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

Makhtar Camara, Assane Dieng and Cheikh Saad Bouh Boye

Laboratory of Bacteriology-Virology, Centre Hospitalier Universitaire Le Dantec, Cheikh Anta DIOP University, Dakar, Senegal.

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, vancomycine, 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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CORRESPONDENCE: camaramakhtar@yahoo.fr

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 trypticase-soya supplemented with 5% sheep blood and incubated in 5% CO_2 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 grouping 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 azythromycin 15 µg), lincosamins (clindamycin 2 µg), streptogramins (pristinamycin 15 µg), ketolids (telithromycin 15 µg), fluoroquinolones (levofloxacin 5 µg), glycopeptides (teicoplanin 30 µg, and vancomycine 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.[6] Briefly, bacterial suspensions at a concentration of 10^5 CFU/mL were inoculated on sheep blood Mueller-Hinton agar plates and incubated in 5% CO_2 for 24 to 48 hours at 37°C. The ATCC 29213 strain of *Staphylococcus aureus* was used as control. MIC endpoints and percentage susceptibilities were calculated based on Clinical Laboratory Standards Institute (CLSI) break points.[7]

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 MIC_{50} and MIC_{90} values of 10 antibiotics tested are shown in Table 2.

Susceptibility to β-lactams. The β-lactam antibiotics showed high activity with low MIC_{90} ranging from 0.016 to 0.094 mg/L. Penicillin remains effective with an MIC_{90} 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. Azythromycin remains fully active as all 40 isolates are completely susceptible. Erythromycin and azythromycin had MIC_{90} 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 MIC_{90} value of 4 mg/L.

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

ANTIBIOTICS	MEAN VALUES ± SD (mm)	R (%)	I (%)	S (%)
β-lactamins				
Penicillin G	30.43 ± 2.3	0	5	95
Amoxillin	30.93 ± 2.9	0	0	100
Cefixime	23.7 ± 2.76	0	0	100
Cefpodoxine	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
Spiramycine	20.33 ± 3.2	37.5	40	22.5
Azythromycin	20.36 ± 1.66	0	0	100
Lincosamines				
Clindamycin	21.64 ± 2.45	0	2.6	97.4
Streptogramines				
Pristinamycin	25.53 ± 2.55	2.5	0	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
Vancomycine	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.

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

ANTIBIOTICS	R (%)	I (%)	S (%)	MIC_{50}	MIC_{90}	GEOM MEAN	MIC RANGE
β-lactamins							
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
Phenicols							
Chloramphenicol	0	0	100	3	4	0.723	2–4
Fluoroquinolones							
Levofloxanine	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).

Susceptibility to other antibiotics. *S. pyogenes* isolates showed complete sensitivity to teicoplanin, vancomycin, and levofloxacin. Interestingly, these molecules showed low MIC_{90} values (Table 2). Tetracycline resistance was observed with all strains.

Discussion

Susceptibility to β-lactam antibiotics. In our study, we observed that all strains were susceptible to penicillin G with only 5% of strains presenting an intermediate susceptibility, which could be the result of poor reading of inhibition diameter (27 mm). This high activity of penicillin G was confirmed by the E-test with a susceptibility of 100%. A similar result was obtained in a previous study conducted in Dakar from patients with acute tonsillopharyngitis.[6] The same high activity of penicillin G had been reported in many countries, namely Morocco,[8] France,[9] and Germany.[10] However, penicillin treatment failures have been reported in patients with tonsillopharyngitis,[11] which has suggested a possible emergence of penicillin-resistant strains, probably by β-lactamase production or copathogens interference and alteration of microbial balance. Diversification of molecules used could reduce this phenomenon. The teicoplanin (glycopeptides) and pristinamycin (streptogramins) may represent a safe alternative.

In addition to penicillin, amoxicillin and cephalosporins were fully active against *S. pyogenes*, as previously reported.[6] 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 K. 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 France[16] 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).

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 stains 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.[21] However, low rates of resistance to clindamycin have been recently observed in Japan (1.4%)[22] and in Germany (1.1%).[10] By contrast, a high percentage of resistance to clindamycin was previously observed in 90% of the erythromycin-resistant isolates in 2000 in Berlin.[23]

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).[24] 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 250 mg twice daily or phenoxymethylpenicillin 500 mg 3 times daily.[25]

Susceptibility to others antibiotics. In this present study, levofloxacin showed very good activity with 100% of strains susceptible to this molecule. This is in agreement with high susceptibility rates (90% to 100%) reported in North African countries,[26] in France,[27] and also in the USA.[28] By contrast, resistance to levofloxacin was observed in Poland in 2001.[29] However, based on our results, levofloxacin may represent an effective alternative choice for treatment of patients infected with *S. pyogenes* in case of penicillin allergy and resistance to macrolides. In this study, complete resistance to tetracycline (100%) was observed, as previously reported in Dakar in 2009,[6] and in other countries such as Poland,[29] Iran,[30] and South Korea,[31] contrasting with a low rate of tetracycline-*S. pyogenes* resistance (6.6%) recently detected from Nepali school children in 2009.[14] All strains tested in this study were susceptible to vancomycin. These results are consistent with findings reported in other countries.[32,33] The teicoplanin tested showed complete activity on all strains of *S. pyogenes* with a low MIC_{90} of about 1 µg/mL. Similar to vancomycin, teicoplanin acts by disrupting the cell wall synthesis after inhibition of transglycosidase. These data suggest that glycopeptides (teicoplanin and vancomycin) and fluoroquinolones (levofloxacin) could be an effective alternative choice in *S. pyogenes* infections.

Conclusion

Group A β-haemolytic streptococci is one of the major causes of acute respiratory tract infections. Emergence of penicillin-resistant and other antibiotic-resistant clinical isolates of *S. pyogenes* underscores the need for continuous surveillance of antimicrobial resistance patterns. In this study, we investigated antimicrobial activity of 17 widely used antibiotics against 40 *S. pyogenes* isolates. Penicillin remains fully active. In addition to penicillin, amoxicillin and cephalosporin were very effective. Azithromycin and erythromycin were very active with susceptibility rates greater than 95% and 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, vancomycine, 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.

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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