Clonal Spread of emm Type 28 Isolates of Streptococcus pyogenes That Are Multiresistant to Antibiotics

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Fifty-three pharyngitis-related and invasive isolates of Streptococcus pyogenes that are resistant to bacitracin were collected. They were also resistant to streptomycin, kanamycin, macrolides, lincosamides, and streptogramin B. These multiresistant isolates were of emm type 28 and clonally related as shown by pulsed-field gel electrophoresis.

Streptococcus pyogenes (Lancefield group A beta-hemolytic streptococcus) is responsible for suppurative local infections such as pharyngitis and severe invasive infections, such as necrotizing fasciitis and streptococcal toxic shock syndrome (7). The M protein is a major virulence factor, and a limited number of emm types, mainly 1, 3, 4, 6, 12, and 28, have been associated with invasive isolates of S. pyogenes (8, 13, 20). Besides emm typing, pulsed-field gel electrophoresis (PFGE) is also currently used for clonality studies (1, 21). Erythromycin or clindamycin are recommended as an alternative treatment of streptococcal pharyngitis in case of allergy to beta-lactams (3). Moreover, clindamycin is used in combination with penicillin for treatment of necrotizing fasciitis or streptococcal toxic shock syndrome (17). However, resistance of S. pyogenes to macrolides and lincosamides has been reported (2, 9, 10, 15). Susceptibility to bacitracin has been used as a preliminary laboratory test for the differentiation of S. pyogenes from other beta-hemolytic streptococci. Strains of S. pyogenes resistant to bacitracin are uncommon but, since 2000, we collected 53 strains of S. pyogenes resistant to bacitracin.

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Thirty bacitracin-resistant isolates of S. pyogenes out of 247 were obtained from November 2000 to May 2001 during a prospective survey of pharyngitis in Bourgogne, France. They were compared to 23 bacitracin-resistant invasive strains of S. pyogenes isolated in different regions of France from 2001 to 2003. Invasive strains were responsible for puerperal sepsis (n = 7), necrotizing fasciitis (n = 7), peritonitis of genital origin (n = 4), bacteremia (n = 3), endocarditis (n = 1), or meningitis (n = 1). Strains were identified as S. pyogenes by morphology, beta-hemolysis, absence of catalase, presence of pyrrolidonyl arylamidase, and presence of group A antigen. Biotype was determined by the presence of beta-glucuronidase and results of carbohydrate fermentation when tested on the strip Rapid ID32 STREP (bioMérieux, Marcy l’Etoile, France) as reported previously (4). Serotype T was determined by slide agglutination with type-specific antisera (Institute of Sera and Vaccines, Prague, Czech Republic). Antimicrobial susceptibility was studied by the disk diffusion method on Mueller-Hinton agar with 5% sheep blood according to the guidelines of the Antibiogram Committee of the French Society for Microbiology (http://www.sfm.asso.fr) (12). Eleven antimicrobial agents were tested: penicillin G, amoxicillin, vancomycin, teicoplanin, rifampin, tetracycline, streptomycin, kanamycin, gentamicin, erythromycin, and clindamycin. High-level resistance to aminoglycosides was tested with disks containing 500 μg of streptomycin, 1 mg of kanamycin, or 500 μg of gentamicin. Resistance to bacitracin was determined by an absence of inhibition of growth around the disk of bacitracin (0.02 IU). Disks were from Bio-Rad, Marnes-la-Coquette, France. Erythromycin-resistant isolates were further studied by the determination of MICs (erythromycin, azithromycin, josamycin, and clindamycin) using the agar dilution method (12) and by the triple-disc test (erythromycin, clindamycin, and josamycin) as previously described (9). Macrolide resistance genes [erm(B), emm(A), and mef(A)] were identified by PCR in all isolates resistant to erythromycin according to previously described procedures (16, 18). Clonality of isolates was studied by emm typing and PFGE. emm types were determined by sequencing the variable 5′ end of the emm gene after amplification by PCR according to the Centers for Disease Control and Prevention recommendations (http://www.cdc.gov/ncidod/biotech/strep/doc.htm). PFGE was performed with a CHEF-DR III apparatus (Bio-Rad). Chromosomal DNA was digested with SmaI by a method described elsewhere (1). PFGE patterns were compared according to the criteria of Tenover et al. (19). Isolates were considered to be clonally related if they showed differences of three or fewer bands between strains. They were considered to be possibly clonally related if they showed differences of four to six bands between strains.

The 53 pharyngitis-related and invasive isolates of S. pyogenes resistant to bacitracin were of biotype 1 (4), serotype T28, and emm type 28. They were susceptible to penicillin G, amoxicillin, vancomycin, teicoplanin, rifampin, and tetracycline. They showed a low level of resistance to gentamicin and a high level of resistance to streptomycin and kanamycin. They were also resistant to erythromycin and clindamycin. MIC re-
Susceptibility to bacitracin has been used as a preliminary test for the differentiation of *S. pyogenes* from other beta-hemolytic streptococci. Because this test lacks specificity, most laboratories currently use the presence of pyrrolidonyl arylamidase as a better test to identify *S. pyogenes*. Our results confirm the importance of using an alternative to bacitracin susceptibility testing. The emergence of strains of *S. pyogenes* resistant to macrolides has been observed, but they have not been multiresistant to antibiotics (2, 9, 10, 15). In this study, 53 pharyngitis-related and invasive isolates of *S. pyogenes* resistant to bacitracin were shown to also be resistant to streptomycin, kanamycin, macrolides, lincomamide, and streptogramin B. The constitutive resistance to macrolides was due to the presence of the gene *erm*(B). All these multiresistant isolates were of *erm* type 28 and clonally related as shown by PFGE. The high prevalence of 12% (30 of 247 isolates) of this multiresistant clone among pharyngitis isolates contrasts with the rare cases of bacitracin-resistant isolates reported previously (11, 14, 21). The results reported previously and those described in this study suggest a clonal relationship between all the bacitracin-resistant isolates. The multiresistant clone of *S. pyogenes* of *erm* type 28 was not only responsible for pharyngitis but also for invasive infections. *erm* type 28 is frequently associated with invasive infections and has even been reported as the second-most-common invasive type (13). Moreover, *erm* type 28 is predominant in invasive postpartum *S. pyogenes* infections, which may be due to tropism for vaginal tissue (5, 6). Our results also demonstrate that PFGE is a valuable epidemiological marker for differentiation of epidemiologically related *erm*28 *S. pyogenes* isolates. PFGE was performed on all *erm*28 *S. pyogenes* strains isolated during the prospective survey of pharyngitis. Twenty-one bacitracin-susceptible isolates were compared to the 30 bacitracin-resistant clonal isolates. The 21 bacitracin-susceptible isolates were susceptible to all antibiotics tested, including erythromycin and clindamycin. They had heterogeneous PFGE patterns, distinct from those of the bacitracin-resistant clone, and were thus not clonally related (data not shown). The emergence of this multiresistant *S. pyogenes* clone may be of importance in clinical practice as macrolides or clindamycin may be used for treatment of *S. pyogenes* infections.

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REFERENCES


