Personalized antifungal susceptibility testing


Pramod M. Shah

Medizinische Klinik III, Schwerpunkt Infektiologie, Klinikum der Johann Wolfgang Goethe-Universität, Theodor-Stern-Kai 7, 60590 Frankfurt, Germany

Tel: +49-69-6301-6614; Fax: +49-69-6301-7717; E-mail: shah@em.uni-frankfurt.de

Sir,

In a recent issue of the Journal, Conti et al. concluded that so-called personalized antifungal susceptibility testing might be superior to the standardized reference method in terms of predicting the outcome of antifungal therapy. They rightly point out that a wide range of factors can significantly affect the reproducibility of the test method. In this sense, antifungal agents are no different to antibacterials.

The findings of Conti et al. that the in-vitro activities of antifungal agents when determined in plasma are markedly different from those determined with a synthetic medium (RPMI 1640) come as no surprise, similar observations having been reported by several investigators in respect of antibacterials. With regard to the latter, it was shown, albeit in only a few instances, that the reduced activities of these drugs in body fluids influence their clinical efficacy. We have evaluated the activities of amphotericin B and fluconazole, alone and in combination, both in a synthetic medium (broth) and in human blood and showed that fluconazole, at certain concentrations, was fungistatic when susceptibility testing was carried out in broth, but fungicidal, at the same concentrations, when tested in blood. This might explain why the results of in-vitro testing failed to predict the efficacy of fluconazole in vivo. For amphotericin B, we observed a marked decrease in its activity in blood which could be accounted for by the binding of the drug to the outer membranes of the cellular components of blood and the consequent reduction in the concentrations of free drug.

Sir,

Metronidazole is frequently included in regimens used to eradicate Helicobacter pylori. However, the rates of

References


Setting the standard for determining the in-vitro susceptibility of Helicobacter pylori to metronidazole


Tanittha Chatsuwan* and Sebastian G. B. Amyes

Department of Medical Microbiology, The Medical School, University of Edinburgh, Teviot Place, Edinburgh EH8 9AG, Scotland, UK

*Corresponding author. Tel: +44-131-650-8270; Fax: +44-131-650-6531; E-mail: Tanittha.Chatsuwan@ed.ac.uk

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metronidazole resistance amongst *H. pylori* isolates are highly variable—from 11% to 70% in developed countries and even higher in developing countries. As susceptibility testing of *H. pylori* has not been standardized, it may be that variations in the rates of resistance can be accounted for by differences in the methods of determining susceptibility. For example, Smith & Edwards demonstrated that *H. pylori* NCTC 11637 is resistant to metronidazole when tested under microaerophilic growth conditions, but susceptible when tested under anaerobic conditions. This raises an important question: are strains which are resistant to metronidazole when in-vitro susceptibility testing is carried out under microaerophilic conditions also resistant in vivo? The methodology of determining the susceptibility of this bacterium to metronidazole is crucial to the ability to predict the efficacy of eradication therapy. The present study was therefore undertaken to evaluate variations in methodology on the outcome of susceptibility testing.

The MICs of metronidazole for 21 clinical isolates of *H. pylori* were determined by the Etest method (AB Biodisk, Solna, Sweden) according to the manufacturer’s instructions; the medium used was Columbia agar (Oxoid, Basingstoke, UK) supplemented with 7% horse blood and *H. pylori* NCTC 11637 was included as a control. The inocula were prepared by suspending colonies from 48 h growth on Columbia blood agar in Brain Heart Infusion (BHI) broth (Oxoid) and adjusting the suspensions to give turbidities equivalent to that of a No. 3 McFarland standard. The plates were incubated at 37°C in a microaerophilic atmosphere (Campylobacter System Gas Generating Kit, Oxoid) and MICs were recorded after 72 h. The majority (12) of the 21 strains, as well as the control, were resistant to metronidazole on the basis of a lower MIC breakpoint of ≥0.5 mg/L recommended by AB Biodisk; the MICs for five strains were ≥256 mg/L.

The effects of varying the conditions of susceptibility testing were evaluated with the control strain which was shown by us to be highly resistant to metronidazole (MIC ≥256 mg/L), although other investigators have reported it to be susceptible. Firstly, the inoculated Etest plates were incubated under anaerobic conditions for 24 h before being incubated under microaerophilic conditions for a further 72 h. With the introduction of a pre-incubation phase, the MIC for *H. pylori* NCTC 11637 fell to 0.016 mg/L which is below the breakpoint defining susceptibility. This suggests that metronidazole is activated, i.e. a nitro group is reduced to a radical anion which damages DNA, only under strictly anaerobic conditions. The requirement for an anaerobic atmosphere of incubation for the activation of metronidazole may explain why some centres have reported high rates of resistance to this drug amongst *H. pylori* isolates.

The next step was to compare the outcome of incubating *H. pylori* NCTC 11637 in an anaerobic jar with that in an anaerobic chamber before the plates were incubated in a microaerophilic atmosphere. Either no growth or only poor growth was observed following pre-incubation in an anaerobic chamber (Don Whitley Scientific Ltd, Shipley, UK), whereas the strain grew well when an anaerobic jar was used, suggesting that growth of *H. pylori* depends on a high level of humidity which is not provided in an anaerobic chamber.

Finally, we also investigated the effects of varying the medium, inoculum size and duration of incubation on the MICs of metronidazole for the control strain. The media evaluated were Columbia and Mueller–Hinton agar (Oxoid), both supplemented with 7% horse blood. Colonies from a 48 h culture were suspended in BHI broth and the turbidities of the suspensions were adjusted so that they were equivalent to that of either a 0.5 or a No. 3 McFarland standard. The inoculated plates were then incubated in an anaerobic atmosphere for 24 h, followed by incubation for either 48 h or 72 h in a microaerophilic atmosphere. Growth on Mueller–Hinton agar was poor, while that on Columbia agar was profuse. The MICs were difficult to interpret when the lower inoculum was used, but were clear and reproducible with the higher inoculum. After 48 h of incubation, it was not possible to record the MICs accurately because growth was too poor. On the other hand, following incubation for 72 h, the MICs were easy to read and unequivocal.

Susceptibility testing of all 21 *H. pylori* isolates to metronidazole was then repeated under optimal conditions, i.e. pre-incubation of Columbia agar plates inoculated with the higher inoculum for 24 h in an anaerobic jar, followed by incubation for 72 h in a microaerophilic atmosphere. Under these conditions, the MIC90 and MIC50 were 0.016 mg/L and 0.19 mg/L respectively; only one strain was truly resistant to metronidazole (MIC >256 mg/L).

We have identified the optimal conditions for determining the susceptibility of *H. pylori* to metronidazole. In particular, pre-incubation under anaerobic conditions, ideally in an anaerobic jar, is regarded by us as being essential if accurate results are to be obtained. Our interpretation of the findings of this study is that *H. pylori* strains are inherently susceptible to metronidazole—a view that is in contrast with that of earlier investigators. If the drug is pre-reduced, as it must be when undertaking susceptibility testing with other bacteria, then most *H. pylori* isolates will appear susceptible to it. It has been suggested previously that *H. pylori* is resistant to metronidazole when susceptibility is determined under microaerophilic conditions. We believe that, on the basis of current evidence, this conclusion is unwarranted as there is no means of confirming that the conditions that prevail during susceptibility testing are the same as those at the site of infection. None the less, the success achieved with metronidazole when it is used as treatment of patients with *H. pylori* infection suggests that most strains are susceptible to this drug, as our susceptibility test results indicate.
The Stokes’ disc diffusion method was performed and MIC breakpoint of Trimethoprim has the smallest colony with DST agar. Antimicrobial susceptibility testing of Helicobacter pylori has been recently introduced into our laboratory as the principal means of identifying clinical isolates and performing susceptibility testing of staphylococci and methicillin-resistant Staphylococcus aureus (MRSA). Eighty-seven of the 100 isolates were identified as resistant—a major error rate of 37%. The susceptibilities of 100 non-replicate clinical isolates of MRSA to trimethoprim were determined in duplicate by three different methods. Vitek GPS-AK cards were inoculated, susceptibilities read according to the manufacturer’s instructions and susceptibility categories assigned on the basis of MIC breakpoints recommended by the National Committee for Clinical Laboratory Standards (NCCLS), i.e. susceptible, MIC ≤2 mg/L and resistant, MIC ≥4 mg/L. The Stokes’ disc diffusion method was performed according to a method described previously with DST agar (Oxoid, Basingstoke, UK) supplemented with 2% lysed horse blood, discs (Oxoid) containing 2.5 μg of trimethoprim and inocula with turbidities equivalent to that of a 0.5 McFarland standard. The Oxford strain of S. aureus was used as the control and the zones of inhibition were read after overnight incubation at 37°C. Susceptibility was defined as a zone of inhibition greater than or equal to that of the control or not >3 mm smaller and resistance as a zone of inhibition ≥3 mm smaller than that of the control. Finally, MICs were determined by an agar dilution method recommended by the NCCLS, an MIC breakpoint of ≤2 mg/L was used to define susceptibility.

Eighty-seven of the 100 isolates were identified as susceptible to trimethoprim by both the disc diffusion and agar dilution methods; MICs ranged from 0.25 to 1 mg/L. Of these 87, 36 (41%) were classified as susceptible and 32 (37%) as resistant with the Vitek card. Susceptibility testing of the remaining 19 strains by this method was inconclusive, the isolates being susceptible on one of the duplicate testings and resistant on the other, i.e. either 1 mg/L or 2 mg/L and ≥4 mg/L respectively. Thirteen isolates were found to be resistant by all three methods. Preliminary investigations with coagulase-negative staphylococci and methicillin-resistant strains of S. aureus revealed patterns similar to that observed with the MRSA strains (data not shown).

In summary, the susceptibilities of 100 clinical isolates of MRSA to trimethoprim, as determined by the disc diffusion and agar dilution methods, were identical, with 87 strains being categorized as susceptible. The Vitek GPS-AK card, on the other hand, incorrectly designated 32 of these 87 strains as resistant—a major error rate of 37%. Moreover, the results of susceptibility testing of a further 19 strains were not reproducible and, therefore, inconclusive. These observations suggest that the Vitek GPS-AK card is an unreliable method of determining the susceptibilities of staphylococci to trimethoprim.

References


Errors associated with determining the susceptibilities of staphylococci to trimethoprim by the Vitek GPS-AK card

*Corresponding author. Tel: +44-1382-660111.

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Ian C. Carmichael*, Valerie Godfrey and Gary Nicholson

Department of Medical Microbiology, Level 6, Medical School, Ninewells Hospital, Dundee DD1 9SY, UK

*Corresponding author. Tel: +44-1382-660111.

Sir,

The Vitek system (bioMérieux, Hazelwood, MO, USA) was recently introduced into our laboratory as the principal means of identifying clinical isolates and performing susceptibility testing. However, concerns about the system’s ability to accurately detect susceptibility to trimethoprim arose when the number of strains of methicillin-resistant Staphylococcus aureus (MRSA) resistant to this agent increased. Although trimethoprim is not normally regarded as first-line treatment of patients with infections caused by MRSA, it has been used successfully in combination with various other agents as oral therapy and may also have some value as monotherapy, owing to its in-vitro activity against most staphylococci, including MRSA. Trimethoprim has the added advantages of being inexpensive, being available as an oral formulation, being well absorbed and having an excellent safety profile. In common with antibiotics in general, any apparent increase in the incidence of resistance to trimethoprim will limit its potential as treatment and the investigations described here were undertaken in an attempt to identify an explanation for this phenomenon.

The susceptibilities of 100 non-replicate clinical isolates of MRSA to trimethoprim were determined in duplicate by three different methods. Vitek GPS-AK cards were inoculated, susceptibilities read according to the manufacturer’s instructions and susceptibility categories assigned on the basis of MIC breakpoints recommended by the National Committee for Clinical Laboratory Standards (NCCLS), i.e. susceptible, MIC ≤2 mg/L and resistant, MIC ≥4 mg/L. The Stokes’ disc diffusion method was performed according to a method described previously with DST agar (Oxoid, Basingstoke, UK) supplemented with 2% lysed horse blood, discs (Oxoid) containing 2.5 μg of trimethoprim and inocula with turbidities equivalent to that of a 0.5 McFarland standard. The Oxford strain of S. aureus was used as the control and the zones of inhibition were read after overnight incubation at 37°C. Susceptibility was defined as a zone of inhibition greater than or equal to that of the control or not >3 mm smaller and resistance as a zone of inhibition ≥3 mm smaller than that of the control. Finally, MICs were determined by an agar dilution method recommended by the NCCLS, an MIC breakpoint of ≤2 mg/L was used to define susceptibility.

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In summary, the susceptibilities of 100 clinical isolates of MRSA to trimethoprim, as determined by the disc diffusion and agar dilution methods, were identical, with 87 strains being categorized as susceptible. The Vitek GPS-AK card, on the other hand, incorrectly designated 32 of these 87 strains as resistant—a major error rate of 37%. Moreover, the results of susceptibility testing of a further 19 strains were not reproducible and, therefore, inconclusive. These observations suggest that the Vitek GPS-AK card is an unreliable method of determining the susceptibilities of staphylococci to trimethoprim.

References


**Mycobacterium tuberculosis rpoB gene DNA sequencing: implications for detection of rifampicin resistance**


Vitali Sintchenko*, Peter J. Jelfs, William K. Chew and Gwendolyn L. Gilbert

Centre for Infectious Diseases and Microbiology Laboratory Services, Institute of Clinical Pathology and Medical Research, Westmead Hospital, Westmead, New South Wales, Australia

*Corresponding author. Tel: +61-2-9845-6255; Fax: +61-2-9893-8659; E-mail: vitalis@icpmr.wsahs.nsw.gov.au*

Sir,

There is a growing demand from clinicians for rapid molecular diagnostic tests for multidrug-resistant *Mycobacterium tuberculosis* (MDRTB) detection. In their recent paper, Yang et al. have drawn attention to the contribution of different insertion, deletion and missense mutations within a hypervariable region of *rpoB*, the gene encoding the β-subunit of the DNA-dependent RNA polymerase of *M. tuberculosis*, in the development of resistance to rifamycins. They confirmed the observation of Bodmer et al. that the activity of rifabutin in rifampicin-resistant isolates depends on both the mutation position and the type of amino acid change in the *rpoB* gene. High-frequency mutations in codons 531, 526 and 516 may serve as predictors of resistance to rifampicin, which itself is a useful surrogate marker for MDRTB. The authors have further extended the pool of low-frequency genetic alterations in this region associated with in-vitro resistance to rifampicin and rifabutin. However, the clinical implications and the diagnostic utility of their detection remain controversial.

To illustrate this point we now report a previously uncharacterized mutation in the *rpoB* gene of a multidrug-resistant clinical isolate of *M. tuberculosis* from the sputum of a 31-year-old man who had recently migrated to Australia from Pakistan. He had a long history of chest infections and diabetes mellitus. His pulmonary tuberculosis failed to respond clinically and bacteriologically to fully supervised standard first-line antituberculous treatment and subsequent therapy with ethambutol, pyrazinamide, capreomycin, amikacin and ciprofloxacin. Clinical recovery and sputum culture conversion occurred only after radical surgical lobectomy.

Susceptibility testing on the isolate was performed by the BACTEC 460 (Becton Dickinson, Towson, MD, USA) radiometric method. The isolate was resistant to rifampicin (MIC ≥2 mg/L), isoniazid and streptomycin, and sensitive to rifabutin (MIC 0.25 mg/L). The commercially available line probe assay (INNO-LiPA Rif.TB; Innogenetics NV, Haven, Belgium), which was performed according to the manufacturer’s instructions, showed a wild-type pattern suggesting in-vitro sensitivity to rifampicin. To resolve this contradiction the rifampicin resistance-determining 69 bp region of the *rpoB* gene was amplified as described by Ohno et al. except that 1U of AmpliTaq DNA polymerase (Perkin–Elmer, Norwalk, CT, USA) per reaction mixture was used. The cycling protocol was: initial denaturation, 95°C for 5 min following by 30 cycles of 1 min at 95°C, 1 min at 55°C and 1 min at 72°C, with a final cycle of 10 min at 72°C. The presence of a 260-bp product in 2% agarose gel indicated a successful amplification of mycobacterium DNA. Amplified DNA was directly sequenced on an automated sequencer with fluorescence-labelled dideoxynucleotide terminators (Model 373, ABI Prism, Perkin–Elmer, Foster City, CA, USA) with IP1 and IP2 primers. DNA sequence analysis revealed a single-point nucleotide substitution affecting the codon of Gln516 (Escherichia coli *rpoB* codon numbering system) (CAG→CAA) (GenBank accession number AF112973). Neither insertion nor deletion mutations were found.

This case provides evidence to support the opinion that rapid molecular susceptibility testing of *M. tuberculosis* must be confirmed by conventional rifampicin susceptibility testing. At the same time, it also demonstrates the role of DNA sequence analysis for the detection of new genetic alterations. As the activity of rifamycins in *M. tuberculosis* largely depends on both the mutation position and the type of substitution in the *rpoB* gene, this region of clinical isolates with rifampicin-resistant phenotype should be sequenced to identify resistant alleles. Other clusters of mutations in the *rpoB* gene or other target sites can be associated with resistance to rifampicin and rifabutin.

In addition, there are many opportunities for different kinds of mutations that are not critical for the binding
activity of RNA polymerase. We believe that the recent successful sequencing of the entire genome of *M. tuberculosis* will facilitate our understanding of its drug resistance.

Detection of low-frequency *M. tuberculosis* mutants that remain susceptible to rifabutin is important, considering the limited management options for MDRTB infection. Our analysis of 22 MDRTB isolates in Australia in 1996–1998 revealed that four (18%) rifampicin-resistant MDRTB isolates were rifabutin-sensitive. All of them carried low-frequency *rpoB* gene alterations in codons 516 and 522.8

Several PCR-based methods including heteroduplex formation, single strand conformation polymorphism, direct DNA sequencing and LiPA can be used for rapid detection of rifamycin resistance in clinical isolates.7 INNO-LiPA can detect only high-frequency mutations3 and most other PCR-based methods detect only the presence of a mutation, not the actual substitution in the *rpoB* gene. This limits our ability to estimate the level of resistance to rifamycins and may lead to false-positive and false-negative reports. Nevertheless, there is a need to quantify antibiotic resistance in order to manage and control MDRTB.

In conclusion, we suggest that PCR analysis should be supported by DNA sequencing of relevant MDRTB target genes. Cumulative data concerning the relationship between the phenotype, clinical response, and type and position of mutations are recommended to facilitate the rapid response required to limit the extent and severity of MDRTB transmission and infection.

References


In-vitro activity of gatifloxacin, a novel fluoroquinolone, compared with that of ciprofloxacin against *Legionella* spp.

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Susan L. Pendland*, Karen J. Losnedahl and Christopher A. Schriever

The University of Illinois at Chicago, Department of Pharmacy Practice, Microbiology Research Laboratory, 833 South Wood Street, Chicago, IL 60612, USA

*Corresponding author. Tel: +1-312-996-8639; Fax: +1-312-413-1797; E-mail: Pendland@uic.edu

Sir,

Gatifloxacin, also known as BMS-206584, AM-1155 and CG-5501, is a novel 8-methoxy fluoroquinolone with activity against a broad-spectrum of bacteria. In the present study we have evaluated the in-vitro activity of this agent against *Legionella* spp. and compared it with that of the older fluoroquinolone, ciprofloxacin, which has previously been shown to be active in-vitro against these pathogens and to be effective when used as treatment of patients with infections caused by them.1,2

Gatifloxacin was obtained from Bristol-Myers Squibb (Wallington, CT, USA) and was prepared according to the manufacturer’s instructions with dimethylsulphoxide (DMSO) as the solvent and sterile distilled water as the diluent. Ciprofloxacin, which was obtained from United States Pharmacopeia (Rockville, MD, USA), was prepared according to guidelines of the National Committee for Clinical Laboratory Standards (NCCLS).3 The organisms used in the study were provided by the University of Illinois Hospital (Chicago, IL, USA), Northwestern University Hospital (Chicago, IL, USA), Abbott Laboratories (Chicago, IL, USA), the Centers for Disease Control (Atlanta, GA, USA) and the American Type Culture Collection (ATCC) (Rockville, MD, USA), and included 41 non-replicate clinical isolates and five ATCC strains (*Legionella pneumophila* 33152, *Legionella bozemanii

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The MICs of gatifloxacin and ciprofloxacin are summarized in the Table. The MIC$_{50}$s of both quinolones for the *L. pneumophila* and non-*pneumophila* *Legionella* spp. strains were 1 mg/L on BCYE$_\alpha$ and 0.03 mg/L on BSYE. The lower MICs obtained with the non-charcoal-containing medium are believed to more accurately reflect the susceptibilities of *Legionella* spp. isolates. Unfortunately, however, BSYE and other less antagonistic media do not support the growth of all strains.$^{4,5}$

The data reported here demonstrate that gatifloxacin is active in vitro against *Legionella* spp. (both *L. pneumophila* and non-*pneumophila* *Legionella* spp.), the MIC$_{50}$s of this agent being identical to those of ciprofloxacin. On the basis of MIC breakpoints of <2 mg/L for gatifloxacin$^{7}$ and <1 mg/L for ciprofloxacin,$^{6}$ all of the isolates were considered highly susceptible to both agents when MICs were determined with BSYE. When susceptibility testing was performed with BCYE$_\alpha$, all of the strains were susceptible to gatifloxacin and all but one strain of *L. pneumophila*, which exhibited intermediate susceptibility (MIC, 2 mg/L), were susceptible to ciprofloxacin. In a study recently published in this Journal, Croco et al., who used the Etest method with supplemented BCYE$_\alpha$ agar, reported the MIC$_{50}$ of gatifloxacin for 103 *Legionella* spp. isolates to be 0.38 mg/L (range 0.125–0.5 mg/L).$^{6}$ This value is approximately three-fold lower than those obtained in the present study with the agar dilution method and the same medium, but >10-fold higher than those obtained with BSYE.

In the light of the good in-vitro activity of gatifloxacin against *Legionella* spp. isolates, as demonstrated by a range of susceptibility testing methods, we believe that this antibiotic warrants further investigation as treatment of patients with infections caused by these organisms.

Acknowledgement

This work was supported by a grant from Bristol-Myers Squibb.

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<th>Table. MICs of gatifloxacin and ciprofloxacin for <em>Legionella</em> spp. strains, as determined with two different media</th>
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<td><strong>Medium</strong></td>
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*Only 33 and six strains of *L. pneumophila* and non-*pneumophila* *Legionella* spp., respectively, were tested on BSYE, the remaining seven strains having failed to grow on this medium.*
Polyhexamethylene biguanide (PHMB), a contact lens disinfectant, has been shown to have excellent in-vitro activity against a broad range of fungal pathogens and to be effective and well tolerated at concentrations of 200 mg/L (0.02%) when used as treatment of patients with keratitis caused by Acanthamoeba spp. The present study was undertaken to evaluate the in-vitro activity of PHMB against fungal isolates associated with infective keratitis.

The organisms used in the study included 25 isolates of C. albicans which were isolated from patients at the University of Illinois Hospital, Chicago, IL, USA (n = 10), Columbia Wesley Medical Center, Wichita, KS, USA (n = 10) and St Vincent’s Mercy Medical Center, Toledo, OH, USA (n = 5), a single isolate of Aspergillus niger which was recovered from a patient at the University of Illinois Hospital, and the following four strains which were provided by the American Type Culture Collection, Rockville, MD, USA: C. albicans 24433; Candida parapsilosis 22019; Candida krusei 6258 and Fusarium solani 44366. PHMB 20% was obtained from Zeneca Biocides, Wilmington, DE, USA.

References


In-vitro activity of polyhexamethylene biguanide (PHMB) against fungal isolates associated with infective keratitis


Chad R. Messick, Susan L. Pendland, Majid Mosshifard, Richard G. Fiscella, Karen J. Losnedahl, Christopher A. Schriever and Paul C. Schreckenberger

*a*Veterans Affairs Cooperative Studies Program, *Clinical Research Pharmacy Coordinating Center, Albuquerque, NM; b*Microbiology Research Laboratory and Departments of *c*Pharmacy Practice, *d*Ophthalmology and *e*Pathology, *University of Illinois at Chicago, Chicago, IL; *f*John A. Moran Eye Center, Salt Lake City, UT, USA

*Correspondence address. University of Illinois, College of Pharmacy, Department of Pharmacy Practice (M/C 886), 833 South Wood Street, Chicago, IL 60612, USA. Tel: +1-312-996-8639; Fax: +1-312-413-1797; E-mail: pendland@uic.edu

Sir,

Fungal keratitis is an infection that is difficult both to diagnose and to treat. *Candida albicans*, other yeasts and the septate moulds, *Fusarium* spp. and *Aspergillus* spp., are common causes of this disease. Treatment options are limited and include topical formulations of natamycin (5%), amphotericin B (0.15%) and fluconazole (1%). However, the efficacies of these agents are compromised by poor ocular penetration, poor tolerability and/or poor in-vitro activity. Polyhexamethylene biguanide (PHMB), which is currently used as an environmental biocide and contact lens disinfectant, has been shown to have excellent in-vitro activity against a broad range of fungal pathogens and to be effective and well tolerated at concentrations of 200 mg/L (0.02%) when used as treatment of patients with keratitis caused by *Acanthamoeba* spp. The present study was undertaken to evaluate the in-vitro activity of PHMB against fungal isolates associated with infective keratitis.

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Antimicrobial resistance levels of enterobacteria isolated from minced meat


M. Österblad, E. Kilpi, A. Hakanen, L. Palmu and P. Huovinen

Antimicrobial Research Laboratory, National Public Health Institute, PO Box 57, FIN-20521 Turku; Municipal Food Laboratory of Turku, Kirkkötie 13, 20540 Turku, Finland

*Corresponding author. Tel: +358-2-2519255; Fax: +358-2-2519254.

Sir,

Antibiotic resistance among the members of the family Enterobacteriaceae is often high and can cause major clinical problems. In the normal flora they have also been found to harbour resistant strains at high frequencies. Some of this resistance could originate from food. In a study of enterobacteria on vegetables, we found very low...
resistance levels, including plasmid-mediated.\(^1\) We hypothesized that the corresponding population on meat might be different, since the risk of faecal contamination is much greater, and resistance in animal faecal flora could be high as a consequence of antibiotic use in animal husbandry. In contrast to vegetables, meat is not normally consumed raw, but can easily cross-contaminate other foods.

Samples of minced meat, collected by the local food surveillance laboratory (six beef, 20 beef–pork, locally produced), were plated onto MacConkey agar plates for the selective cultivation of enterobacteria. All colonies of different morphology were streaked for purity. Gram-negative, oxidase-negative, glucose-fermenting bacilli were further identified to at least genus level by a set of 22 standard biochemical tests. MICs were done by a standard agar dilution method, using NCCLS breakpoints. Since most enterobacteria have intrinsic resistance to penicillins and older cephalosporins, results for these are not presented. In all, 150 isolates were tested. Duplicate isolates from a sample which appeared to be the same based on biochemical and MIC profiles, were excluded from the analysis, leaving 131 isolates.

The most common genera were *Serratia* (*n* = 37), *Hafnia* (*n* = 22), *Yersinia* (*n* = 13) and 10 each of *Citrobacter*, *Klebsiella* and unnamed\(^2\) Enteric Groups. Only four *Escherichia coli* were found, which would indicate that the Enterobacteriaceae found were of environmental, not animal faecal, origin.

The resistance levels were of the same magnitude as in our earlier study on vegetables.\(^1\) Resistance was not found to piperacillin/tazobactam, cefotaxime, aztreonam, imipenem, gentamicin, nalidixic acid and ciprofloxacin. Most of the cefuroxime resistance found (Table) was caused by endogenous chromosomal \(\beta\)-lactamases; 15 of 18 resistant, and 15 of 17 intermediately resistant isolates were among *Serratia* spp. *Serratia* spp. have an AmpC enzyme that, when produced at high levels, also gives resistance to newer cephalosporins. We also saw very little evidence of the kind of transferable plasmid-mediated multi-resistance that is common in human strains. Only three strains with probable plasmid-mediated multiresistance were found (sulphamethoxazole-trimethoprim-streptomycin-tetracycline, *Klebsiella oxytoca*; sulphamethoxazole-chloramphenicol-tetracycline, *Hafnia alvei*; sulphamethoxazole-trimethoprim, *Enterobacter* sp.).

One reason for the low levels of resistance in this study might be the technique employed; selective plating with antibiotics is more sensitive. However, other workers have found high levels of resistance in ‘coliforms’ isolated from meat using methods corresponding to ours,\(^3\) and we have employed a similar method in the study of human faecal flora; in that study, 14% of samples demonstrated resistance to trimethoprim, 19% to sulphamethoxazole and 25% to tetracycline.\(^4,5\) A possible explanation is the species distribution. The small numbers of *E. coli* found here, indicating a low level of faecal contamination, could also have affected the overall resistance levels: in one study of human faecal flora, *E. coli* was found to be more resistant than other enterobacteria.\(^6\) This difference between species is usually not seen with clinical isolates, however.

Enterobacteria from minced meat do not seem to contribute to the relatively high levels of antimicrobial resistance of enterobacteria we have found in human faecal flora in Finland. Thus excluding food, and indirectly the environment, as an important source of resistance, the evidence points ever more strongly towards the human use of antibiotics as the most important factor maintaining high resistance levels in our normal flora. The resistance might arise elsewhere too, e.g. in animal husbandry, and occasionally transfer to humans, where antibiotic use would favour its spread. In countries where the use of antimicrobials in animal husbandry is greater than in Finland, and contamination of meat by faecal matter at the time of slaughter perhaps more common, the results of a study such as this might be very different.

### References


### Table

Percentage resistant enterobacteria in isolates from minced meat

<table>
<thead>
<tr>
<th>Antibiotic (resistance breakpoint)</th>
<th>% resistant strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefuroxime (≥16 mg/L)</td>
<td>13.7</td>
</tr>
<tr>
<td>Chloramphenicol (≥32 mg/L)</td>
<td>0.8</td>
</tr>
<tr>
<td>Streptomycin (≥32 mg/L)</td>
<td>3.8</td>
</tr>
<tr>
<td>Sulphamethoxazole (≥512 mg/L)</td>
<td>1.5</td>
</tr>
<tr>
<td>Tetracycline (≥16 mg/L)</td>
<td>1.5</td>
</tr>
<tr>
<td>Trimethoprim (≥16 mg/L)</td>
<td>2.3</td>
</tr>
</tbody>
</table>
Continuing high prevalence of methicillin resistance amongst Staphylococcus aureus blood culture isolates

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Department of Clinical Microbiology, Beaumont Hospital and Royal College of Surgeons in Ireland, Dublin 9, Ireland

*Tel: +353-1-809-2667; Fax: +353-1-809-2995.

Sir,

We read with interest a recent article in the *Journal* about the increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) blood culture isolates referred to the Public Health Laboratory Service in the UK.\(^1\) Since the computerization of our laboratory in 1993, we too have been monitoring blood culture isolate data at Beaumont Hospital, Dublin, a 600 bed teaching hospital with a busy intensive care unit, and national neurosurgical and renal transplant reference centres. *S. aureus* was the second most common bloodstream isolate, accounting for, on average, 20% of positive blood cultures during this period. Our data reveal a relatively stable percentage of strains of MRSA among all episodes of bacteraemia caused by *S. aureus*, ranging from 26% in 1997 to 42% in 1994; the percentage for 1998 was 34%. These figures are similar to those reported by centres in Spain and the USA.\(^2,3\) A national survey of MRSA isolates conducted in Ireland in 1995 showed that the period prevalence was 16.5/1000 hospital patient discharges and highlighted the endemicity of this bacterium in many Irish hospitals.\(^4\)

In common with Johnson *et al.*,\(^5\) we are concerned about the emerging threat of glycopeptide resistance among isolates of MRSA, as well as the controversy regarding the relative virulences of MRSA and methicillin-susceptible *S. aureus* (MSSA). Comparing the mortality rate in patients with MRSA bacteraemia with that in patients with MSSA bacteraemia can be unreliable, as patients with MRSA infection tend to be older and more debilitated, and the administration of appropriate antimicrobial therapy is often delayed if the initial empirical therapy is, as is often the case, a β-lactam. None the less, the authors of a recent report from Spain demonstrated a higher mortality rate amongst patients with MRSA bacteraemia and we have also documented a higher mortality rate in patients with hospital-acquired MRSA bacteraemia (22% compared with 3% in patients with MSSA bacteraemia).\(^5\)

The cost of a 1 week course of treatment of a patient with MSSA bacteraemia in Ireland, i.e. iv flucloxacillin 1 g qds, is IR£136.08, compared with IR£293.16 (excluding drug assay costs) for a comparable course of therapy of a patient with a bloodstream infection caused by MRSA, i.e. vancomycin 1 g bd. Therefore, bacteraemic episodes caused by MRSA have important cost implications and, in our hospital, therapy and preventative measures consume considerable financial resources. In contrast, the prevalences of MRSA bacteraemia in other countries such as Denmark (0.2%)\(^6\) are strikingly low. This may be the result of higher standards of infection control, more appropriate use of antibiotics and/or better medical facilities. Those countries in which the prevalences of MRSA are high or increasing should learn from those in which MRSA is rarely encountered. Therefore, while allowing for contrasting demographics and risk factors, a comparison of surveillance data from various countries should help to highlight differences in approach and facilitate greater efforts to prevent bacteraemias and other serious infections caused by MRSA.

References


Determination of sparfloxacin and its degradation products by HPLC-PDA


H. R. N. Marona*ª, J. A. S. Zuanazziª and E. E. S. Schapovalª

ªFaculdade de Ciências Farmacêuticas, UNESP, Araraquara; ªCurso de Pós-Graduação em Ciências Farmacêuticas, UFRGS, Av. Ipiranga, 2752, Porto Alegre, CEP. 90610-000, Brazil

*Corresponding author.
E-mail: hmarona@hotmail.com

Sparfloxacin, a quinolone carboxylic acid derivative is active, as an antimicrobial agent, against a wide range of Gram-positive and Gram-negative organisms including mycobacteria1–2. A drawback of fluoroquinolones is their photoreactivity3–5, and though sparfloxacin has been studied in terms of therapeutic activities1–2, few reports about its physicochemical analysis are available in the literature6.

Due to the photosensitivity of sparfloxacin3, the aim of this work was to develop an easy, rapid and sensitive method to determine the presence of any photodegradation products in powder. An accelerated study of stability in aqueous solution was carried out by subjecting a solution of sparfloxacin to UV light (peak wavelength 290 nm) for 5 h at room temperature. Sparfloxacin powder (purity 99.5%) was supplied by Dainippon Pharmaceutical Co., Osaka, Japan and Rhone-Poulenc Rorer, USA. All other chemicals used were of analytical grade.

HPLC analysis was performed on a Waters SCL-6A chromatograph equipped with a model LC-10AS pump; SPD-10A variable-wavelength detector (set at 292 nm); SCL-10A system controller; C-R6A integrator and Rheodine injection valve with a 20 L loop. A Shim-pack CLC-ODS column (250 mm × 4.6 mm I.D., 5 µm particle size, 100 pore diameter) was used with aqueous acetic acid 5%:methanol:acetonitrile (80:10:10, v/v/v) isocratic as mobile phase at a flow-rate of 1.0 mL/min. The mobile phase was filtered by membrane filter (Supelco) 0.45 m × 47 mm and degassed with helium sparge for 15 min. To photodegrade sparfloxacin, a fresh solution (1mg/mL) was submitted to UV light (290 nm) for 24 h and 36 h in a chamber (10 × 10 × 90cm). Sparfloxacin tablets (200 mg): Ten tablets were ground up and five times the average weight were transferred to prepare 1000 mg/L solution. This solution was placed in Petri dishes in the UV light chamber for 24 h.

The applicability of the proposed method for the determination of sparfloxacin and its degradation products was demonstrated by analysing six aliquots of reference substance. The HPLC data show that sparfloxacin is sensitive to photodegradation under the conditions used in this study. A 200–400 nm scan HPLC chromatogram, five main degradation products were detected, along with several other minor peaks with poor resolution between 1 and 3 min. The total area was made with the area for the five peaks. The UV-spectra (200–400 nm) of these peaks have an absorption profile similar to the reference substance. The second largest peak corresponded to sparfloxacin (10.3 min, 32%), followed by another more apolar at 11.5 min (Peak IV, 41%). The UV spectra for the peaks IV and II (6.7 min, 13%) are similar and show a bathochromic shift (c. 8 nm) from sparfloxacin. However, photoproducts I and III, 5.9 min (11%) and 7.6 min (4%), respectively, show a hypsochromic shift (c. 4nm) of the absorption maximum in the UV spectrum from sparfloxacin. Peak II also shows a shoulder which possibly indicates the existence of two products. The results of these analyses are shown in the Figure. The isolation of these photoproducts will be performed by preparative HPLC and their chemical structures determined by NMR, MS, UV and IR spectra.

The results have shown that the substance studied is sensitive to photodegradation. Finally, a large decrease in the concentration of sparfloxacin after exposure to UV light was observed and detected in HPLC assay. This decrease is an important source of concern and suggests further studies about its photodegradation mechanism. The existence of photoproducts can induce side effects and toxicity as well as loss of activity expected for the treatment.

Figure 1. HPLC chromatogram of sparfloxacin and its degradation products. Detection UV- Photodiode array (scan UV total 200–400 nm).
Acknowledgement

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References


