Emergence of mefA and mefE genes in beta-haemolytic streptococci and pneumococci in France

J Antimicrob Chemother 1999; 44: 133–134

Corinne A rpin, M arie-H élène Canon, Patrick N oury and Claudiq Quentin*

L aboratoire de M icrobiologie, U niversité V ictor Segal e Bordeaux 2, 146 rue L éo Saignat, 33076 B ordeaux cedex, France

*Corresponding author. T el: +33-5-57-57-10-75; Fax: +33-5-56-90-90-72; E-mail: claudine.quentin@bacterio.u-bordeaux2.fr

Sir,

Since the early 1990s, most developed countries have experienced a rapid and substantial increase in erythromycin resistance among isolates of Streptococcus pyogenes and Streptococcus pneumoniae, probably related to the increased prescribing of this antibiotic or its novel semisynthetic derivatives. Until recently, the unique mechanism of macrolide resistance identified in streptococci was target modification by 23S rRNA methylases encoded by erm genes, which convey cross-resistance to macrolides, lincosamides and the streptogramin B compounds (MLSb phenotype). With the increase of erythromycin resistance, a new phenotype designated M, consisting of resistance to 14- and 15-membered macrolides, but susceptibility to 16-membered macrolides, lincosamides and type-B streptogramins, has emerged. The mechanism of this resistance is a proton-dependent efflux system encoded by mef genes: mefA in S. pyogenes, and mefE in S. pneumoniae. S. pyogenes and S. pneumoniae with an M phenotype and/or mef genes have been reported in a number of countries, where their prevalence among erythromycin-resistant isolates has reached very high rates; for S. pyogenes, mainly in Europe (38–97%) and for S. pneumoniae, principally in North America (41–85%). Subsequently, mef genes have been detected in Group C streptococci in Finland, where they represented 95% of the erythromycin-resistant isolates.

In France, such an increase in erythromycin resistance has not been observed, at least in our geographical area. The current rate of erythromycin resistance in the South West of France appears to be stabilized around 10% of S. pyogenes and Group C streptococci, 15% of Streptococcus agalactiae and Group G streptococci, and 45% of S. pneumoniae, with high local and temporal variations. Between 1993 and 1998, we collected 18 isolates of S. agalactiae (<1%) with an M phenotype, which have been demonstrated to possess either an mefA or an mefE gene. Over the same period, we have recovered only two S. pyogenes isolates, one Group C streptococcus and two pneumococci with an M phenotype (<0.1%). By the disc diffusion method, these five isolates exhibited a reduced zone of inhibition (diameter 10–20 mm) around the erythromycin disc (15 IU), with neither blunting zones around the discs of spiramycin (100 µg), lincomycin (15 µg) or pristinamycin (15 µg) placed nearby, nor zonal lincomycin resistance, even after prolonged incubation (72 h). MICs of 12 MLS drugs, determined by a agar dilution method in Mueller-Hinton medium supplemented with 5% horse blood, confirmed that these isolates were low-level resistant to erythromycin (2–8 mg/L) and other 14- and 15-membered macrolides, although the intrinsically more active new ketolide HMR3647 retained a significant activity; in contrast, they remained fully susceptible to 16-membered macrolides, lincosamides, and A (pristinamycin I) and B (pristinamycin I) streptogramin components (Table). The DNA of the five isolates was amplified using primers specific to the mefA or mefE gene as previously described. A II isolates except for one (S. pneumoniae No. 4) yielded a PCR product of the expected size (1.2 kb), whereas no amplification was obtained with the DNA of negative control isolates (sensitive or MLSb phenotype isolates). The amplicons were analysed by enzymatic restriction using four endonucleases designed to differentiate mefA and mefE: the Clal and HindIII enzymes cleave only the mefA gene in two fragments (0.701 and 0.517 kb for Clal, and 0.89 and 0.328 kb for HindIII), whereas A ccl and H hal enzymes cleave only the mefE gene in two fragments (0.651 and 0.567 kb for Accl, and 0.8 and 0.418 kb for H hal). The results showed that four isolates carried an mefA gene, whereas the remaining one (S. pyogenes No. 2) possessed an mefE gene (Table). Thus, there is no species-specific distribution of the mefA or mefE gene, and mefE appears to be predominant among streptococci with an M phenotype as previously observed for S. agalactiae and pneumococci. The S. pneumoniae No. 4 isolate carried neither an mefA gene nor an ermA gene, using PCR with primers specific for ermA (erm1, 5'-TCTAAAGGAGATGTAAAAGAA-3'; erm2, 5'-CTTCCGATGTATTAAATAATTAGT-3'), ermA (erm3, 5'-GAATACATCTCAACCAAATA-3'; erm4, 5'-AGTACCGGTCTTAAATTGTTTACT-3'), ermA (erm5, 5'-TCAAACATAATAATGATAA-3'; erm6, 5'-GCTAAAATTTTAAATCTCGTCAAT-3').
These results suggest the existence of a novel erythromycin resistance gene or mechanism in pneumococci. This is the first description of mefE in *S. pyogenes*, and the first report of *S. pyogenes*, Group C streptococci and *S. pneumoniae* from France with an M phenotype and mef genes. The reason why this mechanism of erythromycin resistance remains more uncommon in France than in other European countries, and why *S. agalactiae* is more frequently implicated than other streptococcal species, remains to be explained. Our findings emphasize the importance of preparing cultures and carrying out susceptibility testing whenever streptococcal infections are suspected. Our findings also suggest the existence of a novel erythromycin resistance gene in *S. agalactiae*. These results support the need for additional studies to better understand the role of this gene in the epidemiology of streptococcal infections.

References


Carbapenem-hydrolysing β-lactamase from clinical isolates of Pseudomonas aeruginosa in Portugal

J A ntimicrob Chemother 1999; 44: 135

Olga Cardoso\textsuperscript{a,c}, João Carlos Sousa\textsuperscript{b}, Rui Leitão\textsuperscript{a} and Luísa Peixe\textsuperscript{b}

\textsuperscript{a}Laboratório de Meficrobiologia da Faculdade de Farmácia da Universidade de Coimbra, Couraça dos A postolos 51 r/c 3030 Coimbra; \textsuperscript{b}Laboratório de Microbiologia da Faculdade de Farmácia do Porto, Rua A níbal Cunha 164 4050-Porto, Portugal

\textsuperscript{c}Corresponding author. Tel: +35-1-39-852567; Fax: +35-1-39-852569; E-mail: ocardoso@ci.uc.pt

Sir,

Although the carbapenem antibiotics have the broadest antibacterial activity of all β-lactams, strains of Pseudomonas aeruginosa resistant to these agents have been reported. The mechanisms of the acquired imipenem resistance in this species may result from a decrease in the uptake of carbapenems and from an increased expression of the pump efflux. In P. aeruginosa imipenem proved to be capable of penetrating cells via an outer membrane protein, the OprD porin, and the absence of this channel resulted in the acquisition of imipenem resistance, reducing meropenem susceptibility, but not affecting the activity of other β-lactams.\textsuperscript{1} Recently, plasmid mediated carbapenemase, which confers high-level resistance to several β-lactams including carbapenems, was found.\textsuperscript{2} The report of these enzymes was restricted to Japan, although recently, a carbapenemase producer in a colonizer isolate in the UK was detected.\textsuperscript{3}

In the present work we describe the carbapenemase activity observed in three imipenem-resistant P. aeruginosa isolates clinically responsible for urinary and lower respiratory tract infections in patients in a Portuguese hospital. MICs for these isolates were determined by Etest. Isoelectric focusing and β-lactamase activity were evaluated as detailed previously.\textsuperscript{4}

High-level MICs of imipenem (>32 mg/L), piperacillin-tazobactam (48–256 mg/L) and ceftazidime (64–256 mg/L), and susceptibility to aztreonam (6–8 mg/L) were found. In contrast to the previous reports of carbapenemases,\textsuperscript{3,4} meropenem and ceftazidime were more active, 0.5–0.75 mg/L and 24–48 mg/L, respectively. A multiresistant phenotype, which includes amikacin, tobramycin, gentamicin, ciprofloxacin and chloramphenicol, was shared by all the isolates.

Carbapenemase activity was demonstrated in the crude sonicates of cell suspensions by UV spectrophotometry with 0.1 mM of imipenem in 50 mM phosphate buffer, pH 7.0. Imipenem was hydrolysed (36.5–59.6 U/mg protein) and when the suspension was incubated with EDTA 2 mM, the activity of the enzyme was completely lost (0.7–1 U/mg protein). A ctitivity was restored by addition of divalent cations, such as Zn\textsuperscript{2+} (9.4–19.6 U/mg protein). One unit of enzyme activity was defined as the amount of enzyme needed to hydrolyse 1 μmol of imipenem per min at 30°C. These results were further confirmed by a biological method. The extracts and imipenem 10 mg/L were incubated together for 15 min before being transferred to wells cut into a bioassay plate containing Mueller–Hinton agar, the surface of which was inoculated with Escherichia coli ATCC 25922. The extracts of all isolates hydrolysed the imipenem. Inhibition of the activity over imipenem was obtained when the extracts were incubated with EDTA 2 mM. This metallo-β-lactamase with a pl >9.0, as predicted by isoelectric focusing, was observed in the three nosocomial P. aeruginosa isolates. A specific OprD expression has been implicated as a resistance mechanism in imipenem-resistant isolates, a normal expression of this protein in these clinical isolates allowed us to exclude the contribution of this porin in the behaviour observed.

A part from Japan, this is the first report of evidence of carbapenemase production by P. aeruginosa isolates responsible for nosocomial infections. As the previously reported carbapenemases showed different MICs from this one, a molecular and enzymatic characterization of this new enzyme is being undertaken.

The emergence of this new carbapenemase is of great concern, as carbapenems are often used as treatment of last resort against infections caused by Gram-negative bacteria that are resistant to other antimicrobial agents.

References

Spectrophotometric determination of sparfloxacin in tablets


Hérida R. N. Marona a, b * and Elfrides E. S. Schapoval b

a Faculdade de Ciências Farmacêuticas, UNESP, Rod. A raraquara-J au, km 1, CEP. 14801-902, A raraquara, SP; b Curso de Pós-graduação em Ciências Farmacêuticas, UFRGS, A v. Ipiranga, 2752, Porto A legre, CEP. 90610-000, R S, Brazil

*Corresponding author. Fax: +55-51-316-5437; E-mail: hmarona@hotmail.com

Sir,

Sparfloxacin is a fluoroquinolone antibacterial agent active against Gram-positive and Gram-negative bacteria. 1, 2 It has been reported to be more active in vitro than other quinolones against some microorganisms including staphylococci and mycobacteria, 3, 4 and has a 16 h plasma half-life. 5 High-performance liquid chromatography methods for the analysis of sparfloxacin in body fluids have been reported, 6, 7 but there is no information on the quantification of sparfloxacin in tablets. This report describes a sensitive and accurate spectrophotometric method for determination of sparfloxacin in raw material and tablets.

Sparfloxacin powder (99.5% potency) was supplied by Dainippon Pharmaceutical Co. (Osaka, Japan). Each sparfloxacin tablet contained 200 mg of the active drug. The sparfloxacin powder and tablets were stored protected from light. Solutions of sparfloxacin powder in methanol (1 mg/mL) were prepared by accurately weighing 10 mg of sparfloxacin into a 50 mL volumetric flask, adding 10 mL methanol and shaking for 30 min by mechanical shaker, followed by sterile distilled water up to volume. The sparfloxacin standard solution was diluted in 50 mL volumetric flasks with sterile distilled water to give a range of solutions with final concentrations of 2–12 mg/L. The absorbance value of each solution at 292 nm was determined in a 10 mm quartz cell using a UV–VIS spectrophotometer (Shimadzu, Kyoto, Japan). To analyse the concentration of sparfloxacin in tablets, each tablet was weighed to obtain the average tablet weight. The tablets were ground up and 960 mg (representing three times the average weight of each tablet) was transferred to a 1000 mL volumetric flask; 200 mL methanol was added and the flask was shaken for 30 min by mechanical shaker followed by addition of sterile distilled water to volume. A aliquots of 10mL of this solution were transferred to a 100 mL volumetric flask and sterile distilled water was added to volume to give an estimated concentration of 60 mg/L. Aifier rotation in a centrifuge flask, the solution was diluted 1:10 to give a final estimated concentration of 6.0 mg/L. This solution was prepared six times and the absorbance of each solution was determined at 292 nm. In addition, to verify the accuracy of the tablet solutions, a standard solution of 200 mg/L made from sparfloxacin powder was added in 1, 2 and 3 mL aliquots to sample solutions with 600 mg/L to give a final estimated concentration of 8, 10 and 12 mg/L, respectively. The most powerful statistical tool to verify the internal validity of an analytical procedure, a criterion of accuracy, is the analysis of variance (ANOVA). Therefore, the data were analysed by linear simple regression by the least-squares method using Excel 5.0.

The aim of this study was to develop a spectrophotometric procedure for the analysis of sparfloxacin in raw material and tablets. The linear regression equation for sparfloxacin was calculated to be y = 0.0725x + 0.0008, where x and y are concentration and absorbance, respectively. The method had excellent reproducibility for the standard solution of 100 mg/L, 97.08 ± 1.07% (n = 6). The recovery test is an experimental design to verify the relationship between the amount of substance added and the amount quantified by this assay. In this test the observed concentrations of pure sparfloxacin in the powdered tablets were not significantly different from the stated concentrations by Student’s t test, P = 0.05% (100.04 ± 1.39%, n = 3). The detailed accuracy and precision data are shown in the Table. The method gave rise to linear data in the range 2–12 mg/L with accuracy and precision in the range 0.56–3.01%. Therefore, this UV-spectrophotometric assay was accurate, and may be recommended for the simple quantification of sparfloxacin.

A cknowledgements

The authors are grateful to Dr Sayuri Kitada (Dainippon Co., Japan) and Dr Michael Pease (Rhône-Poulenc Rorer,

<table>
<thead>
<tr>
<th>Estimated (mg/L)</th>
<th>Observed concentration (mg/L)</th>
<th>% of stated concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.00</td>
<td>1.9606 ± 0.000884</td>
<td>98.03 ± 0.0442</td>
</tr>
<tr>
<td>4.00</td>
<td>4.0932 ± 0.000240</td>
<td>102.33 ± 0.0060</td>
</tr>
<tr>
<td>6.00</td>
<td>5.9856 ± 0.000540</td>
<td>99.76 ± 0.0090</td>
</tr>
</tbody>
</table>

Table. Experimental values obtained in the recovery test for sparfloxacin in tablets
A cute pancreatitis with severe lactic acidosis in an HIV-infected patient on didanosine therapy


B. Ailaouchichea, F. Duflo, L. Cotte, M. Mathon and D. Chassard

aIntensive Care Unit, and bHepato-gastroenterology and AIDS Unit, Hôtel-Dieu Hospital, 69288 Lyon, France

Sir,

Didanosine (ddI) is a member of the nucleoside analogue class (2’,3’-dideoxyinosine) which inhibits the reverse transcriptase of the human immunodeficiency virus (HIV) in vitro and improves surrogate markers of HIV infection in vivo. Major clinical adverse effects are nowadays well known: pancreatitis, painful neuropathic syndrome, fulminant hepatic failure.1 Chattha et al.,2 Freiman et al.3 and Lai et al.4 have reported an uncommon but potentially fatal adverse effect similar to Reye’s syndrome with hepatic steatosis and aerobic (type B) lactic acidosis during the course of ddI therapy. We report the case of acute pancreatitis with severe lactic acidosis in an HIV-infected patient on ddI therapy.

A 58-year-old HIV-1-infected homosexual man had been diagnosed with AIDS 8 months earlier after he had presented with Kaposi’s sarcoma. He had been treated with ddI (400 mg/day), d4T (80 mg/day) and indinavir (1200 mg/day). He had no past history of liver or biliary disease, blood transfusion, drug addiction or alcohol abuse. He had been admitted to the hospital with a 10 day history of abdominal pain. Admission laboratory values were significantly increased for amylase (1059 IU/L), bilirubin (124 µmol/L), aspartate aminotransferase (107 IU/L), alanine aminotransferase (75 IU/L), alkaline phosphatase (172 IU/L), γ-glutamyl transpeptidase (453 IU/L) and arterial blood lactate concentration (13 mmol/L). There was no sign of hepatic failure. A abdominal computed tomography confirmed the pancreatitis and showed vesicular sludge in the gall bladder. A abdominal ultrasound did not show biliary tract abnormalities and revealed hepatomegaly. Endoscopic ultrasonography of the biliary tract did not show abnormalities. A liver biopsy showed inflammation without necrosis or fibrosis. A II microbial cultures of blood, bone marrow and cerebrospinal fluid were negative. There were no other obvious causes of lactic acidosis such as diabetic ketoacidosis, malignancy, sepsis, uraemia, thyrotoxicosis, exogenous intoxication, myopathy or thiamine deficiency.5 Therefore, ddI, d4T and indinavir were stopped.

References


Correspondence