Serotype 19F Multiresistant Pneumococcal Clone Harboring Two Erythromycin Resistance Determinants [erm(B) and mef(A)] in South Africa

LESLEY MCGEE,1* KEITH P. KLUGMAN,1 AVRIL WASAS,1 THORA CAPPER,1 ADRIAN BRINK,2 AND THE ANTIBIOTICS SURVEILLANCE FORUM OF SOUTH AFRICA

MRC/SAIMR/WITS Pneumococcal Diseases Research Unit, South African Institute for Medical Research,1 and Dre Buisson, Bruinette and Partners,2 Johannesburg, South Africa

Received 12 October 2000/Returned for modification 30 November 2000/Accepted 24 January 2001

One hundred eighteen erythromycin-resistant Streptococcus pneumoniae (ERSP) strains (MICs of $\geq 0.5 \mu g/ml$) from five laboratories serving the private sector in South Africa were analyzed for the genes encoding resistance to macrolides. Sixty-seven ERSP strains (56.8%) contained the ermA gene, and 15 isolates (12.7%) contained the mef(A) gene. Thirty-six isolates (30.5%) harbored both the ermA and mef(A) genes and were highly resistant to erythromycin and clindamycin. DNA fingerprinting by BOX-PCR and pulsed-field gel electrophoresis identified 83% of these strains as belonging to a single multiresistant serotype 19F clone.

Resistance to erythromycin in Streptococcus pneumoniae has been observed since 1967 (3) and was first reported for South African multiresistant pneumococcal strains in 1978 (7). Macrolide resistance in the pneumococcus has increased considerably over the last 5 years in several geographic areas (1, 4, 5, 9, 15). Until recently, the unique mechanism of macrolide resistance in streptococci was target modification by 23S rRNA (15). Highly over the last 5 years in several geographic areas (1, 4, 5, 9, 15).

Resistance to erythromycin in Streptococcus pneumoniae has been observed since 1967 (3) and was first reported for South African multiresistant pneumococcal strains in 1978 (7). Macrolide resistance in the pneumococcus has increased considerably over the last 5 years in several geographic areas (1, 4, 5, 9, 15). Until recently, the unique mechanism of macrolide resistance in streptococci was target modification by 23S rRNA (15). Highly over the last 5 years in several geographic areas (1, 4, 5, 9, 15).

60. The unique mechanism of macrolide resistance in streptococci was target modification by 23S rRNA (15). Highly over the last 5 years in several geographic areas (1, 4, 5, 9, 15).

The unique mechanism of macrolide resistance in streptococci was target modification by 23S rRNA (15). Highly over the last 5 years in several geographic areas (1, 4, 5, 9, 15).

We have recently documented the emergence of the M phenotype in erythromycin-resistant S. pneumoniae (ERSP) strains isolated from the public sector in South Africa (24). In the work described here we determined the prevalence of mef(A) and ermA genes in 118 ERSP isolates from the private sector in South Africa, where macrolides are more frequently prescribed than in the public sector (24).

S. pneumoniae clinical isolates isolated between July and September 1999 in five laboratories serving the private sector in four cities in South Africa were initially screened for erythromycin resistance by disk diffusion according to NCCLS criteria (16). Clinical isolates collected from the following sites were included in the study: cerebrospinal fluid (CSF), middle ear, blood, sinus tract, and sputum. Based on the NCCLS criteria, 118 ERSP strains were identified and included in the study (Table 1). MICs of erythromycin (Sigma, St. Louis, Mo.), penicillin (Sigma), and clindamycin (Sigma) were determined for all strains by the broth dilution method according to NCCLS guidelines (17). Additional MICs of chloramphenicol (Sigma), tetracycline (Sigma), trimethoprim (Glaxo Wellcome, Greenford, United Kingdom)-sulfamethoxazole (Sigma), rifampin (Sigma), and levofloxacin (Hoechst Marion Roussel, Romainville, France) were determined for strains carrying both ermA(B) and mef(A) genes. All 118 isolates were resistant (MICs, $\geq 1 \mu g/ml$) to erythromycin, with 92 (78%) of these isolates exhibiting high-level resistance ($> 64 \mu g/ml$). One hundred three isolates (87.3%) were resistant to clindamycin (MICs, $\geq 1 \mu g/ml$), and 102 (86.4%) were resistant to penicillin (MICs, $\geq 0.12 \mu g/ml$).

Serotyping showed that the majority (78.8%) of isolates belonged to serotypes 19F (46.6%), 6B (18.6%), and 14 (13.6%). The majority of strains were isolated from the upper respiratory tract, with 34.7% from sputum and 33.8% from the ear. Two strains were isolated from the CSF and blood. Overall, 22% of the strains were from adults and 75.4% of the strains were from children under 12 years of age. For three isolates the patient’s age was unknown.

All 118 ERSP strains were screened for the presence of the ermA(B) and mef(A) resistance determinants using PCR (21, 24). The ermA(B) gene alone was amplified in 67 isolates.
(56.8%), and the \textit{mef}(A) gene was amplified in 15 isolates (12.7%). We identified 36 isolates (30.5%) in which both the \textit{erm}(B) and \textit{mef}(A) genes were amplified, which was confirmed by sequencing (20). Isolates carrying the \textit{erm}(B) gene alone were all highly resistant to both erythromycin (MICs, $\geq 8 \mu g/ml$) and clindamycin (MICs, $\geq 4 \mu g/ml$), while those carrying the \textit{mef}(A) gene alone showed low-level resistance to erythromycin (MICs, 1 to 4 $\mu g/ml$) and no resistance to clindamycin, which is typical of the M phenotype. The isolates harboring both resistance genes showed resistance patterns identical to those of strains carrying the \textit{erm}(B) gene alone, i.e., high-level resistance to both erythromycin (MICs, $\geq 8 \mu g/ml$) and clindamycin (MICs, $\geq 4 \mu g/ml$). These \textit{erm}(B) \textit{mef}(A) strains were multiply resistant, showing in addition high-level penicillin resistance as well as resistance to chloramphenicol, tetracycline and trimethoprim-sulfamethoxazole.

DNA fingerprinting by BOX-PCR (11) and pulsed-field gel electrophoresis (PFGE) (13) identified 43 types among the 118 strains. Thirty of the 36 isolates identified as having both the \textit{erm}(B) and \textit{mef}(A) genes, which were isolated in all four cities in South Africa (Fig. 1), were shown to belong to a single multiply resistant 19F clone (Fig. 2).

Resistance to erythromycin in the pneumococcus was first reported in South Africa in the late 1970s (7), and although the rates of resistance of strains isolated from the public sector have increased from 2.5% in 1987 to 1991 (A. Wasas, R. E. Huebner, and K. P. Klugman, 7th Joint Biennial Congr. STD ID Soc. South. Afr., abstr. IDP18, 1999) to 4.9% in 1995 to 1998 (24), these rates are relatively low. In South Africa, macrolide use in the public sector is estimated at 56% of that in the private sector (24). Erythromycin resistance rates in the private sector have increased from 13.3% in 1986 (10) to 38.8% in 1999 (6), a rate far higher than that observed in the public sector. An essential factor in the increase of resistance is the availability and use of pediatric services and antibiotics for children in the private sector.

Virtually all clinical isolates of macrolide-resistant pneumococcal strains that have been examined for macrolide resistance mechanisms have contained either \textit{mef}(A) or \textit{erm}(B), with the \textit{mef}(A) gene predominant in some countries (McDougall and Tenover, 37th ICAAC) and the \textit{erm}(B) gene the major resistance determinant in others (Lantero et al., Int. Conf.

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|c|c|}
\hline
City and lab & No. of isolates & \textit{erm}(B) & \textit{mef}(A) & \textit{erm}(B) \textit{mef}(A) \\
\hline
Durban & 20 & 7 (35) & 1 (5) & 12 (60) \\
\hline
Pretoria & & & & \\
A & 22 & 13 (59) & 6 (27.3) & 3 (13.7) \\
B & 29 & 18 (62) & 7 (24.2) & 4 (13.8) \\
Johannesburg & 26 & 19 (73) & 1 (3.8) & 6 (11.2) \\
Bloemfontein & 21 & 10 (47.6) & 0 (0) & 11 (52.4) \\
\hline
Total & 118 & 67 (56.8) & 15 (12.7) & 36 (30.5) \\
\hline
\end{tabular}
\caption{Distribution and resistance mechanisms of 118 ERSP isolates from five private laboratories in South Africa}
\end{table}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{FIG. 1. Geographic locations of laboratories and distribution of isolates belonging to a serotype 19F clone in South Africa.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{FIG. 1. Geographic locations of laboratories and distribution of isolates belonging to a serotype 19F clone in South Africa.}
\end{figure}
Macrolides Azalides Streptogramins Ketolides). Pneumococcal strains that contain both genes have occasionally been reported (2, 14) and a recent study from Tokyo, Japan (18), reports that 16.1% of macrolide-resistant strains isolated from a single hospital harbored both genes simultaneously. In the present study, a surprisingly high percentage (30.5%) of isolates harbored both genes. In a recent study conducted on strains isolated in the public sector in South Africa, the M phenotype was reported as having increased significantly as a percentage of macrolide-resistant strains from 1987 to 1991 (0.8%) compared with 1992 to 1996 (19.7%) in blood and CSF isolates (24). No strains harboring both genes were detected in that study, however.

Susceptibilities to erythromycin and clindamycin for the \textit{erm}(B) \textit{mef}(A) strains were identical to those for strains carrying \textit{erm}(B) alone, which suggests that \textit{erm}(B) is sufficient on its own to express resistance and that the presence of the \textit{mef}(A) gene cannot be inferred from the phenotypic expression of MIC. The clinical impact of \textit{erm}(B) plus \textit{mef}(A) is likely to be similar to that of \textit{erm}(B) alone, as the high-level resistance phenotype is the same. DNA fingerprinting revealed that 25.4% of all ERSP strains in this study belonged to a single multiresistant serotype 19F clone. The clone appears to have disseminated in both the adult and child populations, suggesting that this clone is circulating in the community and contributing to macrolide resistance in the private sector, where macrolide consumption is high. Corso et al. (2) describe a serogroup 19 macrolide-resistant clone (3.3% of macrolide-resistant strains), harboring \textit{erm}(B) and \textit{mef}(A) genes, in the United States which appears to be related to this clone based on PFGE patterns.

The results from this study suggest that although the MLS\textsubscript{B} phenotype is still predominate in macrolide resistance in South Africa, the M phenotype, which is relatively new, appears to be emerging as an important factor in erythromycin-resistant pneumococci. The majority of \textit{erm}(B) \textit{mef}(A) strains belong to a multiresistant serotype 19F clone which is circulating throughout South Africa, contributing to high levels of resistance to erythromycin and clindamycin, especially in children under 5 years of age, and may be present in the United States. The molecular relatedness of the resistant \textit{erm}(B) and \textit{mef}(A) strains should be determined, and if these strains are confirmed to be an identical clone, this would represent the global emergence of this clone.

Collection of \textit{S. pneumoniae} isolates from the five laboratories was made possible by a grant from Abbott Laboratories.

REFERENCES