JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 2003, p. 5282–5284 Vol. 41, No. 11
0095-1137/03/$08.00+0 DOI: 10.1128/JCM.41.11.5282–5284.2003

Bacitracin-Resistant Clone of *Streptococcus pyogenes* Isolated from Pharyngitis Patients in Belgium

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Received 6 June 2003/Returned for modification 28 July 2003/Accepted 14 August 2003

We report 16 bacitracin-resistant *Streptococcus pyogenes* isolates recovered from pharyngitis patients in Belgium, 14 of which belonged to a particular *emm* type (*emm*28). All 16 isolates were constitutively resistant to macrolides and carried *erm*(B). The emergence of a bacitracin-resistant *S. pyogenes* clone raises questions about the continued reliability of bacitracin susceptibility testing for *S. pyogenes* identification.

Streptococcus pyogenes, a Lancefield group A streptococcus, is a common pathogen in humans, causing tonsillo-pharyngitis and serious invasive infections, such as necrotizing fasciitis and toxic shock syndrome. The prevalence of *S. pyogenes* infections has increased drastically in the last decade (4, 8) and correlates with increasing resistance to macrolide and tetracycline groups of antibiotics, which act by disrupting prokaryotic protein synthesis (2, 3, 6). However, *S. pyogenes* has remained uniformly susceptible to antibiotics that disrupt cell wall synthesis: i.e., penicillins, glycopeptides, and bacitracin. In fact, susceptibility to bacitracin is one of the preliminary laboratory tests employed in the presumptive differentiation of *S. pyogenes* from other beta-hemolytic streptococci. We report here 16 bacitracin-resistant isolates recovered from tonsillo-pharyngitis patients as part of a national surveillance study conducted in Belgium during 2002. These isolates were investigated further for clonality, as well as resistance to macrolides and other antibiotic groups.

A total of 1,572 presumptive *S. pyogenes* isolates were collected from 10 Belgian provinces. Of these, 1,229 isolates were confirmed to be *S. pyogenes* on the basis of a battery of tests: beta-hemolysis on blood agar, Gram stain, catalase, pyrrolidonyl aminopeptidase (PYR), group A antigen, and the bacitracin disk diffusion test (0.4 U; Rosco, Taastrup, Denmark). All *S. pyogenes* isolates showed the expected results, except for 16 isolates that showed resistance to bacitracin (disk diffusion zone diameters of 0 mm). Macrolide resistance for these isolates was determined by the conventional double-disk diffusion test with erythromycin (78 μg) and clindamycin (25 μg) Neo-Sensitab disks (Rosco), and the results were interpreted as reported previously (7). MICs of erythromycin, clarithromycin (Abbott, Ottignies, Belgium), azithromycin (Pfizer, Groton, Conn.), clindamycin and telithromycin (Aventis, Romainville, France), penicillin and ciprofloxacin (Bayer AG, Leverkusen, Germany), and tetracycline were determined by the agar dilution method. The inoculum (10^4 CFU/spot) was incubated under aerobic conditions at 37°C for 18 to 24 h, and the results were interpreted according to National Committee for Clinical Laboratory Standards guidelines. For telithromycin, breakpoints of susceptibility and resistance were taken as ≤1 and ≥4 μg/ml, respectively. Unless specifically mentioned, antibiotics were purchased from Sigma Chemical Co. (St. Louis, Mo.). In addition, the presence of the macrolide resistance determinants *erm*(B), *mef*(A), and *erm*(A) was detected by PCR. Genomic DNA was extracted by the alkaline lysis method (0.25% sodium dodecyl sulfate, 0.05 N NaOH). PCR was performed with a DNA thermal cycler (9600 GeneAmp PCR system; Perkin-Elmer, Zaventem, Belgium). The primers described previously for *erm*(B) and *mef*(A) give PCR products of 639 and 348 bp (9), respectively, while a 590-bp product was obtained with the following primers for *erm*(A): 5′ CCCGAA AAATACGCAAAATTTCAT 3′ and 5′ CCCTGTTTACCCA TTTATAAACG 3′ (G. Cornaglia, personal communication). The PCR mix and cycling conditions for *erm*(B), and *mef*(A) were described previously (2). For *erm*(A), each 50-μl PCR mixture contained 50 mM KCl, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 0.01% gelatin, 200 μM deoxynucleoside triphosphates (dNTPs), 1.5 mM $MgCl_2$, 20 pmol of primers, 0.45 U of *SuperTaq* polymerase (Enzyme Technologies Ltd., United Kingdom), and 2 μl of template DNA. The cycling conditions were an initial cycle of 5 min at 94°C; 35 cycles of 30 s of denaturation at 90°C, 60 s of annealing at 60°C, and 90 s of extension at 72°C; and finally 1 cycle of 5 min of elongation at 72°C. Positive controls used for *erm*(A), *erm*(B), and *mef*(A) were *S. pyogenes* strains UR1092, STP016, and STP046, respectively. Clonality was studied by pulsed field gel electrophoresis (PFGE) as well as *emm* typing as described previously (2, 5).

All 16 bacitracin-resistant *S. pyogenes* strains demonstrated constitutive resistance to erythromycin and clindamycin, explained by the uniform presence of the *erm*(B) gene. Neither *mef*(A) nor *erm*(A) was detected in any isolate. For these 16 isolates, the MICs at which 90% of the isolates tested are inhibited of erythromycin, clindamycin, clarithromycin, azithromycin, telithromycin, tetracycline, penicillin, and ciprofloxacin were >512, >512, 512, >512, 8, 0.125, 0.01, and 0.5 μg/ml, respectively. The PFGE clusters correlated completely with the *emm* typing results. Most interestingly, of the 16 isolates, 14 belonged to one PFGE cluster (10) and 1 particular *emm* type (*emm*28), and 2 isolates belonged to a distinct non-*emm*-typeable PFGE cluster (Fig. 1). Of the 14 clonal isolates, 13 were isolated from patients residing in the southern Belgian

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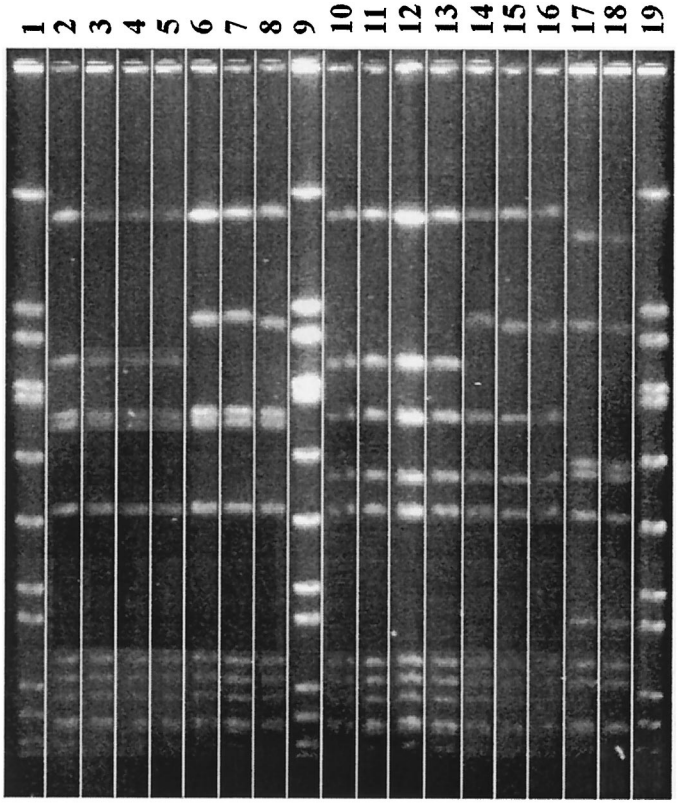

FIG. 1. PFGE pattern of the bacitracin-resistant *S. pyogenes*. Lanes 2 to 8 and 10 to 16 correspond to cluster 1 (*emm*28), and lanes 17 and 18 correspond to cluster 2 (nontypeable isolates) Chromosomal DNA was digested with *Sma*I. Lanes 1, 9, and 19 correspond to control strain *Staphylococcus aureus* NCTC 8325, a reference type strain for *Sma*I digests. The PFGE profiles were obtained by analysis with the computer software Gelcompar, version 4.0 (Applied Maths, Kortrijk, Belgium).

provinces of Hainaut, Luxembourg, and Liège, while 1 isolate was from the northern province of Vlaams-Brabant.

So far, there has only been a single published report of a bacitracin-resistant *S. pyogenes* clone that was recovered from invasive infections (11), indirectly suggesting that bacitracin resistance could be related to invasiveness. However, our findings of bacitracin-resistant *S. pyogenes* from pharyngitis patients suggest that there is no link between bacitracin resistance and invasiveness. Interestingly, both the earlier report (11) and this study show that the majority of the bacitracin-resistant *S. pyogenes* isolates are clonal. Although no gene was ascribed to macrolide resistance in the previous study (11), macrolide resistance in our clone was explained by the presence of *erm*(B). Moreover, the *emm*28 bacitracin-resistant clone was concentrated in southern Belgian provinces. We are currently investigating whether the presence of such strains could be related to the use of non-prescription-based bacitracin-containing throat lozenges. Also, efforts to elucidate the mechanism of bacitracin resistance in the *emm*28 clone are under way. Since bacitracin acts by preventing dephosphorylation and recycling of a lipid carrier (undecaprenol pyrophosphate), resistance to bacitracin, although not definitively characterized, is believed to result from an overproduction of undecaprenol kinase encoded by the *bacA* gene (reviewed in reference 1). Regardless of the precise reason for the resistance, a further search for bacitracin-resistant *S. pyogenes* isolates warrants that a preliminary screening for *S. pyogenes* should not rely on susceptibility to bacitracin.

We thank the following Belgian centers for their participation in this study: AML BVBA, Antwerp; Laboratoire de Biologie Clinique et Hormonale-S.P.R.L., Couillet; Centraal Laboratorium, Hasselt; Medisch Centrum Huisarten, Leuven; Centre Hospitalier de L'Ardenne Laboratoire de Biologie Clinique et de Ria, Libramont; and Laboratoire Marchand, Liège.

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