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Antimicrobial Susceptibility Patterns among Viridans Group Streptococcal Isolates from Infective Endocarditis Patients from 1971 to 1986 and 1994 to 2002

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To determine whether changes in antimicrobial resistance have occurred among viridans group streptococci, we retrospectively examined 50 viridans group streptococcal isolates recovered from patients with infective endocarditis over 3 decades. Resistance rates (percent resistant isolates 1971 to 1986 and 1994 to 2002) were as follows: levofloxacin, 0 and 9; penicillin and clindamycin, 0 and 4; and erythromycin and azithromycin, 11 and 26, respectively.

Viridans group streptococci cause 20 to 40% of breakthrough bacteremia episodes in neutropenic patients (2, 4, 9, 14, 21) and 30 to 40% of native valve infective endocarditis cases (1). In hematology patients, fluoroquinolones are used as antibacterial prophylaxis agents during the neutropenic period after hematopoietic stem cell transplantation or chemotherapy alone (24). Many institutions have reported episodes of breakthrough bacteremia with fluoroquinolone-resistant viridans group streptococci during fluoroquinolone prophylaxis (5, 10). Previously, our group reported a 16% rate of breakthrough bacteremia with fluoroquinolone-resistant viridans group streptococci during levofloxacin prophylaxis in hematology patients undergoing autologous stem cell transplantation (24). In recent years, several case reports of infective endocarditis due to penicillin-resistant viridans group streptococci have been published (15–18, 26). In light of these reports, we retrospectively examined antimicrobial susceptibility patterns, including fluoroquinolone susceptibility, in a collection of viridans group streptococcal isolates spanning 3 decades, to see if changes in antimicrobial resistance had occurred.

Since 1971, selected blood isolates from bacterial endocarditis patients have been collected and stored at −70°C in the Infectious Diseases Research Laboratory as part of the Mayo Clinic Endocarditis Registry. These organisms have clinical relevance from the antimicrobial susceptibility perspective because they are of proven pathogenic potential in immunocompetent hosts. Twenty-eight isolates collected from the years 1971 to 1986 and 24 isolates collected from the years 1994 to 2002 were studied. These two periods represent times before and after the U.S. Food and Drug Administration approval of ciprofloxacin in 1987. MICs were determined by broth microdilution in cation-adjusted Mueller-Hinton broth supplemented with 2.5% lysed horse blood and interpreted in accordance with National Committee for Clinical Laboratory Standards (NCCLS) guidelines (19, 20). *Gemella morbillorum* ATCC 27825 was used as a quality control strain. The concentration ranges tested were 0.125 to 128 μg/ml (in doubling dilutions) for penicillin (Sigma-Aldrich Co., St. Louis, Mo.), erythromycin (USP, Rockville, Md.), azithromycin (Pfizer, Groton, Conn.), clindamycin (Sigma-Aldrich Co.), vancomycin (Sigma-Aldrich Co.), levofloxacin (Pharmaceutical Research Institute, Spring House, Pa.), gatifloxacin (Bristol-Myers Squibb, Plainsboro, N.J.), ciprofloxacin (USP), moxifloxacin (Bayer Corporation Pharmaceutical Division, West Haven, Conn.), and garenoxacin (BMS-284756; Bristol-Myers Squibb). The NCCLS has not published susceptibility breakpoints for the last four quinolones. Due to the initial identification date of many of the isolates, they were biochemically reidentified and classified into one of five viridans streptococcal species groups—*mitis*, *anginosus*, *mutans*, *salivarius*, or *sanguinis*—according to criteria set forth by Facklam (11). For isolates where identification was unclear following biochemical testing, 16S rRNA sequence analysis was performed (24); one isolate from 1971 to 1989 was identified as *Gemella* species, and another isolate from 1994 to 2002 was identified as *Streptococcus gallolyticus* subspecies *gallolyticus*. Among the remaining isolates from 1971 to 1986 and from 1994 to 2002, there were seven and eight of the *S. mitis* species group, two and seven of the *S. anginosus* species group, eight and five of the *S. mutans* species group, three and two of the *S. salivarius* species group, and seven and one of the *S. sanguinis* species group, respectively. The two non-viridans group streptococcal isolates were excluded from the analysis.

In isolates from 1994 to 2002 high rates of nonsusceptibility were measured to erythromycin (26%), azithromycin (26%), levofloxacin (22%), and penicillin (13%) (Table 1). The lowest rates of nonsusceptibility (for the same time period) were those to vancomycin (9%) and clindamycin (4%).

The newer fluoroquinolones, moxifloxacin and gatifloxacin, demonstrated better in vitro activity than did levofloxacin and ciprofloxacin, a finding shared by other studies (6, 7, 12, 13).

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TABLE 1. MIC_{90}s (in micrograms per milliliter) for and susceptibility patterns of viridans group streptococci for the time periods 1971 to 1986 and 1994 to 2002[a]

Antimicrobic	1971–1986 (n = 27)			1994–2002 (n = 23)			
	MIC_{90} (range)	% not S	% R	MIC_{90} (range)	% not S	% R	P^b
Penicillin[c]	0.125 (0.125–0.125)	0	0	0.5 (0.125–4)	13	4	0.09
Clindamycin[d]	0.125 (0.125–0.25)	0	0	0.125 (0.125–128)	4	4	0.46
Erythromycin[d]	4 (0.125–8)	11	11	16 (0.125–128)	26	26	0.27
Azithromycin[e]	8 (0.125–32)	11	11	16 (0.125–128)	26	26	0.27
Vancomycin[f]	1 (0.5–2)	4	4	2 (0.5–2)	9	9	0.59
Levofloxacin[g]	4 (0.125–4)	11	0	8 (0.25–32)	22	9	0.44
Moxifloxacin	0.25 (0.125–2)	NA	NA	0.25 (0.125–4)	NA	NA	
Gatifloxacin	0.5 (0.125–0.5)	NA	NA	0.5 (0.125–8)	NA	NA	
Ciprofloxacin	8 (0.5–16)	NA	NA	8 (0.5–64)	NA	NA	
Garenoxacin	0.25 (0.125–1)	NA	NA	0.25 (0.125–1)	NA	NA	

[a] Abbreviations: S, susceptible; I, intermediate; R, resistant; NA, not applicable.
[b] Fisher's exact test for percent not susceptible, 1971 to 1986 compared to 1994 to 2002 period.
[c] NCCLS breakpoints: S, ≤0.12 μg/ml; I, 0.25–2 μg/ml; R, ≥4 μg/ml.
[d] NCCLS breakpoints: S, ≤0.25 μg/ml; I, 0.5 μg/ml; R, ≥1 μg/ml.
[e] NCCLS breakpoints: S, ≤0.5 μg/ml; I, 1 μg/ml; R, ≥2 μg/ml.
[f] NCCLS breakpoints: S, ≤1 μg/ml.
[g] NCCLS breakpoints: S, ≤2 μg/ml; I, 4 μg/ml; R, ≥8 μg/ml.

Nevertheless, elevated MICs of levofloxacin or ciprofloxacin were associated with elevated MICs of the newer fluoroquinolones. There was no statistically significant difference (by Fisher's exact test) in levofloxacin nonsusceptibility rates and MIC_{90}s (MICs at which 90% of the isolates tested are inhibited) between the two time periods (Table 1).

Among 1994 to 2002 isolates, higher MIC_{90}s were found for ciprofloxacin (8 μg/ml) and levofloxacin (8 μg/ml) compared with other studies, wherein MIC_{90}s ranged from 2 to ≥4 μg/ml for ciprofloxacin and 1 to 2 μg/ml for levofloxacin (6, 7, 12). In the same studies, MIC_{90}s of gatifloxacin (0.25 to 0.5 μg/ml), moxifloxacin (0.25 μg/ml), and garenoxacin (0.06 μg/ml) were comparable to ours (Table 1) (6, 7, 12). In contrast, the level of penicillin nonsusceptibility among the 1994 to 2002 isolates (13%) was lower than the 28 to 56% level reported in recent surveillance blood culture studies (6–8, 12, 13, 22, 28). Three of our isolates (two of *S. mitis* and one of the *S. sanguinis* species group) from 1994 to 2002 were penicillin nonsusceptible. The level of erythromycin (26%) and clindamycin (4%) nonsusceptibility among the 1994 to 2002 isolates in our study was comparable to rates of 40 to 44% (6–8, 12, 13, 22, 28) and 4.2 to 10% (6, 7, 12, 13), respectively, in these same studies.

For the three vancomycin-nonsusceptible viridans group streptococcal isolates in the present report, the MICs were 2 μg/ml, a one-dilution difference from susceptibility (MIC ≤ 1 μg/ml) and within the margin of error of the assay (20). There have been previous reports of viridans group streptococci for which vancomycin MICs were slightly elevated (3, 23, 25, 27).

There are limitations to our study. First, the small sample size did not provide the power to detect statistically significant differences, if present, in susceptibility between the two time periods. Second, the viridans group streptococcal isolates were from a tertiary care center and may not be reflective of endocarditis isolates seen at community hospitals. Third, since the patient population from which isolates are recovered may influence antimicrobial susceptibility patterns, isolates from endocarditis patients may not reflect the fluoroquinolone susceptibility pattern of isolates from other populations. Diekema et al., for example, found a trend of reduced susceptibility to ciprofloxacin (34 versus 46%) in patients with a diagnosis of cancer versus those without such a diagnosis (P = 0.07) (7). Fourth, the antimicrobial use history of these patients was unknown, a factor which may have influenced antimicrobial susceptibility. Fifth, not all viridans group streptococcal strains were archived from endocarditis patients diagnosed at our institution during the time periods of the study, resulting in potential selection bias. The levels of nonsusceptibility reported must therefore be interpreted in the context of these limitations.

The strength of the data presented lies in the uniqueness of this strain collection. Most published studies do not report antimicrobial susceptibility patterns of viridans group streptococci based on clinical diagnosis. To our knowledge, there are no published antimicrobial susceptibility pattern surveys of viridans group streptococci that include strains collected from both recent and past endocardial infections. Larger prospective surveillance studies are needed to monitor antimicrobial resistance in viridans group streptococci from defined patient populations such as neutropenic hematology and endocarditis patients. Increasing levels of antimicrobial resistance could impact the rate of breakthrough bacteremia with viridans group streptococci in neutropenic patients receiving fluoroquinolone prophylaxis and may also influence antimicrobial prevention and management of infective endocarditis.

REFERENCES

1. **Bayer, A. S., and W. M. Scheld.** 2000. Endocarditis and intravascular infections, p. 857–884. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases, 5th ed. Churchill Livingstone, Inc., New York, N.Y.
2. **Bochud, P. Y., P. Eggiman, T. Calandra, G. Van Melle, L. Saghafi, and P. Francioli.** 1994. Bacteremia due to viridans streptococcus in neutropenic patients with cancer: clinical spectrum and risk factors. Clin. Infect. Dis. **18:**25–31.
3. **Bourgault, A. M., W. R. Wilson, and J. A. Washington.** 1979. Antimicrobial susceptibilities of species of viridans streptococci. J. Infect. Dis. **140:**316–321.
4. **Broun, E. R., J. L. Wheat, P. H. Kneebone, K. Sundblad, R. A. Hromas, and G. Tricot.** 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.
5. **Classen, D. C., J. P. Burke, C. D. Ford, S. Evershed, M. R. Aloia, J. K. Wilfahrt, and J. A. Elliott.** 1990. *Streptococcus mitis* sepsis in bone marrow

transplant patients receiving oral antimicrobial prophylaxis. Am. J. Med. **89:**441–446.
6. **de Azavedo, J. C., L. Trpeski, S. Pong-Porter, S. Matsumura, and D. E. Low.** 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.
7. **Diekema, D. J., M. L. Beach, M. A. Pfaller, R. N. Jones, and SENTRY Participants Group.** 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.
8. **Doern, G. V., M. J. Ferraro, A. B. Brueggemann, and K. L. Ruoff.** 1996. Emergence of high rates of antimicrobial resistance among viridans group streptococci in the United States. Antimicrob. Agents Chemother. **40:**891–894.
9. **Donnelly, J. P., E. C. Dompeling, J. F. Meis, and B. E. de Pauw.** 1995. Bacteremia due to oral viridans streptococci in neutropenic patients with cancer: cytostatics are a more important risk factor than antibacterial prophylaxis. Clin. Infect. Dis. **20:**469–470.
10. **Elting, L. S., G. P. Bodey, and B. H. Keefe.** 1992. Septicemia and shock syndrome due to viridans streptococci: a case-control study of predisposing factors. Clin. Infect. Dis. **14:**1201–1207.
11. **Facklam, R.** 2002. What happened to the streptococci: overview of taxonomic and nomenclature changes. Clin. Microbiol. Rev. **15:**613–630.
12. **Gershon, A. S., J. C. de Azavedo, A. McGeer, K. I. Ostrowska, D. Church, D. J. Hoban, G. K. Harding, K. Weiss, L. Abbott, F. Smaill, M. Gourdeau, G. Murray, D. E. Low, and Canadian Bacterial Surveillance.** 2002. Activities of new fluoroquinolones, ketolides, and other antimicrobials against blood culture isolates of viridans group streptococci from across Canada, 2000. Antimicrob. Agents Chemother. **46:**1553–1556.
13. **Gordon, K. A., M. L. Beach, D. J. Biedenbach, R. N. Jones, P. R. Rhomberg, and A. H. Mutnick.** 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.
14. **Haahr, V., N. A. Peterslund, and J. K. Moller.** 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.
15. **Hall, G. E., and L. M. Baddour.** 2002. Apparent failure of endocarditis prophylaxis caused by penicillin-resistant *Streptococcus mitis*. Am. J. Med. Sci. **324:**51–53.
16. **Levitz, R. E.** 1999. Prosthetic-valve endocarditis caused by penicillin-resistant *Streptococcus mitis*. N. Engl. J. Med. **340:**1843–1844.
17. **Levy, C. S., P. Kogulan, V. J. Gill, M. B. Croxton, J. G. Kane, and D. R. Lucey.** 2001. Endocarditis caused by penicillin-resistant viridans streptococci: 2 cases and controversies in therapy. Clin. Infect. Dis. **33:**577–579.
18. **Lonks, J. R., B. P. Dickinson, and V. Runarsdottir.** 1999. Endocarditis due to *Streptococcus mitis* with high-level resistance to penicillin and cefotaxime. N. Engl. J. Med. **341:**1239.
19. **National Committee for Clinical Laboratory Standards.** 2002. Performance standards for antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard, 6th ed. M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
20. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility testing; 12th informational supplement. MS100-S12. National Committee for Clinical Laboratory Standards, Wayne, Pa.
21. **Persson, L., T. Vikerfors, L. Sjoberg, P. Engervall, and U. Tidefelt.** 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.
22. **Pfaller, M. A., R. N. Jones, S. A. Marshall, M. B. Edmond, and R. P. Wenzel.** 1997. Nosocomial streptococcal blood stream infections in the SCOPE program: species occurrence and antimicrobial resistance. Diagn. Microbiol. Infect. Dis. **29:**259–263.
23. **Piper, K. E., M. S. Rouse, K. L. Ronningen, J. M. Steckelberg, W. R. Wilson, and R. Patel.** 2000. Trovafloxacin treatment of viridans group streptococcus experimental endocarditis. Antimicrob. Agents Chemother. **44:**2554–2556.
24. **Razonable, R. R., M. R. Litzow, Y. Khaliq, K. E. Piper, M. S. Rouse, and R. Patel.** 2002. Bacteremia due to viridans group streptococci with diminished susceptibility to levofloxacin among neutropenic patients receiving levofloxacin prophylaxis. Clin. Infect. Dis. **34:**1469–1474.
25. **Rolston, K. V., L. S. Elting, and G. P. Bodey.** 1995. Bacteremia due to viridans streptococci in neutropenic patients. Am. J. Med. **99:**450.
26. **Sabella, C., D. Murphy, and J. Drummond-Webb.** 2001. Endocarditis due to *Streptococcus mitis* with high-level resistance to penicillin and ceftriaxone. JAMA **285:**2195.
27. **Spanik, S., J. Trupl, A. Kunova, R. Botek, D. Sorkovska, E. Grey, M. Studena, J. Lacka, E. Oravcova, A. Krchnakova, V. Rusnakova, J. Svec, I. Krupova, S. Grausova, K. Stopkova, P. Koren, and V. Krcmery, 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.
28. **Teng, L. J., P. R. Hsueh, Y. C. Chen, S. W. Ho, and K. T. Luh.** 1998. Antimicrobial susceptibility of viridans group streptococci in Taiwan with an emphasis on the high rates of resistance to penicillin and macrolides in *Streptococcus oralis*. J. Antimicrob. Chemother. **41:**621–627.