JOURNAL OF CLINICAL MICROBIOLOGY, Apr. 2008, p. 1558–1560 Vol. 46, No. 4
0095-1137/08/$08.00+0 doi:10.1128/JCM.02066-07

Fatal Levofloxacin Failure in Treatment of a Bacteremic Patient Infected with *Streptococcus pneumoniae* with a Preexisting *parC* Mutation▽

M. de Cueto,[1,2]* J. M. Rodríguez,[2] M. J. Soriano,[3] L. López-Cerero,[1,2] J. Venero,[3] and A. Pascual[1,2]

Department of Microbiology, University Hospital Virgen Macarena, Seville, Spain[1]; Department of Microbiology, School of Medicine, University of Seville, Seville, Spain[2]; and Department of Internal Medicine, University Hospital Virgen Macarena, Seville, Spain[3]

Received 24 October 2007/Returned for modification 13 December 2007/Accepted 9 February 2008

The fatal outcome of levofloxacin treatment in a patient with bacteremic pneumonia caused by *Streptococcus pneumoniae* with a preexisting *parC* mutation is reported. Failure was due to the emergence of a *gyrA* mutation after 4 days of therapy. Problems encountered in detecting first-step mutation isolates are discussed.

CASE REPORTS

A 79-year-old man with a history of chronic obstructive lung disease, hypertension, and transient ischemic attacks with residual dysarthria and right hemiparesis, was admitted to the emergency room with abrupt-onset fever, increased coughing, and dyspnea. On admission, the patient's temperature was 38.6°C and his white blood cell count was 18,000/μl (88.2% neutrophils). Arterial gasometry showed pH = 7.48, pO_2 = 59 mm Hg, and pCO_2 = 41 mm Hg, and chest radiography revealed condensation in the lower right lobe. The patient was hospitalized with a diagnosis of community-acquired pneumonia (CAP).

Blood cultures were taken before commencing empirical treatment with levofloxacin (500 mg [given intravenously]/24 h). At 24 h, growth of gram-positive cocci arranged in pairs was observed in the blood culture bottles. After subculturing, the microorganism was identified as *Streptococcus pneumoniae* susceptible to penicillin (Etest MIC, 0.06 μg/ml) and levofloxacin (Etest MIC, 1 μg/ml).

In view of the susceptibility results and because the patient's condition showed some clinical improvement (decrease in fever and total leukocyte number), treatment continued with levofloxacin at the same dosage. However, the fever reappeared on day 4 of hospitalization with the patient showing hypotension and tachycardia. A further blood culture was obtained, and it was decided to change the treatment to intravenous amoxicillin (1 g/8 h). The patient's clinical condition failed to improve, and he died in the hospital on day 5.

The *S. pneumoniae* isolate grown in the second blood culture was susceptible to penicillin (E-test MIC, 0.06 μg/ml) and resistant to levofloxacin (E-test MIC, >32 μg/ml). Both isolates were susceptible to trimethoprim-sulfamethoxazole, tetracycline, vancomycin, erythromycin, and clindamycin. No changes in the MICs were observed.

S. pneumoniae is the most common bacterial pathogen in CAP. The emergent resistance of these isolates to beta-lactams and macrolides has caused some concern about their use in the empirical treatment of CAP (11). Fluoroquinolones such as levofloxacin, with increased activity against *S. pneumoniae*, are now recommended for the empirical treatment of this infection (11, 13). The rate of fluoroquinolone-resistant *S. pneumoniae* infection remains low in most parts of the world, although an increase in fluoroquinolone use also implies a greater risk that resistant isolates will appear (10). Resistance to newer fluoroquinolones in *S. pneumoniae* may be emerging as a result of their increased use. In Spain, the reported resistance rate rose from 0.9% in the 1990s to 7.1% after the year 2000 (6, 14, 15), and significant increases have been noted in other countries such as the United States (7) and Canada (1). Higher rates of quinolone resistance have been found in isolates from the respiratory tracts of adult patients (14, 15).

Fluoroquinolone resistance in pneumococci has been reported previously as a cause of treatment failure, although in only a few cases has an in vivo selection for resistance been demonstrated (3, 8, 15). We describe here the emergence of a *gyrA* mutation and failure of levofloxacin treatment leading to death in a patient with bacteremic CAP caused by *S. pneumoniae* with a preexisting *parC* mutation.

Fluoroquinolone resistance in *S. pneumoniae* is the result of target mutations in the quinolone resistance-determining regions (QRDRs) of *parC* and *gyrA*, which encode topoisomerase IV and DNA gyrase, respectively (1, 10, 18). Low-level resistance occurs after a first-step mutation in one of the target genes, and high-level resistance occurs after a subsequent mutation in the other one. Isolates with a single first-step *parC* mutation are frequently susceptible to fluoroquinolones because the MICs are at or below the Clinical and Laboratory Standards Institute (CLSI) breakpoints (4). However, a first-step *parC* mutation increases the likelihood of a subsequent *gyrA* mutation, high-level resistance, and therapeutic failure (1, 18). At the same time, it has also been observed that once a *parC* mutation has taken place in an *S. pneumoniae* strain, the mutation prevention concentration (MPC) increases dramatically for all fluoroquinolones (17). Reports by Croisier et al. and Allen et al. showed that after levofloxacin or moxifloxacin treatment, mutants occur in vivo if there is a preexisting *parC*

* Corresponding author. Mailing address: Departamento de Microbiología, Hospital Virgen Macarena, Avda. Dr. Fedriani s/n, 41009 Seville, Spain. Phone: 34-955008138. Fax: 34-954377413. E-mail: m@marinadecueto.e.telefonica.net.

▽ Published ahead of print on 20 February 2008.

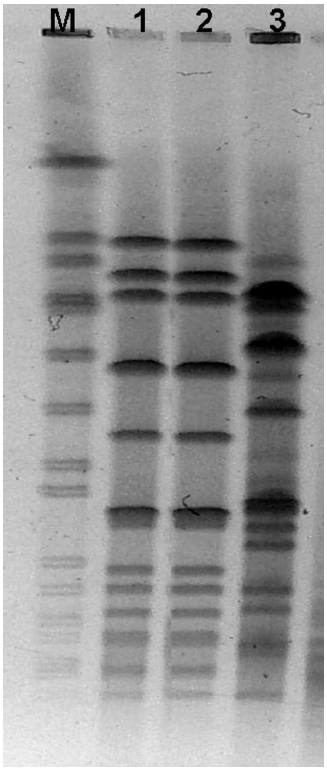

FIG. 1. PFGE of *S. pneumoniae* isolates. Lane M, molecular weight marker; lane 1, levofloxacin-susceptible *S. pneumoniae* (MIC = 1 μg/ml); lane 2, levofloxacin-resistant *S. pneumoniae* (MIC, >32 μg/ml); lane 3, unrelated clone.

mutation since the drug concentrations fall below the MPCs of the strains. Increasing both the drug concentration and exposure to exceed the MPC may prevent mutant strains from emerging, although this strategy did not prevent the selection of secondary mutants in strains with preexisting mutations (2, 5).

According to the recommendations of the CLSI, levofloxacin susceptibility in *S. pneumoniae* is defined as a MIC of ≤2 μg/ml (4). This breakpoint, however, only allows the detection of high-level resistant mutants affecting at least two targets; the prevalence of pneumococci which are not fluoroquinolone susceptible may therefore be underestimated when the present CLSI breakpoints are used. This case report provides disturbing evidence that fluoroquinolone-resistant pneumococci are able to emerge within only a few days of the start of treatment.

Both of the blood culture isolates belonged to serotype 8 (a non-seven-valent vaccine serotype), and their clonal relationship was demonstrated by pulsed-field gel electrophoresis (PFGE) (Fig. 1) with the SmaI endonuclease (6). By PCR and sequencing (12), the first isolate showed a mutation in the QRDR of *parC* (Ser79Phe), and the second one had an additional *gyrA* mutation (Ser81Phe). These mutations are most commonly found in fluoroquinolone-resistant *S. pneumoniae* strains (1, 18). In addition, genetic relatedness was supported by several polymorphisms identified in the QRDRs of the two isolates: Met1Leu in *parC* and Ile460Val in *parE*. Using PCR, we investigated the presence of the *qnrA*, *qnrB*, and *qnrS* genes that cause plasmid-mediated quinolone resistance in gram-negative rods (16). The result was negative for both isolates.

There is no currently available test for accurately identifying isolates with low-level fluoroquinolone resistance. In common with other authors, we consider it an urgent task to develop a phenotypic test which will permit the accurate detection of first-step mutants associated with treatment failure in some pneumonia cases.

The Comité de l'Antibiogramme of the Societé Française de Microbiologie (CA-SFM) recommends the use of norfloxacin, similar to the use of the oxacillin disk test, as a marker for detecting low-level fluoroquinolone-resistant pneumococci. According to the CA-SFM, when the inhibition zone around a disk containing 5 μg of norfloxacin is <10 mm or the MIC is >16 μg/ml, the risk of "in vivo" selection of fluoroquinolone-resistant mutants and of therapeutic failure is high (CA-SFM [http://www.sfm.asso.fr/publi/general.php?pa=1]).

Another proposed screening test is the MIC for ciprofloxacin, where an MIC of ≥4 μg/ml defines nonsusceptible isolates. However, about 30% of the isolates in the susceptible category harbor first-step mutations (10).

A number of risk factors have been identified as identifying patients who are likely to be colonized or infected with fluoroquinolone-resistant pneumococci, i.e., patients who are >64 years of age and have a history of chronic obstructive lung disease and/or prior fluoroquinolone exposure (9). In our case, we do no know whether the patient had previously received fluoroquinolone treatment. However, the risk factors associated with infections caused by first-step quinolone-resistant *S. pneumoniae* mutants remain to be studied so that future research into the effects of these factors may well determine the full clinical implications of this trend.

Because currently available phenotypic diagnostic tests are unable to identify pneumococci with a first-step mutation, empirical monotherapy using fluoroquinolones may be inappropriate for patients with CAP whose risk factors include infection with fluoroquinolone-resistant pneumococci.

This study was supported by the Ministerio de Sanidad y Consumo, Instituto de Salud Carlos III—FEDER, Spanish Network for the Research in Infectious Diseases (REIPI RD06/0008).

We thank Carmen Ardanuy for collaboration in the PFGE analysis.

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