Antibiotic susceptibility profile of *Streptococcus pneumoniae* isolated from acute respiratory infection in Dakar: a cross sectional study

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Summary

Streptococcus pneumoniae is a pathogen causing pneumonia, meningitis, otitis and bacteraemia. Nowadays, *S. pneumoniae* is developing antibacterial resistance, particularly for those with reduced susceptibility to penicillin. The objective of this study was to assess the susceptibility profile of *S. pneumoniae* strains isolated from acute respiratory infections (ARIs) in children younger than 5 years of age in Dakar, Senegal. *S. pneumoniae* strains were isolated from broncho-alveolar lavages (BALs), nasopharyngeal swabs, and middle ear secretion from children in the Paediatric Department of Abass Ndao University Teaching Hospital and Paediatric Department of Roi Baudouin Hospital in Dakar, Senegal. The strains were cultivated on Columbia agar supplemented with 5% of horse blood and gentamicin (6 mg/L). Antibiotic susceptibility testing was performed using E-test method. A total of 34 strains of *S. pneumoniae* were isolated and identified in this study, among them 7 strains (20.58%) showed penicillin-resistance. Antibiotics such as amoxicillin/clavulanic acid (MIC_{90}=0.036 µg/mL), cefuroxim (MIC_{90}=0.38 µg/mL), cefixim (MIC_{90}=1.5 µg/mL), as well as macrolides (azithromycin MIC_{90}=1.5 µg/mL, clarithromycin MIC_{90}=0.125 µg/mL) and fluoroquinolone (levofloxacin MIC_{90}=1 µg/mL, ofloxacin MIC_{90}=2 µg/mL) were mostly active. However, all *S. pneumoniae* strains were resistant to sulfamethoxazole/trimethoprim (MIC_{90}: 32 µg/mL). Except of *S. pneumoniae* strains penicillin-resistance or reduced susceptibility, most strains were susceptible to β-lactams antibiotics commonly used in ARI treatment. Continuous surveillance of antimicrobial resistance patterns of pneumococcus strains is still crucial for effective control of ARIs in children.

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Introduction

Streptococcus pneumoniae is one of the most frequent causes of serious invasive infections, such as meningitis, bacteremia and pneumonia and it is the major cause of morbidity and mortality worldwide (7). Pneumonia accounts for 15% of all deaths of children younger than 5 years of age and it is the single largest infectious cause of death in children worldwide (14). In 2015, the pneumonia killed 920,000 children and is a source of distress, suffering and debilitating long-term health problems for many children (17).

Pneumonia affects children and families everywhere, but is most prevalent in South Asia and sub-Saharan Africa (24).

In Senegal, the mortality rate of children younger than 5 years of age due to pneumonia accounted for 13% of the total rate (22). *S. pneumoniae* was susceptible to many antibiotics including the β-lactams, macrolides, fluoroquinolones and vancomycin (12). However, acquired resistance has emerged in recent decades, especially against penicillin and macrolides. The emergence of *S. pneumoniae* strains resistant not only to penicillin, but also to

other antibiotics, has been reported in the last two decades worldwide and can pose a serious public health challenge (13). Antimicrobial resistance of *S. pneumoniae* is not only a local but also a global problem (11).

The objective of this study was to assess the susceptibility profile of *S. pneumoniae* strains isolated from ARI's cases in children younger than 5 years of age in Dakar.

Materials and Methods

Isolation and culture of *S. pneumoniae*

S. pneumoniae strains were isolated between January 2015 and December 2016, from the Paediatric Department of Abass Ndao University Teaching Hospital and Paediatric Department of Roi Baudouin Hospital in Dakar (Senegal). The strains were isolated from broncho-alveolar lavages (BAL), nasopharyngeal swabs and middle ear secretion in children younger than 5 years of age.

Samples were cultivated using Columbia agar supplemented with 5% of horse blood and gentamicin (6 mg/l) (Biorad, Hercules, California, USA).

Identification of the isolated strains

S. pneumoniae strains were identified if bacterial load was at least 10^5 CFU/mL.

The identification method was performed using morphology characters, optochin susceptibility, bile solubility and the presence of capsule (10).

Antibiotic susceptibility testing

The antibiotic susceptibility was carried out using strips E-Test® (bioMerieux SA, Lyon) and minimum inhibitory concentration (MIC) was determined according European Committee of Antimicrobial Susceptibility Testing guideline (EUCAST) (5).

Penicillin, amoxicillin/clavulanic acid, cefuroxim, cefixim, azithromycin, clarithromycin, clindamycin, levofloxacin, ofloxacin and sulfamethoxazole/trimethoprim were tested. The quality control for antimicrobial susceptibility testing was performed using the ATCC 49619 strains of *S. pneumoniae*.

Analysis of results

The geometric mean values of MIC_{50} and MIC_{90} obtained from the antibiotic susceptibility testing were calculated and analyzed using the Whonet 5.6 software (WHO Collaborating Centre for Surveillance of Antimicrobial Resistance, Boston, Massachusetts).

Results

A total of 34 strains of *S. pneumoniae* were isolated and identified from children under 5 years of age. These strains of *S. pneumoniae* were tested for antibiotics susceptibility.

Antibiotic susceptibility testing

Table 1 summarizes the results of the susceptibility testing of *S. pneumoniae* to commonly used antibiotics in ARIs treatment.

Susceptibility to β-lactam antibiotics

β-lactams were the antimicrobial class mostly active towards *S. pneumoniae;* 20.58% tested strains showed penicillin G resistance (MIC_{90}: 0.125 µg/mL), while amoxicillin and clavulanic acid (AMC) showed a high activity with a very low MIC_{90} (0.032 µg/mL). In addition, a high cephalosporin activity (97.05%) was observed (cefuroxime MIC_{90}: 0.38 µg/mL and cefixim MIC_{90}: 1.5 µg/mL).

Susceptibility to macrolides

Clarithromycin (MIC_{90}: 0.125 µg/mL) and azithromycin (MIC_{90}: 1.5 µg/mL) showed activity rates against *S. pneumoniae* (82.35% and 73.53% respectively). Clarithromycin was the most active molecule among all tested macrolides.

Susceptibility to fluoroquinolones

Levofloxacin was active in all tested strains with lower MIC_{90} (1 µg/mL) compared to ofloxacin (MIC_{90}: 2 µg/mL).

Susceptibility to other antibiotics

Clindamycin was active in 97.0% of strains with low MIC_{90} (0.19 µg/mL). However, all isolates were resistant to sulfamethoxazole/trimethoprim.

Table 1. Susceptibility rates of *S. pneumoniae* and MIC values (E-test).

Antibiotics	MIC range	Obtained MIC ranges	Critical values (µg/mL) R	Critical values (µg/mL) S	R (%)	S (%)	MIC_{50}	MIC_{90}
Penicillin G	0.002-32	0.03-16	R≥2	S≤0.064	20.58	79.42	0.032	0.125
Amoxicillin/clavulanic acid	0.016-256	0.016-16	R≥8	S≤2	2.95	97.05	.016	0.032
Cefuroxim	0.016-256	0.016-48	R≥4	S≤1	2.95	97.05	0.047	0.38
Cefixim	0.016-256	0.094-256	R≥4	S≤1	2.95	97.05	0.38	1.5
Levofloxacin	0.002-32	0.19-2	R≥8	S≤2	0	100	0.5	1
Ofloxacin	0.002-32	1-16	R≥8	S≤2	23.53	76.47	1.5	2
Sulfamethoxazole/Trimethoprim	0.016-256	8-256	R≥4	S≤.5	100	0	3	32
Clindamycin	0.016-256	0.032-1	R≥1	S≤0.25	2.95	97.05	0.094	0.19
Azithromycin	0.016-256	0.094-32	R≥2	S≤0.5	26.47	73.53	0.5	1.5
Clarithromycin	0.016-256	0.094-3	R≥1	S≤.25	17.65	82.35	0.064	0.125

S: susceptibility; R: resistance; MIC: Minimum Inhibitory Concentration; MIC_{50}: Minimum Inhibitory Concentration at which 50% of the strains are inhibited; MIC_{90}: Minimum Inhibitory Concentration at which 90% of the strains are inhibited.

Discussion

In our study, antibiotic susceptibility patterns of *S. pneumoniae* strains isolated in children younger than 5 years of age with ARIs was assessed. Antimicrobial resistance patterns have been detected with antibiotics used in the clinical practice including β-lactams, macrolides, fluoroquinolone and sulfamethoxazole/trimethoprim.

The emergence of high-level penicillin-resistance of S. *pneumoniae* strains complicated seriously infections treatment in the recent years (2).

In our study, 20.58% of *S. pneumoniae* strains showed resistance to penicillin G.

A similar result was reported from an antibiotic resistance surveillance study carried out between 2002 and 2003 in Turkey (19) showing 25.3% of penicillin reduced susceptibility rate. Higher prevalence rates of penicillin G resistance were reported from studies performed in Senegal in 2009 (31.3 %) and in 2017 (33.3%) (3,6).

Penicillin resistance rates vary widely between African countries, with 57% prevalence rates in Algeria (6), 48.5% in Morocco (4), 50.4% in Tunisia (21), 0.5% in South Africa (20), 12% in Ghana (8).

In our study, amoxicillin/clavulanic acid (MIC_{50}: 0.016 µg/mL and MIC_{90}: 0.032 µg/mL) and levofloxacin (MIC_{50}: 0.5µg/mL and MIC_{90}: 1 µg/mL) were the most active antibiotic against *S. pneumoniae*.

According a study performed in Turkey in 2007, AMC was the most active antibiotic tested against *S. pneumoniae* (98.7%), following by cephalosporins cefprozil (90.6%) and cefaclor (78.7%) (18). In our study, 97.05% of activity were observed with cefuroxim (MIC_{90}: 0.38µg/mL) and cefixim (MIC_{90}: 1.5 µg/mL).

Our results are in agreement with data reported in 2017, showing 81.3% and 75.5% activity of cefuroxim and cefixim, respectively (3). Ceftaroline fosamil is a broad-spectrum cephalosporin antibiotic with activity against many bacteria, including *S. pneumoniae* (both penicillin-non-susceptible and multi-drug-resistant strains) (14).

In our study, susceptibility to fluoroquinolones showed a full activity of levofloxacin (100%) with low MIC_{90} (1 µg/mL) and ofloxacin activity was 76.47% with MIC_{90} equal to 2 µg/mL.

Fluoroquinolones were associated with higher success of treatment for severe cases of pneumonia (23). This is similar to results reported from study performed in Dakar in 2017 showing 100% of susceptibility to levofloxacin (3). Fluoroquinolones demonstrated extremely excellent activity against *S. pneumoniae* in China (25). Ciprofloxacin and moxifloxacin were more active than other fluoroquinolones (16). The worldwide prevalence of *S. pneumoniae* fluoroquinolones resistance is low, although it varies over time, geographic region, age group, and origin of isolate (1).

Therefore, fluoroquinolones could be used as alternative choice in the treatment of *S. pneumoniae* infections (25).

Macrolide resistance frequency varies from a country to another, and a worldwide increase is observed: from 8% to 22% over a 10-year period in Canada and from 7.4% to 53.7% over a 20-year period in Greece (15). In our study, clarithromycin showed good activity with 82.35% of isolates susceptible, while azithromycin was active in 73.53% of the strains tested with high MIC_{90} (1.5 µg/mL). In Tunisia, the rates of reduced susceptibility to penicillin, and erythromycin and tetracycline resistance were 60.2%, 68.1% and 38.4%, respectively (15). In China, macrolides including azithromycin, clarithromycin and erythromycin showed lowest activity both against penicillin-non-susceptible *Streptococcus pneumoniae* (PNSSP) and penicillin-susceptible *Streptococcus pneumoniae* (PSSP) strains, with very high MIC_{90} values (>256 mg/L) (25). Increased macrolides resistant strains could be related to the fact that the resistance to penicillin is generally associated with resistance to other antibiotic families, including macrolides, sulfamethoxazole/trimethoprim, and fluoroquinolones (9).

In our study clindamycin showed very good activity with 97.05% of isolates susceptible; a similar result was obtained in Dakar in 2009 (6). In 2015, a study carried out in the Sarajevo Canton showed high clindamycin and erythromycin resistance (11).

Resistance to sulfamethoxazole/trimethoprim was detected in all isolates tested in our study; this is in agreement with results reported in 2017 in Dakar (3).

Conclusions

Except penicillin-resistance, most of *S. pneumoniae* strains remained susceptible to β-lactams mainly used as primary treatment for ARIs. Fluoroquinolones and macrolides showed good activities and could be used as alternative antibiotic therapy.

Continuous surveillance of *S. pneumoniae* susceptibility patterns is required for a better management and prevention of ARIs, particularly in children younger than 5 years of age.

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