Carbon dioxide requirements of Streptococcus pneumoniae and Haemophilus influenzae


Paul G. Flanagan* and A Ian Paull

Department of Medical Microbiology and Public Health Laboratory, University Hospital of Wales, Heath Park, Cardiff CF4 4XN, UK

* Tel: +44-(0)1222-745343/746580;
Fax: +44-(0)1222-742161;
E-mail: flanaganpg@cf.ac.uk

Sir,

It is common practice for sputum cultures to be incubated in an atmosphere containing 5% CO₂ in order to maximize the isolation rate of Streptococcus pneumoniae and Haemophilus influenzae. Plates on which the susceptibilities of strains of S. pneumoniae are determined by the disc diffusion method are also routinely incubated in such an atmosphere. It has been shown, however, that CO₂ can influence the diameter of the zone of inhibition when the Stokes or comparative method of susceptibility testing is used. In our experience, concentrations of CO₂ lower than 5% are adequate to support the growth of those strains of H. influenzae and pneumococci described as CO₂-dependent. We have also found that most pneumococci will grow in an aerobic atmosphere following initial isolation. In the present study, we investigated the CO₂ growth requirements of both pathogens on primary culture of the samples and on subculture of stored isolates recovered from various body sites. We also evaluated the effect of CO₂ on the results of susceptibility testing of pneumococci by the disc diffusion method.

Samples of sputum submitted to the routine diagnostic laboratory were homogenized with dithiothreitol (Oxoid, Basingstoke, UK) at a concentration of 1 g/L, and 10 µL aliquots were inoculated on to each of two chocolate agar plates by a standard streak method. After incubation for 18 h, the weights of growth of the same isolates incubated in 5% CO₂ were then inoculated on to four chocolate agar plates, each of which was incubated under one of the following atmospheric conditions: (i) an aerobic atmosphere without added CO₂; (ii) a CO₂ incubator (providing an atmosphere containing 5% CO₂); (iii) a candle jar; and (iv) a sealed container into which an investigator had breathed, thereby producing low levels of CO₂ (the ‘huff jar’). After overnight incubation, weights of growth were assessed semiquantitatively as described above. Finally, the susceptibilities of pneumococcal isolates to oxacillin (1 µg disc), erythromycin (5 µg disc), trimethoprim (2.5 µg disc) and tetracycline (10 µg disc) were determined by the comparative method. Each isolate was suspended in peptone water to give a turbidity equivalent to that of a 0.5 McFarland standard and 10 µL aliquots of the suspension were then inoculated on to four agar plates containing 5% lysed blood. After the discs had been applied, one plate was incubated under each of the four atmospheric conditions described above; the Oxford strain of Staphylococcus aureus (NCTC 6571) was used as a control. The diameters of the zones of inhibition were measured following incubation for 18 h.

Forty-five strains of S. pneumoniae and 21 of H. influenzae were isolated on primary culture of the samples of sputum; all of the H. influenzae isolates and 44 of the 45 isolates of S. pneumoniae grew aerobically. For only two strains each of H. influenzae and S. pneumoniae were the differences in the weights of growth between samples incubated aerobically and those incubated in an atmosphere containing 5% CO₂ greater than two units of the scale used. Of the stored isolates, the weights of growth of 39 of 47 pneumococci and 37 of 52 H. influenzae strains incubated aerobically were within one scale unit of the weights of growth of the same isolates incubated in 5% CO₂. Four of 47 pneumococci and seven of 52 strains of H. influenzae required CO₂ for growth on subculture, although all grew adequately in the candle and huff jars.

The susceptibilities of 42 pneumococcal isolates were determined. Discrepant results were observed in respect of seven isolates when the diameters of the zones of inhibition obtained under the four atmospheres of incubation were compared. For two strains, the diameters of the zones around the oxacillin discs following incubation in the CO₂ incubator, the candle jar or the huff jar were 2–5 mm smaller than those obtained under aerobic conditions of incubation. Similarly, the zone diameters produced by erythromycin and tetracycline for...
one strain each and by trimethoprim for three strains were reduced (by 2, 6 and 6–7 mm, respectively) after incubation in a CO₂-containing atmosphere compared with those obtained following incubation in the aerobic atmosphere. None of the seven isolates was CO₂-dependent and in all cases, the susceptibility categories for the relevant antibiotics were downgraded from susceptible in the aerobic atmosphere to intermediate susceptibility in the CO₂-containing atmosphere.

The results of this study demonstrate that most pneumococcal and H. influenzae isolates will grow aerobically on primary culture and that CO₂-dependent pneumococci and H. influenzae grow adequately in either a candle or huff jar. In addition, when determining the susceptibilities of pneumococci to oxacillin, erythromycin, trimethoprim and tetracycline, accurate results are obtained for the majority of pneumococci without relying on incubation in an atmosphere containing 5% CO₂. On the basis of this evidence, it is questionable whether CO₂ incubators are necessary for culturing sputum samples when cheaper and equally effective options are readily available.

**References**


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**Comparative in-vitro activity of levofloxacin against Chlamydia spp.**


M. Donati, F. Rumpianes, F. Marchetti, V. Sambri and R. Cevenini*

*Sezione di Microbiologia, D M CSS, O spedale S. O rso, Bologna, Italy; *Medical Department, Hoechst Marion Roussel, Milan, Italy

*Corresponding author. Tel: +39-51-341652; Fax: +39-51-341632.

Sir,

Chlamydiae are obligate intracellular, Gram-negative bacteria, four species of which have been identified to date: Chlamydia pneumoniae, Chlamydia psittaci, Chlamydia trachomatis and Chlamydia pecorum. C. pneumoniae and C. psittaci cause respiratory tract infections, while C. trachomatis is associated with infections of the upper and lower respiratory tracts, the genitourinary tract and the conjunctivae; the pathogenicity of C. pecorum is uncertain.

Tetracyclines are regarded as the drugs of choice for the treatment of patients with chlamydial infections. However, the well-documented recurrence of symptoms with C. psittaci, the potential for resistance to standard therapy to develop and the existence of strains exhibiting heterotypic resistance to erythromycin, tetracycline and their congeners have stimulated a search for alternative agents. The new fluoroquinolones are characterized by potent in-vitro activities against a broad spectrum of organisms, including Mycoplasma, Legionella and Chlamydia spp. O floxacin, in particular, has been shown to be effective and well-tolerated therapy of patients with infections caused by Chlamydia spp. Levofloxacin, the L-isomer of racemic ofloxacin, is approximately twice as active as ofloxacin and possesses pharmacokinetic properties that allow once-daily dosing. In the present study, we compared the in-vitro activity of levofloxacin with those of ofloxacin and three tetracyclines against strains belonging to three chlamydial species.

Three isolates of C. trachomatis (belonging to serotypes D, E and LGV 2-434/Bu respectively), one of C. psittaci (6B C) and one of C. pneumoniae (IO L-207) were studied. The organisms were propagated in LLC-MK2 cells (a continuous cell line derived from Rhesus monkey kidney tissue) which were grown on coverslip culture in Eagle’s minimum essential medium, supplemented with 10% fetal calf serum, in 24-well microtitre plates. Levofloxacin and ofloxacin were supplied by Hoechst Marion Roussel and tetracycline, minocycline and doxycycline were obtained from Sigma Chemical Co. MICs were determined by inoculating growth medium containing cycloheximide (1 mg/L) and glucose (5 mg/L) with each strain to give a
Correspondence

Table. In-vitro susceptibilities of five strains of Chlamydia spp. to two quinolones and three tetracyclines

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC/MBC (mg/L)</th>
<th>C. trachomatis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C. pneumoniae</td>
<td>C. psittaci</td>
</tr>
<tr>
<td>Levofloxacine</td>
<td>0.5/0.5</td>
<td>1.0/1.0</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>1.0/1.0</td>
<td>2.0/2.0</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.125/0.5</td>
<td>0.125/1.0</td>
</tr>
<tr>
<td>Minocycline</td>
<td>0.125/0.5</td>
<td>0.125/0.5</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>0.25/0.5</td>
<td>0.25/1.0</td>
</tr>
</tbody>
</table>

suspension containing $5 \times 10^6$ inclusion-forming units (ifu)/L. The inoculated plates were then centrifuged at 1700g for 1 h, after which the medium was removed and replaced with fresh medium containing cycloheximide and serial two-fold dilutions of each antibiotic. Following incubation for 48 h at 35°C, the coverslips were removed and the cells fixed with methanol and stained with a monoclonal antibody to the lipopolysaccharide genus-specific antigen as described previously. The cells were examined with a Zeiss UV microscope at 400 magnification and the MIC was defined as the lowest concentration of each antibiotic at which no inclusions were seen. MBCs were determined as described previously and the MBC was defined as the lowest concentration of each antibiotic allowing no inclusions after re-incubation of the monolayers in antibiotic-free medium for a further 48 h.

The results are shown in the Table. The MICs of levofloxacin, which ranged from 0.5 mg/L to 1 mg/L, were between half and quarter than those of ofloxacin. The MBCs of both quinolones were the same as the corresponding MICs. Of the tetracyclines tested, minocycline and doxycycline exhibited comparable activities which were marginally better than that of tetracycline (the MICs of the former being half those of the latter). In contrast to the quinolones, the MBCs of the tetracyclines were two to eight times the corresponding MICs. The MICs of each antibiotic for each strain tested differed by no more than one two-fold dilution.

The results of the present study are in accord with those of earlier investigations and confirm that levofloxacin and ofloxacin exhibit bactericidal activities against chlamydial strains in a single growth cycle at concentrations identical to the MICs. The bactericidal activities of the tetracyclines, on the other hand, were achieved at concentrations up to eight times the MICs. Studies designed to evaluate the efficacy of levofloxacin, and to compare this efficacy with those of the tetracyclines, as treatment of patients with chlamydial infections are under way, controlled clinical trials currently being the only means of comparing the activities of antibiotics belonging to different groups in this clinical setting.

References


Correspondence

In-vitro activities of gatifloxacin, sparfloxacin and trovafloxacin against 103 strains of Legionella spp.

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Matthew A. T. Croco, Douglas J. Biedenbach, Michael A. Pfaller, Gary V. Doern and Ronald N. J. Jones

Medical Microbiology Division, Department of Pathology, University of Iowa College of Medicine, Iowa City, IA 52242, USA

*Corresponding author. Tel: +1-319-356-2990; Fax: +1-319-356-4916.

Sir,
The development of novel fluoroquinolones with enhanced activities against Gram-positive bacteria has produced a valuable class of antimicrobial compounds that cover a broad range of bacterial pathogens, including Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis and the so-called ‘atypical’ pathogens associated with respiratory tract infections. Legionella pneumophila continues to be an important ‘atypical’ cause of both nosocomial and community-acquired pneumonias. Antibiotics such as the macrolides and rifampicin have been regarded as the drugs of choice for the treatment of patients with legionellosis because of their excellent in-vitro activities, good intracellular penetration and favourable pharmacokinetic properties. However, other agents, including the fluoroquinolones, have also been shown to be effective therapies. Indeed, the utilization of newer fluoroquinolones (available since 1997) as treatment of patients with community-acquired respiratory tract infections is becoming more widely accepted as their spectra of activity and potencies against common bacterial respiratory pathogens have increased. The present study was undertaken to evaluate the in-vitro activity of the novel quinolone, gatifloxacin (formerly CG 5501, AM-1155), against 103 isolates of Legionella spp. and to compare this activity with those of trovafloxacin and sparfloxacin.

Gatifloxacin was provided by Bristol-Myers Squibb (Princeton, NJ, USA) and trovafloxacin and sparfloxacin were obtained from Pfizer Inc. (Groton, CT, USA) and Rhône-Poulenc Rorer (Collegeville, PA, USA) respectively. The organisms used in the study were 98 epidemic or endemic clinical isolates of L. pneumophila from the collection at the University of Iowa Hospitals and Clinics and five control strains (L. pneumophila ATCC 33152, Legionella bozemanii ATCC 33217, Legionella micdadei ATCC 33218, Legionella dumoffii ATCC 33219 and Legionella gormanii ATCC 33297); Escherichia coli ATCC 25922 and Staphylococcus aureus ATCC 29212 were also included as controls. The isolates were maintained at −70°C until ready for use and, just before susceptibility testing was carried out, they were subcultured twice on buffered charcoal yeast extract (BCYE) agar containing defined supplements (Remel, Lenexa, KS, USA). MICs were determined by the Etest method (AB Biodisk, Solna, Sweden) according to the manufacturer’s instructions and previous experience. The medium used was BCYE agar and the inoculum was prepared by scraping colonies from a 48 h culture, suspending them in broth and adjusting the suspension to give a turbidity equivalent to that of a 0.5 McFarland standard. The MICs were recorded after incubation for 48 h at 35°C in a candle jar.

The results are summarized in the Table. All three quinolones inhibited the Legionella spp. isolates at concentrations of ≤1 mg/L. Trovafloxacin (MIC<sub>90</sub> = 0.19 mg/L) was twice as active as gatifloxacin and sparfloxacin, the activities of which were identical (MIC<sub>90</sub> = 0.38 mg/L). On the basis of tentative MIC breakpoints, all of the isolates were categorized as susceptible to the three drugs.

The Etest is a simple and reliable method of determining the susceptibilities of Legionella spp. isolates to many antibiotics, including the fluoroquinolones, and the results obtained with it are comparable to those obtained with reference methods. Inactivation of antibiotics by charcoal or other constituents of the preferred medium, BCYE, has been minimal when the MICs for non-

Table. In-vitro activities of gatifloxacin, sparfloxacin and trovafloxacin against 103 isolates of Legionella spp.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt; (mg/L)</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt; (mg/L)</th>
<th>range (mg/L)</th>
<th>% susceptible isolates&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gatifloxacin</td>
<td>0.19</td>
<td>0.38</td>
<td>0.125-0.5</td>
<td>100</td>
</tr>
<tr>
<td>Sparfloxacin</td>
<td>0.19</td>
<td>0.38</td>
<td>0.094-1</td>
<td>100</td>
</tr>
<tr>
<td>Trovafloxacin</td>
<td>0.125</td>
<td>0.19</td>
<td>0.064-0.38</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup>Based on tentative MIC breakpoints of ≤2 mg/L for gatifloxacin<sup>6</sup> and ≤1 mg/L for sparfloxacin and trovafloxacin (US Food and Drug Administration product package insert).
legionella control strains were determined with the
E test.

A ll three fluoroquinolones were shown here to possess
tent in vitro activities against an important group of
respiratory tract pathogens. The MICs of trovafloxacin
and sparfloxacin were similar to those reported previously
by Marco et al. and Rhomberg & Jones, respectively,
who also used the Etest. On the basis of the MIC
susceptibility breakpoints recommended for the quino-
lines (either \( \leq 1 \) mg/L or \( \leq 2 \) mg/L), none of the isolates
was categorized as resistant to the antibiotics tested;
indeed, the highest MIC recorded was 1 mg/L in respect
of sparfloxacin and a single strain. The excellent in vitro
activity of gatifloxacin and its favourable bioavailability
(\( C_{\text{max}} \) of 3.35 mg/L following a 400 mg oral dose) suggest
that it would be effective therapy of patients with
infections caused by Legionella spp.

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Correspondence

G lycopeptide resistance amongst
Staphylococcus spp.

Nicole van den Braak*, A lex van Belkum,
René te Witt, Henri A. V erbrugh and
Hubert P. Endtz

D epartment of Medical Microbiology and Infectious
D iseases, E rasmus University M edical Centre
R otterdam, D r M owaterplein 40,
3015 G D R otterdam, T he N etherlands

*Tel: +31-10-463-5225; Fax: +31-10-463-3875;
E-mail: vandenbraak@bacl.azr.nl

Sir,
The importance of the glycopeptides, vancomycin and
teicoplanin, as treatment of patients with infections caused by
multidrug-resistant Gram-positive bacteria, particu-
larly staphylococci, has increased in recent years. Reports
of staphylococci with reduced susceptibilities to these
antibiotics are therefore cause for concern. Medical micro-
biology laboratories, which represent the first line of
defence against the development of antimicrobial resis-
tance, must be able to identify such strains at an early
stage. The aim of the present study was to determine the
prevalences of Staphylococcus aureus and coagulase-
negative staphylococci (CoNS) blood culture isolates
exhibiting reduced susceptibility to vancomycin.

The susceptibilities to vancomycin of 410 strains of S.
aureus and 400 of CoNS isolated between 1954 and 1997
from hospitalized patients with bacteraemia were deter-
mined by an agar dilution method. The MICs for all
strains were \( \leq 4 \) mg/L and they were therefore categorized
as susceptible on the basis of breakpoints recommended
by the National Committee for Clinical Laboratory Stan-
dards (NCCLS). These results are in accord with recently
published global surveillance data obtained by the same
standard methodology. Eighty-nine (21.7%) of the S.
aureus isolates and 212 (53%) of the CoNS isolates, for
which the MICs of vancomycin were either 2 mg/L or
4 mg/L, were screened for the presence of subpopulations
exhibiting intermediate susceptibility to vancomycin on
Brain Heart Infusion (BHI) agar (Difco, Detroit, MI,
USA) containing the antibiotic at a concentration of
8 mg/L. None of the S. aureus strains, but 20 of the 212
CoNS, grew on the screening plates. Of the 20 CoNS
strains, the MICs of vancomycin for 11 were still 8 mg/L
after nine subcultures on antibiotic-free medium. These 11
strains were subjected to additional population studies.
Approximately \( 10^8 \) CFU of each isolate and two reference
strains (S. aureus HIP 5836 and S. aureus HIP 5827,
provided by Dr F. Tenover) were inoculated on to BHI
agar without vancomycin or containing vancomycin at

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concentrations between 1 mg/L and 10 mg/L. After incubation for 48 h at 37°C, the colonies on plates on which there was growth were counted. The results for two representative strains and the two reference strains are shown in the Figure. The inocula of the S. aureus reference strains and all of the CoNS contained subpopulations that grew in the presence of vancomycin at concentrations ≤8 mg/L and up to 10 mg/L respectively. According to the definition of Tenover et al. and Hiramatsu et al., these 11 strains were classified as heterogeneous glycopeptide-intermediate Staphylococcus spp. (hetero-GISS). Two of the 11 CoNS were identified as Staphylococcus hominis and nine as Staphylococcus epidermidis with the API STAPH system (bioMérieux, Marcy l’Etoile, France). Samples of genomic DNA extracted from the strains were digested with Smal and compared by pulsed-field gel electrophoresis (PFGE) as described previously. The PFGE patterns of the two S. hominis strains were indistinguishable, as were those of six of the nine S. epidermidis strains; the latter clone persisted in our hospital from 1991 to 1997.

We also evaluated the abilities of the following commercial susceptibility test methods to identify heterogeneous intermediate susceptibility to glycopeptides in the 11 isolates: the disc diffusion method, the Etest (AB Biodisk, Solna, Sweden); a BHI agar (BBL Microbiology Systems, Cockeysville, MD, USA) screening plate containing vancomycin at a concentration of 6 mg/L; VITEK 30-well GPS-TA cards (bioMérieux); and Microscan overnight panels (Dade International, West Sacramento, CA, USA). The BHI screening plate and the Etest were the only methods that consistently identified the 11 strains as hetero-GISS. The MICs for all of the strains, as determined by the VITEK system, were 4 mg/L and four of the 11 were categorized as susceptible to vancomycin on the basis of the results obtained with both the Microscan and the disc diffusion method.

Further in-vitro selection of hetero-GISS by a method described previously demonstrated that three strains grew in the presence of vancomycin at concentrations up to 32 mg/L. The MICs of vancomycin and teicoplanin for these isolates were 32 mg/L and 64 mg/L respectively, i.e., resistant to both drugs on the basis of MIC breakpoints recommended by the NCCLS.

In conclusion, in the present study, heterogeneous phenotypes with reduced susceptibilities to vancomycin were detected in 11 (2.8%) of 400 CoNS isolates from blood cultures. Following in-vitro selection, three (0.8%) of the 400 strains exhibited stable subpopulations resistant to both vancomycin and teicoplanin. Commercial susceptibility testing methods may fail to differentiate these hetero-GISS strains from susceptible strains. From 1990 onwards, a single clone of heterogeneous glycopeptide-intermediate S. epidermidis persisted in our hospital. As subclones with reduced susceptibilities to glycopeptides were isolated with ease in the laboratory, this phenomenon may also occur in vivo in patients receiving treatment with glycopeptide antibiotics. Detection of hetero-GISS is a challenge that must be recognized and addressed by all diagnostic laboratories.

References


High prevalence of ciprofloxacin resistance amongst strains of Neisseria gonorrhoeae isolated from commercial sex workers in Bangladesh

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Bahar Uddin Bhuiyan, Ruhul Amin Miah, Motiur Rahman*, Kazi Masihur Rahman and M. John Albert

aInstitute of Postgraduate Medicine and Research, Shahbagh, D haka 1000; bInternational Centre for diarrhoeal Disease Research, Bangladesh, GPO Box 128, D haka 1000, B ngladesh

*Corresponding author. Tel: +880-2-871751; Fax: +880-2-872529; E-mail: motiur@icddrb.org.

Sir,

The control of sexually transmitted diseases (STDs) has assumed greater priority in the public health arena since they were shown to be associated with an increased risk of acquiring infection caused by the human immunodeficiency virus (HIV). Promoting sexual health and reducing the incidence of STDs, including gonorrhoea, are therefore means of preventing HIV infection. However, antibiotic resistance in the gonococcus has itself become an increasingly important public health issue. Because of the emergence of and subsequent increase in the prevalences of strains of penicillinase-producing Neisseria gonorrhoeae (PPNG) and isolates exhibiting chromosomally-mediated resistance to penicillin and tetracycline (CM R N G PT), the Centers for Disease Control in the USA have recommended that third-generation cephalosporins or selected fluoroquinolones, including ciprofloxacin and ofloxacin, should be used as first-line therapy in cases of uncomplicated gonorrhoea. In Bangladesh, ciprofloxacin has been adopted as the drug of choice for the treatment of patients with uncomplicated or suspected gonococcal infection.

Strains of N. gonorrhoeae with reduced susceptibilities to fluoroquinolones have been identified in the Far East, Australia, Africa and Europe and there have been sporadic reports of ciprofloxacin-resistant isolates. Moreover, clinical failures caused by quinolone-resistant gonococci are being described increasingly, although the emergence of these strains has been associated with a decline in the incidence of PPNG. In an attempt to quantify the extent of antimicrobial resistance in gonococci, we have determined the susceptibilities to various antibiotics of 94 strains of N. gonorrhoeae isolated from commercial sex workers in Dhaka between June and November 1997.

The isolates were identified by standard laboratory techniques and by a polymerase chain reaction (PCR) which was used to amplify a 390 bp sequence of the cryptic plasmid. Susceptibilities to penicillin, tetracycline, cefuroxime, ceftriaxone, spectinomycin and ciprofloxacin were determined by disc diffusion and agar dilution methods. The strains were also evaluated for penicillinase production (PPNG), resistance to tetracycline (TR NG), CM R N G PT, chromosomally mediated resistance to penicillin (CM R N G P) and chromosomally mediated resistance to tetracycline (CM R N G T) by methods described previously.

Of the 94 isolates, 11 (11.7%) were resistant (MICs ≥ 1 mg/L), 25 (26.6%) exhibited reduced susceptibility (MICs = 0.125–0.5 mg/L) and 58 (61.7%) were susceptible (MICs ≤ 0.06 mg/L) to ciprofloxacin on the basis of MIC

Table. Susceptibilities to penicillin and tetracycline of gonococcal isolates exhibiting resistance, intermediate susceptibility or susceptibility to ciprofloxacin

<table>
<thead>
<tr>
<th>Susceptibility profile</th>
<th>resistant (n = 11)</th>
<th>intermediate (n = 25)</th>
<th>susceptible (n = 58)</th>
<th>all (n = 94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CM R N G PT</td>
<td>7 (63.6)</td>
<td>6 (24)</td>
<td>15 (25.9)</td>
<td>28 (29.8)</td>
</tr>
<tr>
<td>CM R N G P</td>
<td>1 (9.1)</td>
<td>2 (8)</td>
<td>6 (10.3)</td>
<td>9 (9.6)</td>
</tr>
<tr>
<td>CM R N G T</td>
<td>1 (9.1)</td>
<td>2 (8)</td>
<td>11 (19.0)</td>
<td>14 (14.9)</td>
</tr>
<tr>
<td>TR NG</td>
<td>1 (9.1)</td>
<td>2 (8)</td>
<td>7 (12.1)</td>
<td>10 (10.6)</td>
</tr>
<tr>
<td>PPNG</td>
<td>0</td>
<td>12 (48)</td>
<td>10 (17.2)</td>
<td>22 (23.4)</td>
</tr>
<tr>
<td>Susceptible</td>
<td>1 (9.1)</td>
<td>1 (4)</td>
<td>9 (15.5)</td>
<td>11 (11.7)</td>
</tr>
</tbody>
</table>

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breakpoints recommended by the National Committee for Clinical Laboratory Standards. The MICs of the various antibiotics tested for the 11 ciprofloxacin-resistant strains were as follows: penicillin, 2 mg/L and 2 mg/L, respectively; tetracycline, 2 mg/L and 2 mg/L, respectively; ciprofloxacin, 2 mg/L and 2 mg/L, respectively; cefuroxime, 0.5 mg/L and 1 mg/L, respectively; ceftriaxone, 0.03 mg/L and 0.03 mg/L, respectively; and spectinomycin, 8 mg/L and 16 mg/L, respectively. The distributions of PPNG, TRNG, CMRNG, CMRNG<sup>PT</sup>, CMRNG<sup>P</sup> and CMRNG<sup>T</sup> among the 94 isolates are shown in the Table; all but 11 (11.7%) were resistant to penicillin, tetracycline or both.

To the best of our knowledge, this is the first survey of antimicrobial susceptibility among N. gonorrhoeae strains isolated from commercial sex workers in Bangladesh. The high prevalence of resistance or reduced susceptibility to ciprofloxacin demonstrated in this study undermines confidence in the antibiotics currently regarded as primary therapy of patients with gonorrhoea in Bangladesh. Never the less, the results of this investigation emphasize the need to monitor periodically the antimicrobial susceptibility patterns of gonococcal isolates. Such surveys can provide important information concerning trends in resistance to relevant antibiotics and can thereby facilitate the administration of optimal therapy.

Acknowledgements

We thank Professor J. W. Tapsall, Prince of Wales Hospital, Sydney, Australia, for providing us with N. gonorrhoeae WHO reference strains and other materials. We also thank Mr Manzural Haque of the International Centre for Diarrhoeal Disease Research, Bangladesh for secretarial assistance.

References


Antibiotic usage and methicillin-resistant Staphylococcus aureus: an analysis of causality

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D. A. H III*, T. Herford and D. Parratt

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

*Tel: +44-1382-660111; Fax: +44-1382-641907.

Sir,

Methicillin-resistant Staphylococcus aureus (MRSA), in common with other multidrug-resistant bacteria, is selected by the administration of antibiotics. Asensio et al. demonstrated that patients who had received treatment for > 5 days with cephalosporins were three times more likely to acquire MRSA than those who had not received one of these agents. They also showed that patients who had been given four antibiotics were 15 times more likely to acquire MRSA than those who had not received any of these drugs. The authors concluded that, as MRSA-positive patients had been hospitalized for longer periods than controls, they were more likely both to have received antibiotics and to have acquired MRSA.

The administration to a patient of an antibiotic to which a bacterium is resistant predisposes the patient to colonization by that strain. In the case of MRSA, carriage in the skin folds and anterior nares often precedes the onset of infection. Aquisition of a resistant strain is, therefore, likely to be influenced by the administration of antibiotics that are excreted in high concentrations in sweat. It has recently been demonstrated that ciprofloxacin attains high concentrations in human sweat and promotes colonization of the skin by staphylococci exhibiting resistance to multiple antibiotics. Elimination of the normal skin flora creates a niche which is soon filled by strains tending to be more resistant than the bacteria they have replaced. If MRSA is a particular problem in a hospital, the administration of antibiotics will increase the likelihood of patients becoming colonized with this organism. What has not been determined is precisely how big a role antibiotic administration plays in terms of promoting colonization.
We studied 17 patients newly identified as colonized and/or infected with MRSA (all isolates of which were resistant to ciprofloxacin) and 17 control patients drawn from the same wards. The controls were age-matched within 10 years and had been hospitalized for similar reasons. There was no significant difference between cases and controls in terms of the duration of hospital stay (means of 22.12 days and 20.53 days for controls and MRSA-positive patients respectively) and intravenous lines were present in 15 of 17 patients in both groups. Indwelling urinary catheters were present in 11 (65%) of patients in the MRSA group and seven (41%) of those in the control group; this difference is not statistically significant (two-tailed Fisher’s exact test, P = 0.3). Nine (52.9%) of the patients in the MRSA group and four (23.5%) of the controls had received ciprofloxacin; this difference is not significant (P = 0.16), but not at the 5% level. Five (29.4%) of the MRSA patients, but none of the controls, had been given a cephalosporin; this difference is significant at the 5% level (P = 0.04). Either ciprofloxacin or a cephalosporin had been administered to 14 (82.3%) of the MRSA patients, compared with only four (23.5%) of the patients in the control group; this difference is highly significant (P = 0.01).

The results of this study demonstrate that the administration of either ciprofloxacin or a cephalosporin is significantly associated with the acquisition of MRSA. In contrast, MRSA carriage was not significantly related to the administration of flucloxacillin (four of 17 MRSA-positive patients, compared with five of 17 controls) or co-amoxiclav (seven of 17 MRSA-positive patients, compared with six of 17 controls). It is not clear why some antibiotics to which MRSA strains are resistant influence colonization more than others.

This small pilot study has shown that antibiotic usage promotes colonization and, ultimately, the spread of MRSA in hospital and suggests that limiting the use of cephalosporins and ciprofloxacin is one means of minimizing the selection and dissemination of this bacterium. We are at present undertaking a larger study with the aim of confirming these findings and evaluating the effect of limiting the prescribing of cephalosporins and ciprofloxacin.

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R eferences


C an a single antibiotic policy for the empirical treatment of febrile neutropenic patients be used for all categories of haematology/oncology patient in the same institution?

B. O’Connell*, R. M arcus*, V. B roadbent*, M. V. W illiams and H. L udlam*
Departments of Clinical Microbiology, H haematology, Paediatric Oncology and Oncology, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 2QW, UK

*Correspondence address: Clinical Microbiology and Public Health Laboratory, Box 236, Addenbrooke’s Hospital, Hills Road, Cambridge CB2 2QW, UK. Tel: +44-1223-257036; Fax: +44-1223-242775.

Sir,

A variety of antimicrobial regimens have been adopted as empirical therapy of febrile neutropenic patients. These regimens tend to reflect the spectra and resistance patterns of the pathogens which predominate in a given institution.1,2

A. Addenbrooke’s Hospital is a tertiary referral centre for haematology, oncology and paediatric haematology/oncology patients. The units on which these three types of patient are managed are separated physically, but are located in the same building. A common antibiotic policy has been in use on the three units since 1992. A cording to the policy, ciprofloxacin and fluconazole are administered as prophylaxis if the duration of neutropenia (defined as a neutrophil count <0.5 × 10^9/L) is expected to exceed 7 days and are continued until the neutrophil count rises above 0.5 × 10^9/L; paediatric haematology/oncology patients are also given phenoxymethylpenicillin 4 mg/kg bd. Neutropenic patients who become febrile receive combinations of either azlocillin, gentamicin and vancomycin, ciprofloxacin and benzylpenicillin (if the patient is not already receiving ciprofloxacin as prophylaxis and a central line is not in situ) or ciprofloxacin and vancomycin (if the patient is not already receiving ciprofloxacin as prophylaxis and a central line is in situ). The treatment is reviewed after 72 h and discontinued if there is no obvious site of infection and the blood cultures are sterile. This policy has been evaluated and validated previously.3

In the present study, we reviewed the bacteraemic episodes in patients on each unit for 1996 in order to determine whether or not the protocol was still appropriate.
A bacteraemic episode was defined as the isolation of a microorganism from blood cultures; repeated isolation of the same microorganism within 7 days was regarded as a single episode. Coagulase-negative staphylococci (CoNS) and coryneforms were considered significant if a single strain was recovered from at least two bottles of one blood culture set.

Most of the bacteraemic episodes in patients on all three units were caused by Gram-positive bacteria. However, significantly more episodes in paediatric haematology/oncology patients (42 of 45, 93.3%) were caused by Gram-positive bacteria than in adult haematology patients (68 of 105, 64.8%) and adult oncology patients (22 of 43, 51.2%) (chi-squared test, $P < 0.02$ and $P < 0.001$ respectively). Of 31 bacteraemias caused by a variety of non-fermentative AGNB of environmental origin that were isolated from adult haematology patients ($P < 0.02$), only one bacteraemia caused by an AGNB was detected in the paediatric haematology/oncology patients during the study period.

The susceptibility patterns of the bacteria isolated from the blood cultures of patients on the individual units also differed significantly. The majority of CoNS recovered from both adult haematology and paediatric haematology/oncology patients were methicillin-resistant (68% and 57% respectively), compared with only 37% of CoNS isolated from adult oncology patients ($P < 0.02$). Furthermore, the incidences of resistance to ciprofloxacin, gentamicin and meropenem amongst AGNB isolated from adult haematology patients were significantly higher than those amongst AGNB isolated from adult oncology patients (chi-squared test with Yates correction, $P < 0.001$, $P < 0.01$ and $P < 0.05$ respectively). Of 31 bacteraemias caused by AGNB in adult haematology patients, 20 (64.5%) were caused by ciprofloxacin-resistant strains (Stenotrophomonas maltophilia (eight), P. aeruginosa (two), non-aeruginosa Pseudomonas spp. (two), Agrobacter radiobacter (one), Burkholderia cepacia (one), Alcaligenes sp. (one), Escherichia coli (one) and Klebsiella spp. (four)), ten (32.2%) by gentamicin-resistant strains (E. coli (one), Klebsiella spp. (four), S. maltophilia (one), A. radiobacter (one), Acinetobacter sp. (one), B. cepacia (one) and Comamonas acidovarans (one)) and nine (29.0%) by meropenem-resistant strains (S. maltophilia (eight) and A. radiobacter (one)). Only one ciprofloxacin-resistant strain (Flavobacterium indolgenes), but no gentamicin- or meropenem-resistant AGNB, were recovered from the samples of blood obtained from adult oncology patients.

We believe that the differences between the various haematology/oncology units in our hospital, in terms of both the percentage of bacteraemic episodes caused by AGNB and the susceptibility patterns of these isolates, can be accounted for by parameters that also varied from unit to unit. Included amongst these are the degree and duration of neutropenia, the presence and severity of mucositis, the use of right atrial catheters and cumulative exposure to antibiotics; extensive use of right atrial catheters may explain, at least in part, the relatively high number of non-fermentative AGNB of environmental origin that were isolated from adult haematology patients. A previous report from this centre described a decrease in the incidence of bacteraemia caused by AGNB in neutropenic patients following the introduction of fluoroquinolone prophylaxis in 1987. However, in the present study, although only 29.5% of bacteraemias in adult haematology patients were caused by AGNB, 64.5% of the aetiological agents were resistant to ciprofloxacin. The different incidences of ciprofloxacin-resistant pathogens on each unit are likely to be directly related to the amount of ciprofloxacin administered, in 1996 that being highest on the adult haematology unit, followed by the adult oncology and paediatric haematology/oncology units (3.5 kg, 1.8 kg and 0.3 kg respectively).

In conclusion, we have demonstrated marked differences between units in the same institution caring for neutropenic patients falling into different haematology/oncology categories in terms of the types of bacteria isolated from patients with bloodstream infections and the susceptibility patterns of these organisms. This has caused us to abandon our common antimicrobial policy for the empirical treatment of febrile neutropenic patients and to introduce policies that reflect the specific pathogens on each unit.

References