
Azithromycin (E)	0	1	99	0.023	0.064	≤0.5	≥2
Clarithromycin (E)	4.8	2.9	92.4	0.032	0.19	≤0.25	≥1
Ofloxacin (E)	0	0	100	0.38	1.5	≤2	≥8
Disc-diffusion test							
Erythromycin (D)	3.8	4.8	91.4	-	-	≥21	≤15
Clindamycin (D)	0	1.9	98.1	-	-	≥19	≤15
Cotrimoxazole (D)	21	29.5	49.5	-	-	≥19	≤15
Chloramphenicol (D)	5.7	0	94.3	-	-	≥23	≤18
Tetracycline (D)	28.6	29.5	41.9	-	-	≥23	≤18
***S. pyogenes* (n = 100)**							
	Percentages of isolates			**MICs (mg/L)**		**Break points**	
Antibiotic name	**R**	**I**	**S**	**MIC_{50}**	**MIC_{90}**	**S**	**R**
E-test							
Penicillin G (E)	0	0	100	0.016	0.023	≤0.12	≥0.25
Cefixime (E)	0	0	100	0.094	0.094	≤1	≥4
Cefpodoxime (E)	0	0	100	0.016	0.016	≤2	≥8
Cefotaxime (E)	0	0	100	0.023	0.023	≤0.5	≥1
Erythromycin (E)	0	1	99	0.094	0.125	≤0.25	≥1
Azithromycin (E)	0	1	99	0.38	0.5	≤0.5	≥2
Chloramphenicol (E)	0	0	100	3	4	≤4	≥16
Teicoplanin (E)	0	0	100	0.094	0.094	≤4	≥16
Levofloxacin (E)	0	0	100	0.75	0.075	≤2	≥8
Disc-diffusion test							
Clindamycin (D)	0	1	99	-	-	≥19	≤15
Tetracycline (D)	100	0	0	-	-	≥23	≤18
Amoxicillin (D)	0	0	100	-	-	≥21	≤14

R: Resistant; S: Susceptible; I: Intermediate susceptible

Discussion

The results from this study indicate that a high percentage of *S. pneumoniae* isolates had intermediate susceptibility to penicillin G. All bacteria, except for *M. catarrhalis*, remain susceptible to cephalosporins, fluoroquinolones, and macrolides. For *H. influenzae* and *M. catarrhalis*, beta-lactamase production was the primary reason for the high rates of resistance associated with ampicillin. None of the isolates exhibited multiple resistance. Thus, the antimicrobial class of cephalosporins, fluoroquinolones, and macrolides were useful options to treat RTI.

Overall, 26.7% of *H. influenzae* isolates were resistant to ampicillin. Our results concur with findings from a study conducted on meningitis in the Paediatric Department of Fann Hospital in Dakar [13], and also with studies conducted in other countries in Africa [14-20]. However, this resistance rate was three times higher than the recently reported rate of

9% in a study conducted on meningitis in the same Paediatric Department of the University Hospital of Fann [21].

M. catarrhalis isolates had high susceptibilities towards most of the antibiotics tested. In our study, we observed that beta-lactamase production was the primary mechanism of ampicillin resistance for *H. influenzae*; and *M. catarrhalis;* all isolates that were resistant to ampicillin produced beta-lactamase. The same results were observed in a previous surveillance study conducted in other parts of the world [22]. This proves that beta-lactamase production could be the major mechanism of antibiotic resistance these organisms.

As with other countries, penicillin G resistance in *S. pneumoniae* infections has been reported in Africa as well [2,10]. In the present study, 34.3% of all *S. pneumoniae* isolates were resistant to penicillin G. These rates were lower than those previously reported in a study conducted by Benbachir *et al*. in which 8.6% of pneumococcal isolates developed complete resistance and 53.1% developed intermediate susceptibility towards penicillin G [10]. However, our findings are in line with a study conducted by Camara *et al*. which reported that 1% of all pneumococcal isolates recovered from paediatric patients (0-72 months) were fully resistant to penicillin G [23]. Benbachir *et al*. observed that resistance to other antibiotics (erythromycin, chloramphenicol and cotrimoxazole) is more frequent among pneumococcal isolates with intermediate susceptibility to penicillin G than in susceptible isolates [10]. In our study, differences between penicillin G susceptible and non-susceptible isolates were noted; however, these differences were not statistically significant.

In this study, none of the *S. pyogenes* was resistant to penicillin G. This finding is consistent with the finding from studies conducted in other parts of the world [24,25,26]. Our results confirm the usefulness of penicillin G in treating streptococcal infections. Our study suggests that treatment with macrolides is a suitable alternative for patients allergic to penicillin, as *S. pyogenes* are susceptible to most of the macrolides. However, studies conducted in several countries showed a wide heterogeneity of resistance to macrolides [3,27]. Levofloxacin demonstrated high activity with a 100% susceptibility rate. Our data is consistent with the finding from a study conducted in the United States, which reported less than 1% resistance rate towards levofloxacin [28]. Thus, levofloxacin appears to be an alternative for the treatment of streptococcal infections in case of penicillin allergy and resistance to macrolides.

In summary, most of the bacterial isolates, remain susceptible to cephalosporins, fluoroquinolones, and macrolides. Beta-lactamase production was the primary reason for the high rates of resistance associated with ampicillin for *H. influenzae* and *M. catarrhalis*. The study provides important data, which can help guide physicians in Dakar to choose the appropriate treatment regimen for RTI. This study does not represent a surveillance study for other parts of Senegal, since antibiotic resistance of bacterial pathogens may vary according to geographic location. Further studies in other cities in Senegal as well as in other West African countries are required in order to better clarify the antibiotic susceptibility profile of the major pathogens responsible of RTIs.

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