Diversity of Mutations in the \textit{atpC} Gene Coding for the \textit{c} Subunit of \textit{F}_0\textit{F}_1\textit{ATPase} in Clinical Isolates of Optochin-Resistant \textit{Streptococcus pneumoniae} from Brazil\textsuperscript{\textvis{V}}

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We report the characteristics of four optochin-resistant (Opt\textsuperscript{r}) \textit{Streptococcus pneumoniae} isolates from Brazil. All four Opt\textsuperscript{r} isolates presented mutations in the nucleotide sequence coding for the \textit{c} subunit of \textit{F}_0\textit{F}_1\textit{ATPase}. Two isolates showed mutations in codons 23 (leading to the deduced amino acid substitution isoleucine instead of alanine) and 49 (serine instead of alanine, a novel type of mutation detected at this position), respectively. Two additional novel mutations, both located in codon 45, were detected in the other two isolates, corresponding to leucine or valine (instead of phenylalanine). The data indicate that three previously unrecognized alterations were detected in the \textit{atpC} gene of \textit{S. pneumoniae} and that Opt resistance among Brazilian pneumococcal isolates is not related to a specific pneumococcal serotype, antimicrobial-resistance profile, or clonal group.

\textit{Streptococcus pneumoniae} is one of the most important human pathogens, remaining as a major cause of community-acquired infections, such as pneumonia, bacteremia, meningitis, otitis media, and sinusitis (3). Because of the increasing frequency of antimicrobial resistance, accurate identification and antimicrobial susceptibility testing are essential for correct diagnosis and treatment of patients. Determination of phenotypic characteristics is conventionally used in diagnostic laboratories for the identification of \textit{S. pneumoniae}, including colony morphology on blood agar plates, optochin (Opt) susceptibility, bile solubility, and reactivity with type-specific antisera for detection of capsular polysaccharide antigen (1, 11). Several commercial systems and rapid kits are also available. More recently, a variety of molecular methods, including a DNA probe directed to a section of rRNA (8), PCR assays for detection of genes encoding diverse virulence factors (4, 12, 13), and DNA-DNA reassociation (1), have been applied to identify pneumococcal isolates. Despite the development of newer methods, most routine laboratories still rely on the results of Opt susceptibility testing as the primary or even the only test for the presumptive identification of pneumococci. Occasionally, however, isolates of \textit{S. pneumoniae} exhibiting an optochin-resistant (Opt\textsuperscript{r}) phenotype have been reported (2, 6, 7, 10, 13, 15). The occurrence of such a phenotypic variant is a potential cause of problems in the precise characterization of this agent, leading to misidentification. The Opt\textsuperscript{r} phenotype is attributed to mutations in the \textit{atpC} gene that codes for the molecular target of Opt, the transmembrane \textit{F}_0\textit{F}_1\textit{ATPase}, involved in proton transportation in the respiratory chain (9, 13). Alterations in \textalpha-helix 1, corresponding to codons 14, 20, and 23 (6, 13), and in \textalpha-helix 2, corresponding to codons 48, 49, and 50 (7, 13) of the \textit{c} subunit of the \textit{F}_0 complex of the molecule, have already been described in clinical isolates of \textit{S. pneumoniae}. A mutation located in the \textit{a} subunit of \textit{F}_0\textit{F}_1\textit{ATPase} was also described (13). To date, reports of the occurrence of Opt\textsuperscript{r} pneumococci are still sporadic, and only a few isolates with the Opt\textsuperscript{r} phenotype have had their respective mutations described.

The purpose of this communication is to report the occurrence and characterization of Opt\textsuperscript{r} \textit{S. pneumoniae} clinical isolates from Brazil, illustrating the global diversity among isolates from different geographic areas.

Four \textit{S. pneumoniae} isolates (Sp 910, Sp 913, Sp 917, and Sp 1008) presenting the Opt\textsuperscript{r} phenotype were investigated. Two of them (Sp 910 and Sp 913) were recovered from lower respiratory tract specimens, one (Sp 917) was isolated from blood, and the other (Sp 1008) was isolated from ocular secretion. These isolates were detected among a collection of about 470 pneumococci obtained in the period 1995 to 1999 during studies of the antimicrobial susceptibility, serotype distribution, and molecular epidemiology of pneumococcal isolates recovered from individuals living in Porto Alegre City, Rio Grande do Sul State, located in the southern region of Brazil. Three of them (Sp 910, Sp 913, and Sp 917) were isolated in 1995, and one (Sp 1008) was isolated in 1996. The isolates were initially subjected to conventional identification tests, including observation of colony characteristics on blood agar plates, cellular characteristics as observed after Gram staining, optochin susceptibility, and bile solubility, according to previously described methods (1, 11). Serotyping was based on capsular swelling (the Quellung reaction) with type-specific pneumococcal antisera (Centers for Disease Control and Prevention, Atlanta, Georgia 30333; and Instituto de Microbiologia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-590, Brazil).
Atlanta, GA), using the Danish system of nomenclature. Susceptibility to antimicrobial agents (ceftriaxone, chloramphenicol, erythromycin, penicillin, tetracycline, and vancomycin) was evaluated by an agar dilution method, based on the Clinical and Laboratory Standards Institute (5) guidelines. The presence of the virulence genes *ply* (14), *psaA* and *psaB* was assessed by an agar dilution method, based on the Clinical and Laboratory Standards Institute (5) guidelines. The prevalence of Opt resistance among *S. pneumoniae* isolates is not related to a specific pneumococcal serotype, antimicrobial-resistance profile, or clonal group. Such a finding has also been observed among isolates from the United States (13). When taken in conjunction with the results of previous reports (7, 13) describing an alanine-to-serine amino acid substitution: leucine (instead of phenylalanine) in isolate Sp 917 and valine in isolate Sp 1008. The four isolates belonged to different serotypes, as shown in Table 1. Analysis of PFGE profiles indicated that the isolates were not genetically related to each other (data not shown). The isolates were susceptible to most of the antimicrobial agents tested, except for Sp 910, which was resistant to chloramphenicol and sulfamethoxazole-trimethoprim, and Sp 1008, which was resistant to tetracycline.

This is the first report of the characterization of Opt r *S. pneumoniae* occurring in Brazil. Furthermore, the present communication contains a description of three previously unrecognized alterations occurring in the *atpC* gene of *S. pneumoniae*. Such alterations may be considered putative contributors to the Opt r phenotype, since the results of genetic transformation experiments have demonstrated that point mutations previously detected in the *atpC* gene were associated with Opt r in pneumococcal isolates (9, 13). The overall data indicate that the occurrence of Opt resistance among Brazilian pneumococcal isolates is not related to a specific pneumococcal serotype, antimicrobial-resistance profile, or clonal group. Such a finding has also been observed among isolates from the United States (13). When taken in conjunction with the results of other studies, this investigation additionally illustrates the diverse nature of putative molecular mechanisms of Opt resistance in *S. pneumoniae* and contributes to the global knowledge about the occurrence and diversity of Opt r pneumococci.

The prevalence of Opt resistance among *S. pneumoniae* re-
mains unknown, and it is probably underestimates, as many clinical laboratories still depend on Opt susceptibility testing for screening and identification of this microorganism and therefore may overlook or misidentify *S. pneumoniae* isolates with the Opt<sup>+</sup> phenotype. Clinicians and microbiologists should be aware of the existence of the Opt<sup>+</sup> pneumococcal isolates circulating in various areas and consider them a potential cause of life-threatening infections. Since misidentification of Opt<sup>+</sup> *S. pneumoniae* as viridans streptococci may have significant implications for the management of patients, routine use of at least one additional test, such as the bile solubility test, should be adopted to accurately identify *S. pneumoniae*. As more attention is dedicated to properly detecting and characterizing Opt<sup>+</sup> pneumococcal isolates in the clinical setting, more information will become available on the occurrence and diversity of these atypical variants and their roles as agents of infections.

Nucleotide sequence accession numbers. The sequences of the *atpC* gene reported here have been deposited in the GenBank database under the following accession numbers: EF464066 (isolate Sp 910), EF464067 (isolate Sp 913), EF464068 (isolate Sp 917), and EF464069 (isolate Sp 1008).

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REFERENCES


