Antibiotic Susceptibility of *Streptococcus Pyogenes* Isolated from Respiratory Tract Infections in Dakar, Senegal

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**ABSTRACT:** Group A *Streptococcus* (GAS) is one of the major causes of respiratory tract infections. The objectives of this study were to identify isolates of *S. pyogenes* obtained from respiratory tract infections, and to assess their susceptibility to several antibiotics. A total of 40 strains were isolated and their susceptibility to 17 antibiotics was tested using a standard disk diffusion method. The minimum inhibitory concentrations (MICs) were determined using the E-test. All isolates were sensitive to β-lactam antibiotics including penicillin, amoxicillin, and cephalosporins. Macrolides remain active with the exception of spiramycin, which showed reduced susceptibility. Out of the 40 isolates, 100% of the isolates were resistant to tetracycline. Interestingly, isolates were sensitive to chloramphenicol, teicoplanin, vancomycin, and levofloxacin, providing potential alternative choices of treatment against infections with *S. pyogenes*.

**KEYWORDS:** *Streptococcus pyogenes*, antibiotic susceptibility, respiratory tract infections

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**Introduction**

Respiratory tract infections, such as acute sinusitis, acute otitis media, pharyngitis, community-acquired pneumonia, and acute bronchitis, are widespread and represent a major health concern particularly in low-resource settings. In developing countries, they contribute significantly to morbidity and mortality in the pediatric setting, with an estimated lethality rate of 15% in young children.¹

Group A *Streptococcus* (GAS), or *S. pyogenes*, is one of the major causes of acute respiratory tract infections. This pathogen can lead to severe invasive diseases, including pharyngitis and pyoderma, and to autoimmune post-streptococcal sequelae, such as rheumatic fever (RF) and glomerulonephritis.²

Recently, the increase in the incidence of antibiotic-resistant clinical isolates of *S. pyogenes* underscores the need for continuous surveillance of antimicrobial resistance patterns.³⁻⁵

As in developed countries, studies have been initiated in Dakar to monitor the development of resistance of *S. pyogenes* to current antibiotics. The specific objectives of this study are to identify *S. pyogenes* isolates from respiratory tract infections and to study their susceptibility pattern to 17 widely used antimicrobial agents.

**Materials and Methods**

*S. pyogenes* isolates. From November 2008 to April 2009, clinical specimens from sputum, bronchoalveolar lavage, middle ear, throat swap, and sinus fluids were collected from patients with upper respiratory tract infections (sinusitis, acute otitis media, and pharyngitis) or lower respiratory tract infections (community-acquired pneumonia, acute bronchitis) in Aristide Le Dantec university hospital in Dakar, Senegal. The samples were analyzed as previously described.⁶ Briefly, samples were cultured on agar tryptase-soya supplemented with 5% sheep blood and incubated in 5% CO₂ for 24 to 48 hours at 37°C. *S. pyogenes* strains were phenotypically identified by bacteriological characteristics (including beta hemolysis, Gram-positive cocci grouped into chains, catalase-negative, and growth...
inhibition around a disk containing 0.04 units of bacitracin). S. pyogenes identification was confirmed with a positive latex agglutination group A antigen using streptococcal group B kit (SlideX Strepto A®, BioRad).

**Antibiotic susceptibility testing.** The antimicrobial susceptibility profile of seventeen antibiotics belonging to 9 classes, including β-lactams (penicillin G 10 µg, amoxicillin 25 µg, cefixime 5 µg, cefpodoxime 10 µg, cefotaxime 30 µg, and ceftriaxone 30 µg), macrolides (erythromycin 15 µg, spiramycin 100 µg, and azithromycin 15 µg), lincomasins (clindamycin 2 µg), streptogramins (pristinamycin 15 µg), ketolids (telithromycin 15 µg), fluoroquinolones (levofloxacin 5 µg), glycopeptides (teicoplanin 30 µg, and vancomycin 30 µg), phenicols (chloramphenicol 30 µg), and cyclines (tetracycline 30 µg), was performed using standard disk diffusion method (Oxoid Ltd, Basingstoke, Hampshire, UK), and the minimum inhibitory concentration (MIC) was determined for 10 antibiotics by E-test (AB Biodisk, Solna, Sweden), as described elsewhere. Briefly, bacterial suspensions at a concentration of 10^6 CFU/mL were inoculated on sheep blood Mueller-Hinton agar plates and incubated in 5% CO₂ for 24 to 48 hours at 37°C. The ATCC 29213 strain of Staphylococcus aureus was used as control. MIC endpoints and percentage susceptibility were calculated based on Clinical Laboratory Standards Institute (CLSI) break points.

**Analysis of results.** The WHONET software (version 5.4) was used to analyze the antimicrobial susceptibility test results. Mean values and standard deviation for diameter of inhibition zones, and geometric mean MICs were calculated. The results were expressed as mean values ± SD or as geometric means.

**Results**

**Antimicrobial susceptibility rates of S. pyogenes isolates.** A total of 40 strains of S. pyogenes were isolated from 15 pediatric patients (2–15 years of age) and 25 from adults (18–60 years of age). Table 1 shows the results of susceptibility testing of 40 S. pyogenes isolates against 17 antibiotics with disk diffusion method, while MIC range, geometric means, and the calculated MIC₉₀ and MIC₉₀ values of 10 antibiotics tested are shown in Table 2.

**Susceptibility to β-lactams.** The β-lactam antibiotics showed high activity with low MIC₉₀ ranging from 0.016 to 0.094 mg/L. Penicillin G remains effective with an MIC₉₀ value of 0.023 mg/L, although two strains showed intermediate susceptibility to this molecule. All isolates were found to be susceptible to amoxicillin, cefixime, cefpodoxime, cefotaxime, and ceftriaxone.

**Susceptibility to macrolides, lincosamins, streptogramins-B, and ketolids (MLSB K).** Erythromycin showed good activity with 97.5% of isolates displaying susceptibility and only 2.5% with intermediate susceptibility. Azithromycin remains fully active as all 40 isolates are completely susceptible. Erythromycin and azithromycin had MIC₉₀ values of 0.0125 mg/L and 0.5 mg/L, respectively. In contrast, more than half of the isolates were resistant to spiramycin with 37.5% of resistance and 40% intermediate susceptibility. Clindamycin showed high activity with 97.4% of the strains susceptible. Only 2.5% of the isolates were resistant to pristinamycin. 92.5% of the strains were susceptible to telithromycin, and 7.5% showed reduced susceptibility.

**Susceptibility to chloramphenicol.** Chloramphenicol was sensitive in 82.1% of isolates with the disk diffusion method, and fully sensitive with by the E-test method with MIC₉₀ value of 4 mg/L.

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**Table 1. Susceptibility rates of Streptococcus pyogenes (Disk diffusion).**

| ANTIBIOTICS       | MEAN VALUES ± SD (mm) | R (%) | I (%) | S (%) |
|-------------------|-----------------------|-------|-------|-------|
| **β-lactams**     |                       |       |       |       |
| Penicillin G      | 30.43 ± 2.3            | 0     | 5     | 95    |
| Amoxicillin       | 30.93 ± 2.9            | 0     | 0     | 100   |
| Cefixime          | 23.7 ± 2.7             | 0     | 0     | 100   |
| Cefpodoxime       | 28.5 ± 3.2             | 0     | 0     | 100   |
| Cefotaxime        | 28.73 ± 2.6            | 0     | 0     | 100   |
| Ceftriaxone       | 29.43 ± 2.9            | 0     | 0     | 100   |
| **Macrolides**    |                       |       |       |       |
| Erythromycin      | 24.7 ± 2.1             | 0     | 2.5   | 97.5  |
| Spiramycin        | 20.33 ± 3.2            | 37.5  | 40    | 22.5  |
| Azithromycin      | 20.36 ± 1.66           | 0     | 0     | 100   |
| **Lincomasines**  |                       |       |       |       |
| Clindamycin       | 21.64 ± 2.45           | 0     | 2.6   | 97.4  |
| **Streptogramins**|                       |       |       |       |
| Pristinamycin     | 25.53 ± 2.55           | 2.5   | 97.5  |
| **Ketolides**     |                       |       |       |       |
| Telithromycin     | 25.28 ± 2.34           | 0     | 7.5   | 92.5  |
| **Phenicols**     |                       |       |       |       |
| Chloramphenicol   | 24.03 ± 2.85           | 0     | 17.9  | 82.1  |
| **Glycopeptides** |                       |       |       |       |
| Teicoplanin       | 18.28 ± 2.72           | 0     | 0     | 100   |
| Vancomycin        | 18.85 ± 1.73           | 0     | 0     | 100   |
| **Fluoroquinolones** |                 |       |       |       |
| Levofloxacin      | 19.05 ± 1.65           | 0     | 0     | 100   |
| **Tetracyclines** |                       |       |       |       |
| Tetracycline      | 9.53 ± 2.36            | 100   | 0     | 0     |

*Susceptibility rates have been interpreted according to CLSI breakpoints. Abbreviations: R, resistant; I, intermediate; S, susceptible.*
Our results are in agreement with data reported in Central, Eastern, and Baltic European countries,12 in Turkey,13 as well as in Nepal,14 where no resistance to β-lactams among S. pyogenes isolates has been detected. The advantage of amoxicillin compared to penicillin G is the presence of an OH-radical on the latter, which confers good bioavailability and improves stability and gastrointestinal absorption. On the other hand, cephalosporins are often recommended in patients with penicillin therapy failure to eradicate β-lactamase producing organisms in the pharynx.11

Susceptibility to MLSB. Erythromycin and other macrolides were recommended as initial alternate choices for patients who are allergic to penicillin.15 No erythromycin-S. pyogenes resistance was detected in this study. Only 2.5% of all strains presented an intermediate susceptibility to erythromycin. This result is dramatically different from what has been reported in France16 and Canada,17 where rates of 14.5% of resistance to erythromycin in 2004 and 14.4% in 2001 have been reported. Similar high rates of resistance have been observed in Bavaria in Germany with 13.3%,10 in central Greece with 19.3%,18 in Portugal with 26.6%,19 in Spain with 29.7%,4 and in Italy with 35.8%.20 The increase in resistance to erythromycin detected in many of these countries appears to be predominately associated with Serotype M28 of S. pyogenes (based on the monitoring programs of laboratory LSPQ).

### Table 2. Susceptibility rates of *Streptococcus pyogenes* and MIC values (E-test).*

| ANTIBIOTICS | R (%) | I (%) | S (%) | MIC₉₀ | MIC₉₀ | GEOM MEAN | MIC RANGE |
|-------------|-------|-------|-------|-------|-------|-----------|-----------|
| **β-lactams** |       |       |       |       |       |           |           |
| Penicillin G | 0     | 0     | 100   | 0.016 | 0.023 | 0.012     | 0.004–0.032 |
| Cefpodoxime | 0     | 0     | 100   | 0.016 | 0.016 | 0.016     | 0.0016–0.023 |
| Cefixime    | 0     | 0     | 100   | 0.094 | 0.094 | 0.071     | 0.016–0.125 |
| Cefotaxime  | 0     | 0     | 100   | 0.023 | 0.023 | 0.021     | 0.008–0.047 |
| Ceftriaxone | 0     | 0     | 100   | 0.023 | 0.023 | 0.023     | 0.012–0.064 |

| Macrolides |       |       |       |       |       |           |           |
| Erythromycin | 0     | 0     | 100   | 0.094 | 0.125 | 0.079     | 0.032–0.019 |
| Azythromycin | 0     | 2.5   | 97.5  | 0.38  | 0.5   | 0.355     | 0.125–0.75  |

| phenicol |       |       |       |       |       |           |           |
| Chloramphenicol | 0   | 0     | 100   | 3     | 4     | 0.723     | 2–4       |

| Fluoroquinolones |       |       |       |       |       |           |           |
| Levofoxaniline | 0     | 0     | 100   | 0.75  | 0.075 | 0.738     | 0.38–2    |

| Glycopeptides |       |       |       |       |       |           |           |
| Teicoplanin | 0     | 0     | 100   | 0.094 | 0.094 | 0.206     | 0.023–2   |

*Susceptibility rates have been interpreted according to CLSI breakpoints.
Abbreviations: R, resistant; I, intermediate; S, susceptible; MIC, minimal inhibitory concentration (mg/L).
More than half of strains of *S. pyogenes* obtained in this study showed resistance to spiramycin (37.5% complete resistance and 40% intermediate resistance). Only 22.5% of the strains (8 strains) were susceptible to spiramycin. These results show existence of high level of resistance to spiramycin of group A streptococci in Dakar, having implications for drug treatment policy. In light of this data, we suggest that treatment of *S. pyogenes* infections with spiramycin in the event of penicillin allergy should be reconsidered. Total activity of the azithromycin on group A streptococci has been found in this study, with more than 97% of the strains susceptible by both standard disk diffusion and E-test. These results are consistent with data reported in previous studies in Dakar in 2002 and 2004 (Soumah unpublished data, Hounkponou unpublished data).

In this study, activity of clindamycin for group A streptococci was excellent: 97.4% of strains were susceptible, with 2.6% exhibiting intermediate susceptibility. No resistance has been observed in our study, in contrast to that of Soumah who reported, in 2002 in Dakar, 2.7% resistance (Soumah unpublished data). Our result is similar to the lack of clindamycin resistance of *S. pyogenes* reported in Spain in 2003. However, low rates of resistance to clindamycin have been recently observed in Japan (1.4%) and in Germany (1.1%). By contrast, a high percentage of resistance to clindamycin was previously observed in 90% of the erythromycin-resistant isolates in 2000 in Berlin.

Pristinamycin was very active in our study (97.5% of strains were susceptible, with only 2.5% of strains showing decreased susceptibility). This difference could be due to poor distribution of the strains on the agar and may not reflect true decreased susceptibility.

All strains were susceptible to telithromycin with more than 92% of activity, comparable to observations in Europe (98.5% of the strains tested were susceptible). Such results may justify the use of this antibiotic in the treatment of pharyngitis due to group A streptococci. Indeed, a 5-day regimen of telithromycin is as effective as 10-day regimen of clarithromycin. Such results may be associated with adequate preventive methods that could be used as first alternative choice. Clindamycin and pristinamycin, less used in therapeutic settings, have shown high degree of efficacy on the β-haemolytic *Streptococcus*. With 97% susceptibility, these two molecules could be used as an alternative or second line antibiotic. Interestingly, chloramphenicol, teicoplanin, vancomycin, and levofloxacin were also very active and could be potential alternative choices of treatment against infections with *S. pyogenes*.

Treatment of respiratory streptococcal infections is difficult and there are many factors to consider when choosing an antibiotic regimen. Susceptibility to antibiotics of any isolated strain should be evaluated as this is the only guarantee of prompt and effective treatment. The antibiotic therapy should be associated with adequate preventive methods that must include education of nursing staff in order to avoid as much as possible nosocomial infections, education of the general population for a politic of hygiene and abandonment of the common practice of self-medication, and increased scientific cooperation between clinicians and microbiologists in the interest of improving public health.

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**Author Contributions**

Conceived and designed the experiments: MC, AD and CSBB. Analyzed the data: MC and AD. Wrote the first draft of the manuscript: MC and AD. Contributed to the writing of the manuscript: MC, AD and CSBB. Agree with manuscript results and conclusions: MC, AD and CSBB. Jointly developed the structure and arguments for the paper: MC, AD and CSBB. Made critical revisions and approved final version: MC and CSBB. All authors reviewed and approved of the final manuscript.
Antibiotic susceptibility of *Streptococcus pyogenes*

**DISCLOSURES AND ETHICS**

As a requirement of publication the authors have provided signed confirmation of their compliance with ethical and legal obligations including but not limited to compliance with ICMJE authorship and competing interests guidelines, that the article is neither under consideration for publication nor published elsewhere, of their compliance with legal and ethical guidelines concerning human and animal research participants (if applicable), and that permission has been obtained for reproduction of any copyrighted material. This article was subject to blind, independent, expert peer review. The reviewers reported no competing interests.

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