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R. M. Prabhu · K. E. Piper · M. R. Litzow · J. M. Steckelberg · R. Patel

Emergence of quinolone resistance among viridans group streptococci isolated from the oropharynx of neutropenic peripheral blood stem cell transplant patients receiving quinolone antimicrobial prophylaxis

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Abstract In neutropenic patients receiving quinolone prophylaxis, bacteremia with viridans group streptococci resistant to quinolones is a known complication. The frequency of occurrence of quinolone-resistant organisms colonizing the oropharynx during antibacterial prophylaxis with a quinolone is not well defined. In 48 patients undergoing hematopoietic stem cell transplantation, the prevalence of quinolone resistance in viridans group streptococci colonizing the oropharynx before and during antibacterial prophylaxis with gatifloxacin or moxifloxacin (most with concomitant penicillin) was determined. For quinolone-resistant isolates, mutations in the genes *gyrA* and *parC*, which confer resistance to quinolones, were analyzed. Seventy-four isolates before and 27 isolates during quinolone use were recovered from patients' oropharynxes. The numbers of susceptible isolates recovered before versus during quinolone use were as follows: 52 (70%) versus three (11%) for ciprofloxacin, 66 (89%) versus eight (30%) for levofloxacin, 66 (89%) versus ten (37%) for gatifloxacin, and 67 (91%) versus 11 (41%) for moxifloxacin ($p<0.0001$). Mutations in *gyrA* and/or *parC* were detected in quinolone-resistant isolates. Quinolone-resistant viridans group streptococci are frequently found in the oropharynx of neutropenic patients after a brief (median, 8 days) exposure to gatifloxacin or moxifloxacin.

R. M. Prabhu · K. E. Piper · J. M. Steckelberg · R. Patel
Division of Infectious Diseases,
Department of Internal Medicine,
Mayo Clinic College of Medicine,
200 First Street SW,
Rochester, MN 55905, USA

M. R. Litzow
Division of Hematology,
Department of Internal Medicine,
Mayo Clinic College of Medicine,
Rochester, MN, USA

R. Patel (✉)
Division of Clinical Microbiology,
Department of Laboratory Medicine and Pathology,
Mayo Clinic College of Medicine,
Rochester, MN, USA
e-mail: patel.robin@mayo.edu
Tel.: +1-507-2556482
Fax: +1-507-2557767

M. R. Litzow · R. Patel
Transplant Center,
Mayo Clinic College of Medicine,
Rochester, MN, USA

Introduction

Administration of quinolone antibacterial prophylaxis during the neutropenic period of bone marrow or peripheral blood stem cell transplantation (PBSCT) for hematologic malignancy reduces the incidence of gram-negative bacterial infections [1–9]. Gram-positive bacteria are the most common organisms (29–74%) isolated in breakthrough bacteremia in patients receiving quinolone prophylaxis [10, 11]; viridans group streptococci are second only to coagulase-negative staphylococci [11–13]. Chemotherapy-induced oral mucositis is considered the major source of viridans group streptococcal bacteremia in this patient population [13–15]. Viridans group streptococcal bacteremia can lead to life-threatening conditions such as septic shock, acute respiratory distress syndrome, and endocarditis [10–12, 15–18].

Breakthrough bacteremia with quinolone-resistant viridans group streptococci during quinolone prophylaxis is of concern [19, 20]. We reported a cluster of cases of levofloxacin-resistant viridans group streptococcal bacteremia among autologous PBSCT recipients receiving levofloxacin prophylaxis [19]. The isolates also had reduced susceptibility to gatifloxacin and moxifloxacin. These findings prompted a switch to the combination of gatifloxacin and penicillin as antibacterial prophylaxis in an attempt to reduce the incidence of viridans group streptococcal bacteremia. Other hematology programs have taken similar measures [10, 21]. Gatifloxacin was chosen for its superior in vitro activity against viridans group

streptococci (compared to older quinolones) [22–25]. Quinolones inhibit DNA gyrase (topoisomerase II) and topoisomerase IV, which are tetramers composed of GyrA/GyrB and ParC/ParE subunits, respectively [26]. Mutations in the quinolone resistance-determining region (QRDR) of *gyrA* at codons 81 (Ser) or 85 (Glu) and/or *parC* at codons 79 (Ser) or 83 (Asp) and efflux confer resistance to quinolones in viridans group streptococci [27, 28].

We hypothesized that the prior episodes of quinolone-resistant viridans group streptococcal bacteremia in our autologous PBSCT patients receiving quinolone prophylaxis resulted from selection of these organisms in their oropharynx.

The purpose of this study was to prospectively monitor for the emergence of quinolone resistance in viridans group streptococci recovered from the oropharynx among neutropenic hematology patients receiving quinolone prophylaxis. Quinolone-resistant viridans group streptococci were phenotypically and genotypically characterized.

Patients and methods

Patient selection

Volunteer patients ≥18 years who were expected to undergo autologous or allogeneic bone marrow transplantation or PBSCT at the Mayo Clinic, Rochester, MN, USA, were enrolled from 19 July 2002 to 15 May 2003. Before transplantation and initiation of quinolone prophylaxis and after obtaining informed consent, the subject's oropharynx was swabbed with a BBL CultureSwab Collection & Transport System (Becton Dickinson, Sparks, MD, USA). A repeat oropharyngeal culture was obtained approximately 1 week after commencing quinolone prophylaxis. Follow-up oropharyngeal cultures were not obtained if the subject did not undergo transplantation or developed febrile neutropenia before receiving 7 days of quinolone prophylaxis, or if a quinolone was not prescribed.

Prophylaxis with a quinolone and penicillin VK was initiated during conditioning chemotherapy 2 days prior to transplantation and continued through neutrophil engraftment (absolute neutrophil count [ANC] >500 cells/mm^3) or development of febrile neutropenia. Subjects with multiple myeloma received two courses of quinolone prophylaxis: first, during the neutropenic period (ANC ≤500 cells/mm^3) of stem cell mobilization, and second, during transplantation. Patients were enrolled and sampled only during their first course of quinolone prophylaxis for either stem cell mobilization or transplantation.

Subjects were monitored for infectious complications during the neutropenic period after transplantation while receiving quinolone prophylaxis. The Mayo Clinic Institutional Review Board approved this study.

Oral antimicrobial prophylaxis regimen

From October 2001 until March 2003, gatifloxacin 400 mg once daily was administered to most patients. After this time, moxifloxacin 400 mg once daily was substituted (due to a hospital formulary change). Penicillin VK 500 mg twice daily was included in the prophylaxis regimen during transplantation, but not during stem cell mobilization. Patients received the quinolone alone for a minimum of 7 days after chemotherapy for stem cell mobilization during the expected period of neutropenia. Fluconazole was used as antifungal prophylaxis and valacyclovir as antiviral prophylaxis for the initial 30 days post-transplant. Study subjects were seen daily by the transplant service.

Conditioning chemotherapy

Patients with non-Hodgkin's lymphoma typically received carmustine, etoposide, cytarabine, melphalan, and dexamethasone conditioning chemotherapy. Patients with multiple myeloma or amyloidosis received high-dose melphalan. Allogeneic stem cell transplantation included total body irradiation. For patients with multiple myeloma, stem cell mobilization consisted of high-dose cyclophosphamide and granulocyte-macrophage colony stimulating factor or granulocyte colony stimulating factor. All patients had a tunneled central venous catheter.

Definitions

Neutropenia was defined as an ANC of ≤500 cells/μl. Duration of neutropenia was the number of days the ANC was ≤500 cells/μl. Febrile neutropenia was defined as an ANC of ≤500 cells/μl and an oral temperature of ≥38°C.

Isolation and identification of viridans group streptococci

Each oropharyngeal swab was inoculated onto duplicate sheep blood agar plates and one trypticase soy agar plate supplemented with 5% lysed horse blood and 1 μg/ml of gatifloxacin. After 20–24 h of incubation at 37°C in a 5% CO_2 incubator, unique colonies resembling viridans group streptococci were subcultured and classified by morphological and biochemical criteria into the five major groups of viridans group *Streptococcus* species: *mitis*, *anginosus*, *salivarius*, *sanguinis*, or *mutans* [29].

Antimicrobial susceptibility testing

MIC values were determined by broth microdilution for ciprofloxacin (USP, Rockville, MD, USA), levofloxacin (Pharmaceutical Research Institute, Spring House, PA, USA), gatifloxacin (Bristol-Myers Squibb, Plainsboro, NJ, USA), moxifloxacin (Bayer Pharmaceutical Division, West Haven, CT, USA), garenoxacin (Bristol-Myers Squibb, Plainsboro, NJ, USA), penicillin (Sigma Chemical, St. Louis, MO, USA), erythromycin gluceptate (USP), and vancomycin (Sigma) and interpreted in accordance with

the guidelines of the Clinical and Laboratory Standards Institute [30, 31]. In order to perform an exploratory analysis of susceptibilities, the following breakpoints (in μg/ml) were applied: S≤2, R≥8 for ciprofloxacin, and S≤1, R≥4 for gatifloxacin, moxifloxacin, and garenoxacin, for which there are no established Clinical and Laboratory Standards Institute breakpoints. *Gemella morbillorum* American Type Culture Collection 27825 was used as a quality control strain for susceptibility testing.

Identification of mutations in the quinolone resistance-determining region

Isolates recovered before quinolone use and demonstrating reduced susceptibility to levofloxacin or ciprofloxacin (MIC≥ 8 μg/ml), as well as all isolates recovered during quinolone use, underwent QRDR mutation testing. DNA was prepared for amplification with either the IsoQuick Nucleic Acid Extraction Kit (ORCA Research, Bothell, WA, USA) or with alkaline wash as described previously, with some modifications [32]. For alkaline wash, several fresh colonies were inoculated into 7 ml of Todd–Hewitt broth and grown overnight at 37°C in a 5% CO_2 incubator. The bacterial suspension was centrifuged at 4,000×*g* for 25 min, and the pellet was resuspended in 500 μl of alkaline wash solution (0.05 M sodium citrate, 0.5 M sodium hydroxide), transferred to a 1.5-ml microcentrifuge tube, and incubated at room temperature for 20 min. The suspension was centrifuged at 14,000×*g* for 5 min, and the pellet was resuspended in 500 μl of Tris–HCl buffer (0.5 M, pH 8.0). The suspension was centrifuged again at 14,000×*g* for 5 min, and the pellet was resuspended in 100 μl of sterile water in a screw-cap microcentrifuge tube and placed in a boiling water bath for 10 min. The tube was centrifuged for 10 min at 14,000×*g* and the supernatant used for polymerase chain reaction (PCR).

PCR amplification of *gyrA* was performed either with primers 5′CAGGKGATGTYATGGGTA3′ (forward) and 5′ GAAGCATTTCCARRGCAA3′ (reverse), which amplify a 163 base pair region of *gyrA*, or with previously published primers [33]. For *parC*, primers 5′CAGCGYCGKATTC TTTATTC3′ (forward) and 5′TTCKGTRTCRTCAAAG TTCC3′ (reverse), which amplify a 287 base pair region of the QRDR of *parC*, were used.

PCR was performed in a total reaction volume of 50 μl containing 5 μl of extracted DNA, 1× PCR buffer containing 1.5 mM $MgCl_2$, 200 μmol/l of each deoxyribonucleotide (Boehringer Mannheim, Indianapolis, IN, USA), 0.4 μmol/l of each primer, 1.25 U of Ampli*Taq* Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA), and 10 μl of 50% glycerol. The following cycling parameters were used: initial preheating for 10 min at 95°C, followed by 40 cycles of 1 min at 94°C, 1 min at 46°C for *gyrA* or 50°C for *parC* primers, and 1 min at 72°C. Amplification was confirmed by running the amplified product on a 1.5% agarose gel with 0.5 μg/ml of ethidium bromide. DNA sequencing was performed by ABI Prism Big Dye Terminator cycle sequencing with the ABI Prism 377 automated DNA sequencer (Applied Biosystems). Sequence data were analyzed using Sequencher 3.1.1 (GeneCodes, Ann Arbor, MI, USA). Codon positions 81 and 85 of *gyrA* and 79 and 83 of *parC* were analyzed for mutations in comparison with previously published QRDR sequences of viridans group streptococci [19, 33, 34].

Statistical methods

Differences in antimicrobial susceptibility of viridans group streptococci recovered before and during prophylaxis with a quinolone were analyzed using the two-tailed Fisher's exact test or chi-square test with the statistical software package JMP Version 5.0.1.2 (SAS Institute, Cary, NC, USA). A p value of <0.05 was considered statistically significant.

Table 1 Follow-up microbiological results and patient outcome after hematopoietic stem cell transplantation

Characteristic	No. (%)
Patients with follow-up oropharyngeal culture	38/48 (79)
Positive for viridans group *Streptococcus* spp.[a]	21/38 (55)
Prophylaxis regimen at time of follow-up culture	
Gatifloxacin	9/38 (24)
Gatifloxacin/penicillin	24/38 (63)
Levofloxacin/penicillin	1/38 (3)
Moxifloxacin/vancomycin	1/38 (3)
Moxifloxacin/penicillin	3/38 (8)
Duration of neutropenia	
(absolute neutrophil count ≤500 cells/mm^3), days	
Median	12
Range	6–60
Mean	15
Patients with febrile neutropenia post-transplant	32/46 (70)
Time to febrile neutropenia post-transplant, days	
Median	8
Range	3–11
Breakthrough bacteremia on quinolone±penicillin	
Gram-negative bacillus	2 (4)
Escherichia coli[b]	1
Pseudomonas aeruginosa[b]	1
Gram-positive coccus	8 (17)
Coagulase-negative *Staphylococcus* spp.[c]	6
Enterococcus faecalis[b]	1
Micrococcus spp.	1
Mortality within first 100 days post-transplant	3/46 (7)

[a]One patient isolate (*S. mitis* spp. group) was not amenable to susceptibility testing and is not included in Table 3
[b]Not susceptible to levofloxacin or ciprofloxacin
[c]Four isolates not susceptible to levofloxacin or ciprofloxacin

Table 2 Species groups of viridans group streptococci isolated from patients before and after quinolone prophylaxis

Species group	No. (%) of patients with viridans group streptococci	
	Before prophylaxis: 74 isolates from 47 patients	After commencing prophylaxis: 27 isolates from 20 patients
S. mitis	32 (43)	14 (52)
S. sanguinis	31 (42)	8 (30)
S. salivarius	10 (14)	4 (15)
S. anginosus	1 (1)	1 (4)
S. mutans	0 (0)	0 (0)

Results

Patient characteristics

Forty-eight patients were enrolled. The median patient age was 55 years (range, 23–72 years); 62% were men, and all were Caucasian. Seventeen (35%) patients had non-Hodgkin's lymphoma, 17 (35%) had multiple myeloma, nine (19%) had amyloidosis, two (4%) had Hodgkin's lymphoma, one (2%) had acute lymphoblastic leukemia, one (2%) had chronic lymphocytic leukemia, and one (2%) had POEMS (polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, and skin changes) syndrome.

Twenty-five (52%) enrolled patients had received an antimicrobic within the month preceding their first oropharyngeal culture; however, only five had received a quinolone in this time frame. Forty-three patients underwent PBSCT, three underwent bone marrow transplantation, and two had no transplant performed. Of the 46 transplanted patients, 45 received a quinolone; one received cefixime as antibacterial prophylaxis (because of a potential drug interaction with an antiarrhythmic agent).

Microbiological follow-up investigations

Forty-seven of 48 (98%) patients had viridans group streptococci recovered from the initial oropharyngeal swab. Thirty-eight of the 46 (83%) transplanted patients had a follow-up oropharyngeal culture while receiving antibacterial prophylaxis. Thirty-three of these 38 (87%) patients were receiving a gatifloxacin-based regimen (with or without penicillin) at the time the follow-up throat swab was obtained (Table 1). Eight transplanted patients did not have a follow-up culture because no quinolone was used (n=1), the patient was missed at follow-up (n=2), or the patient developed early febrile neutropenia (n=5).

Twenty of 38 (53%) patients who had a follow-up swab had viridans group streptococci recovered (Table 2). The median duration of quinolone use at the time of repeat oropharyngeal culture was 8 days (range, 6–11 days).

Selection of quinolone resistance

There was a statistically significant decrease in quinolone susceptibility among viridans group streptococcal isolates recovered during quinolone prophylaxis versus those recovered before quinolone prophylaxis (Table 3); 70 versus 11% were susceptible to ciprofloxacin (p<0.0001), 89 vs. 30% were susceptible to levofloxacin (p<0.0001), 89 versus 37% were susceptible to gatifloxacin (p<0.0001), and 91 versus 41% were susceptible to moxifloxacin (p<0.0001). These findings were both similar and statistically significant when the analysis included only the 20 patients who had isolates recovered both before and during quinolone use (data not shown). Among the available quinolones, moxifloxacin or gatifloxacin had lower MIC_{90} values than levofloxacin or ciprofloxacin. There was no statistically significant difference in penicillin or erythromycin susceptibility prior to versus during prophylaxis

Table 3 Antimicrobial susceptibility of viridans group streptococci recovered from 47 patients before and 20 patients after commencing quinolone prophylaxis

Antibiotic[a]	MIC_{50} in μg/ml		MIC_{90} in μg/ml (range)		No. (%) susceptible		No. (%) fully resistant[b]		p value
	Before (n=74)	After (n=27)	Before (n=74)	After (n=27)	Before (n=74)	After (n=27)	Before (n=74)	After (n=27)	
Penicillin	0.25	0.125	4 (≤0.125–32)	2 (≤0.125–4)	28 (38)	15 (56)	8 (11)	1 (4)	0.12
Erythromycin	8	4	16 (≤0.125≥128)	16 (≤0.125≥128)	28 (38)	12 (44)	46 (62)	14 (52)	0.65
Vancomycin	0.5	0.5	0.5 (0.25–1)	0.5 (0.5)	74 (100)	27 (100)	0 (0)	0 (0)	–
Ciprofloxacin[c]	2	32	16 (0.25–128)	128 (1–128)	52 (70)	3 (11)	12 (16)	20 (74)	<0.0001
Levofloxacin	1	16	8 (≤0.125≥128)	64 (0.5≥128)	66 (89)	8 (30)	8 (11)	16 (59)	<0.0001
Gatifloxacin[c]	0.5	4	4 (≤0.125–32)	16 (0.5–128)	66 (89)	10 (37)	7 (10)	15 (56)	<0.0001
Moxifloxacin[c]	0.125	4	2 (≤0.125–16)	16 (≤0.125–128)	67 (91)	11 (41)	6 (8)	15 (56)	<0.0001
Garenoxacin[c]	0.125	1	0.25 (≤0.125–4)	4 (≤0.125–16)	68 (92)	19 (70)	2 (3)	5 (19)	0.006

S susceptible, *I* intermediate, *R* resistant, according to Clinical and Laboratory Standards Institute guidelines

[a]Breakpoints in μg/ml: penicillin S≤0.12, I=0.25–2, R≥4; erythromycin S≤0.25, I=0.5, R≥1; vancomycin S≤1; gatifloxacin, moxifloxacin, garenoxacin S≤1, R≥4; ciprofloxacin S≤2, R≥8; levofloxacin S≤2, I=4, R≥8

[b]Includes only R and not I isolates

[c]No approved Clinical and Laboratory Standards Institute breakpoints

Table 4 Susceptibility patterns of different species groups before and after quinolone prophylaxis

Species group	Levofloxacin[a]				Gatifloxacin[a,b]				Moxifloxacin[a,b]			
	No. (%) susceptible		No. (%) fully resistant[c]		No. (%) susceptible		No. (%) fully resistant[c]		No. (%) susceptible		No. (%) fully resistant[c]	
	Before	After	Before	After	Before	After	Before	After	Before	After	Before	After
S. mitis	29 (91)	5 (36)	3 (9)	9 (64)	29 (91)	5 (36)	3 (9)	9 (64)	29 (91)	5 (36)	3 (9)	9 (64)
S. sanguinis	26 (84)	0 (0)	5 (16)	7 (88)	26 (84)	0 (0)	4 (13)	6 (75)	27 (87)	1 (13)	2 (6)	6 (75)
S. salivarius	10 (100)	2 (50)	0 (0)	0 (0)	10 (100)	4 (100)	0 (0)	0 (0)	10 (100)	4 (100)	0 (0)	0 (0)
S. anginosus	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
S. mutans	–	–	–	–	–	–	–	–	–	–	–	–

S susceptible, *I* intermediate, *R* resistant, according to Clinical and Laboratory Standards Institute guidelines
[a]Breakpoints in μg/ml: gatifloxacin, moxifloxacin S≤1, R≥4; levofloxacin S≤2, I=4, R≥8
[b]No approved Clinical and Laboratory Standards Institute breakpoints
[c]Includes only R and not I isolates

(Table 3). Only isolates from *S. mitis* and *S. sanguinis* species groups demonstrated resistance to quinolones both before and during quinolone prophylaxis (Table 4).

Presence of quinolone resistance-determining region mutations in quinolone-resistant isolates

QRDR regions were successfully amplified in 21 of 40 (53%) isolates for *gyrA* and in 36 of 40 (90%) isolates for *parC*. The following mutations were found in GyrA: Ser 81→Tyr (*n*=3) or Phe (*n*=6), and/or Glu 85→Lys (*n*=2). Mutations found in ParC were Ser 79→Arg (*n*=4), Ile (*n*=3), Phe (*n*=4), or Tyr (*n*=2), and/or Asp 83→Asn (*n*=2) or Glu (*n*=4). Most isolates that were not susceptible to levofloxacin had at least one QRDR mutation in either *gyrA* or *parC*. One *S. mitis* isolate with an MIC value of 16 μg/ml for levofloxacin, 8 μg/ml for gatifloxacin, and 4 μg/ml for moxifloxacin had a one-step mutation in GyrA (Ser 81→Phe) only. For three isolates (one *S. mitis*, two *S. sanguinis*) with levofloxacin MIC values of 16–32 μg/ml, two-step mutations involving GyrA (Ser 81→Phe or Tyr) and ParC (Ser 79→Ile, Tyr, or Arg) were detected. In one *S. mitis* isolate that was highly resistant to levofloxacin, gatifloxacin, and moxifloxacin (MIC 128 μg/ml), three QRDR mutations were detected (GyrA: Ser 81→Phe, Glu85→Lys; ParC: Ser79→Phe).

Clinical outcomes

No episodes of viridans group streptococcal bacteremia occurred. Ten episodes of breakthrough bacteremia, two gram-negative and eight gram-positive, occurred during antibacterial prophylaxis (Table 1).

Discussion

Our study revealed two important clinical findings. First, we found that quinolone-resistant viridans group streptococci were frequently recovered from the oropharynx of patients after only a brief period of exposure to quinolones (primarily gatifloxacin) despite the superior in vitro activity of gatifloxacin against viridans group streptococci compared to levofloxacin or ciprofloxacin. Second, despite the presence of these bacteria in the oropharynx of our study subjects, no episodes of breakthrough bacteremia with viridans group streptococci developed during quinolone–penicillin prophylaxis during the neutropenic period after transplantation.

There have been many published reports of breakthrough bacteremia with quinolone-resistant viridans group streptococci during quinolone prophylaxis [10, 17, 19]. One recently published study has characterized the prevalence of quinolone resistance in viridans group streptococci colonizing the oropharynx of hematology patients during quinolone prophylaxis [35]. In this relatively small (20 study subjects) study, the investigators reported 0% (first week) and 20% (second week) prevalence rates of levofloxacin resistance (MIC≥8 μg/ml) among viridans group streptococci isolated during levofloxacin prophylaxis. In contrast, we observed a much higher prevalence of levofloxacin resistance (59%) among viridans group streptococci recovered after just 6–11 days of quinolone prophylaxis (Table 3) [10, 17, 19]. Methodological differences between this study and ours may explain these discrepancies. The investigators performed twice weekly surveillance throat cultures during levofloxacin prophylaxis [35]. In the eight patients with neutropenic fever, levofloxacin was continued and empiric antimicrobial therapy, typically imipenem, was added. It is unclear in what percentage of patients surveillance throat cultures were performed while levofloxacin was being administered with empiric antimicrobial therapy for febrile neutropenia. This practice may have impacted the recovery of viridans group streptococci and resulted in an underestimation of the prevalence of quinolone resistance. We identified and attempted to determine the presence of QRDR mutations in our viridans group streptococcal isolates, which was not done in their study. The majority (96%) of our patients underwent stem cell transplantation, in contrast to ap-

proximately half of their patients. Notably, both studies reported a similar percentage of bloodstream infections associated with resistance to the quinolone administered (Table 1).

We found low rates of susceptibility to available quinolones (11–41%) among viridans group streptococci isolated during quinolone prophylaxis (Table 3). Gatifloxacin or moxifloxacin did not prevent the selection of resistance in vivo. The specific *gyrA* and *parC* mutations identified in our quinolone-resistant viridans group streptococcal isolates are consistent with those previously reported [27, 28, 36, 37].

During chemotherapy-induced neutropenia, the use of a quinolone with poor in vitro activity against viridans group streptococci has been found to be a risk factor for breakthrough viridans group streptococcal bacteremia. Gatifloxacin and moxifloxacin have better in vitro activity than levofloxacin against viridans group streptococci [36, 38]. However, no definite conclusion can be drawn regarding the role gatifloxacin (or moxifloxacin) played in the absence of breakthrough bacteremia with viridans group streptococci because of the addition of penicillin. Several studies have suggested, however, that the addition of penicillin to a quinolone-based prophylaxis regimen reduces the incidence of viridans group streptococcal bacteremia [1, 10, 16, 21].

There are limitations to our study. First, it was not meant to determine the efficacy of a quinolone–penicillin prophylaxis regimen in preventing viridans group streptococcal bacteremia. The small sample size, along with the fact that consecutive patients undergoing transplantation were not enrolled, may have potentially resulted in an underestimation of the incidence of breakthrough bacteremia with viridans group streptococci; however, the selection of quinolone resistance should not have been affected. Despite the small sample size, we were able to find a statistically significant difference in quinolone susceptibility after quinolone exposure. Second, there were a variety of antibacterial prophylaxis regimens used, including penicillin, which may have affected our findings. Despite this, we observed a high prevalence of quinolone-resistant isolates. Third, the duration of persistent colonization with quinolone-resistant viridans group streptococci was not determined. It was not feasible to repeat oropharyngeal cultures after cessation of quinolone prophylaxis, given that most patients were placed on broad-spectrum intravenous anti-infective agents for the management of febrile neutropenia (Table 1). Colonization may be expected to persist for at least a month [39]. Fourth, there were difficulties in amplifying both *gyrA* and *parC* in many isolates; to our knowledge, one primer set cannot amplify these genes in all five viridans streptococcal species groups [27, 28, 34, 36, 38].

In summary, this study demonstrates that administration of a quinolone to a neutropenic hematology patient for a brief period of time, as short as 1 week, selects for quinolone-resistant viridans group streptococci in the oropharynx.

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References

1. Anonymous (1994) Reduction of fever and streptococcal bacteremia in granulocytopenic patients with cancer. A trial of oral penicillin V or placebo combined with pefloxacin. International Antimicrobial Therapy Cooperative Group of the European Organization for Research and Treatment of Cancer. JAMA 272:1183–1189
2. Bow EJ, Mandell LA, Louie TJ, Feld R, Palmer M, Zee B, Pater J (1996) Quinolone-based antibacterial chemoprophylaxis in neutropenic patients: effect of augmented gram-positive activity on infectious morbidity. National Cancer Institute of Canada Clinical Trials Group. Ann Intern Med 125:183–190
3. Haahr V, Peterslund NA, Moller JK (1997) The influence of antimicrobial prophylaxis on the microbial and clinical findings in patients after autologous bone marrow transplantation. Scand J Infect Dis 29:623–626
4. Patrick CC (1997) Use of fluoroquinolones as prophylactic agents in patients with neutropenia. Pediatr Infect Dis J 16:135–139; discussion 160–162
5. Prentice HG, Hann IM, Nazareth B, Paterson P, Bhamra A, Kibbler CC (2001) Oral ciprofloxacin plus colistin: prophylaxis against bacterial infection in neutropenic patients. A strategy for the prevention of emergence of antimicrobial resistance. Br J Haematol 115:46–52
6. Yeh SP, Hsueh EJ, Yu MS, Wu H, Wang YC (1999) Oral ciprofloxacin as antibacterial prophylaxis after allogeneic bone marrow transplantation: a reappraisal. Bone Marrow Transplant 24:1207–1211
7. Warren RE, Wimperis JZ, Baglin TP, Constantine CE, Marcus R (1990) Prevention of infection by ciprofloxacin in neutropenia. J Antimicrob Chemother 26:109–123
8. Kern W, Kurrle E (1991) Ofloxacin versus trimethoprim-sulfamethoxazole for prevention of infection in patients with acute leukemia and granulocytopenia. Infection 19:73–80
9. Anonymous (1991) Prevention of bacterial infection in neutropenic patients with hematologic malignancies. A randomized, multicenter trial comparing norfloxacin with ciprofloxacin. The GIMEMA Infection Program. Ann Intern Med 115:7–12
10. Bochud PY, Eggiman P, Calandra T, Van Melle G, Saghafi L, Francioli P (1994) Bacteremia due to viridans streptococcus in neutropenic patients with cancer: clinical spectrum and risk factors. Clin Infect Dis 18:25–31
11. Schots R, Trullemans F, Van Riet I, Kaufman L, Hafsia A, Meddeb B, Hadj Ali ZB, Ben Abid H, Lauwers S, Van Camp B (2000) The clinical impact of early gram-positive bacteremia and the use of vancomycin after allogeneic bone marrow transplantation. Transplantation 69:1511–1514
12. Persson L, Vikerfors T, Sjoberg L, Engervall P, Tidefelt U (2000) Increased incidence of bacteraemia due to viridans streptococci in an unselected population of patients with acute myeloid leukaemia. Scand J Infect Dis 32:615–621
13. Yuen KY, Woo PC, Liang RH, Chiu EK, Chen FF, Wong SS, Lau YL, Ha SY, Peiris JS, Siau H, Chan TK (1998) Clinical significance of alimentary tract microbes in bone marrow transplant recipients. Diagn Microbiol Infect Dis 30:75–81
14. Kennedy HF, Morrison D, Kaufmann ME, Jackson MS, Bagg J, Gibson BE, Gemmell CG, Michie JR (2000) Origins of *Staphylococcus epidermidis* and *Streptococcus oralis* causing bacteraemia in a bone marrow transplant patient. J Med Microbiol 49:367–370

15. Bochud PY, Calandra T, Francioli P (1994) Bacteremia due to viridans streptococci in neutropenic patients: a review. Am J Med 97:256–264
16. Broun ER, Wheat JL, Kneebone PH, Sundblad K, Hromas RA, Tricot G (1994) Randomized trial of the addition of gram-positive prophylaxis to standard antimicrobial prophylaxis for patients undergoing autologous bone marrow transplantation. Antimicrob Agents Chemother 38:576–579
17. Classen DC, Burke JP, Ford CD, Evershed S, Aloia MR, Wilfahrt JK, Elliott JA (1990) *Streptococcus mitis* sepsis in bone marrow transplant patients receiving oral antimicrobial prophylaxis. Am J Med 89:441–446
18. Marron A, Carratala J, Gonzalez-Barca E, Fernandez-Sevilla A, Alcaide F, Gudiol F (2000) Serious complications of bacteremia caused by viridans streptococci in neutropenic patients with cancer. Clin Infect Dis 31:1126–1130
19. Razonable RR, Litzow MR, Khaliq Y, Piper KE, Rouse MS, Patel R (2002) Bacteremia due to viridans group streptococci with diminished susceptibility to levofloxacin among neutropenic patients receiving levofloxacin prophylaxis. Clin Infect Dis 34:1469–1474
20. Elting LS, Bodey GP, Keefe BH (1992) Septicemia and shock syndrome due to viridans streptococci: a case-control study of predisposing factors. Clin Infect Dis 14:1201–1207
21. Spanik S, Trupl J, Kunova A, Botek R, Sorkovska D, Grey E, Studena M, Lacka J, Oravcova E, Krchnakova A, Rusnakova V, Svec J, Krupova I, Grausova S, Stopkova K, Koren P, Krcmery V Jr (1997) Viridans streptococcal bacteraemia due to penicillin-resistant and penicillin-sensitive streptococci: analysis of risk factors and outcome in 60 patients from a single cancer centre before and after penicillin is used for prophylaxis. Scand J Infect Dis 29:245–249
22. Diekema DJ, Beach ML, Pfaller MA, Jones RN, Group SP (2001) Antimicrobial resistance in viridans group streptococci among patients with and without the diagnosis of cancer in the USA, Canada and Latin America. Clin Microbiol Infect 7:152–157
23. Gordon KA, Beach ML, Biedenbach DJ, Jones RN, Rhomberg PR, Mutnick AH (2002) Antimicrobial susceptibility patterns of beta-hemolytic and viridans group streptococci: report from the SENTRY Antimicrobial Surveillance Program (1997–2000). Diagn Microbiol Infect Dis 43:157–162
24. Bassetti M, Dembry LM, Farrel PA, Callan DA, Andriole VT (2001) Comparative antimicrobial activity of gatifloxacin with ciprofloxacin and beta-lactams against gram-positive bacteria. Diagn Microbiol Infect Dis 41:143–148
25. de Azavedo JC, Trpeski L, Pong-Porter S, Matsumura S, Low DE (1999) In vitro activities of fluoroquinolones against antibiotic-resistant blood culture isolates of viridans group streptococci from across Canada. Antimicrob Agents Chemother 43:2299–2301
26. Andriole VT (ed) (2000) The quinolones. Academic, San Diego
27. Ferrandiz MJ, Oteo J, Aracil B, Gomez-Garces JL, De La Campa AG (1999) Drug efflux and *parC* mutations are involved in fluoroquinolone resistance in viridans group streptococci. Antimicrob Agents Chemother 43:2520–2523
28. Kaneko A, Sasaki J, Shimadzu M, Kanayama A, Saika T, Kobayashi I (2000) Comparison of *gyrA* and *parC* mutations and resistance levels among fluoroquinolone-resistant isolates and laboratory-derived mutants of oral streptococci. J Antimicrob Chemother 45:771–775
29. Facklam R (2002) What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin Microbiol Rev 15:613–630
30. National Committee for Clinical Laboratory Standards (2001) Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standards Institute (formerly NCCLS), Wayne, PA
31. National Committee for Clinical Laboratory Standards (2003) Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Clinical and Laboratory Standards Institute (formerly NCCLS), Wayne, PA
32. Hall L, Doerr KA, Wohlfiel SL, Roberts GD (2003) Evaluation of the MicroSeq System for identification of mycobacteria by 16S ribosomal DNA sequencing and its integration into a routine clinical mycobacteriology laboratory. J Clin Microbiol 41:1447–1453
33. Janoir C, Zeller V, Kitzis MD, Moreau NJ, Gutmann L (1996) High-level fluoroquinolone resistance in *Streptococcus pneumoniae* requires mutations in *parC* and *gyrA*. Antimicrob Agents Chemother 40:2760–2764
34. Gonzalez I, Georgiou M, Alcaide F, Balas D, Linares J, de la Campa AG (1998) Fluoroquinolone resistance mutations in the *parC*, *parE*, and *gyrA* genes of clinical isolates of viridans group streptococci. Antimicrob Agents Chemother 42:2792–2798
35. Timmers GJ, Dijstelbloem Y, Simoons-Smit AM, van Winkelhoff AJ, Touw DJ, Vandenbroucke-Graul CMJE, Huijgens PC (2004) Pharmacokinetics and effects on bowel and throat microflora of oral levofloxacin as antibacterial prophylaxis in neutropenic patients with haematological malignancies. Bone Marrow Transplant 33:847–853
36. Schmitz FJ, Fisher A, Boos M, Mayer S, Milatovic D, Fluit AC (2001) Quinolone-resistance mechanisms and in vitro susceptibility patterns among European isolates of *Streptococcus mitis*, *Streptococcus sanguis*, and *Streptococcus pneumoniae*. Eur J Clin Microbiol Infect Dis 20:219–222
37. Janoir C, Podglajen I, Kitzis MD, Poyart C, Gutmann L (1999) In vitro exchange of fluoroquinolone resistance determinants between *Streptococcus pneumoniae* and viridans streptococci and genomic organization of the *parE–parC* region in S. mitis. J Infect Dis 180:555–558
38. Schmitz FJ, Milatovic D, Boos M, Mayer S, Fluit AC (2002) In vitro activity of the novel des-F(6) quinolone BMS-284756 against genetically characterized clinical streptococcal isolates, including isolates with reduced quinolone susceptibility. J Antimicrob Chemother 49:698–701
39. Erickson PR, Herzberg MC (1999) Emergence of antibiotic-resistant *Streptococcus sanguis* in dental plaque of children after frequent antibiotic therapy. Pediatr Dent 21:181–185