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Short Communication

Species-level assessment of the molecular basis of fluoroquinolone resistance among viridans group streptococci causing bacteraemia in cancer patients

Pranoti Sahasrabhojane[a], Jessica Galloway-Peña[a], Luis Velazquez[a], Miguel Saldaña[a], Nicola Horstmann[a], Jeffrey Tarrand[b], Samuel A. Shelburne[a,c,*]

[a] Department of Infectious Diseases, MD Anderson Cancer Center, Houston, TX 77030, USA
[b] Department of Laboratory Medicine, MD Anderson Cancer Center, Houston, TX 77030, USA
[c] Department of Genomic Medicine, MD Anderson Cancer Center, Houston, TX 77030, USA

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ABSTRACT

Viridans group streptococci (VGS) are a major cause of bacteraemia in neutropenic cancer patients, particularly those receiving fluoroquinolone prophylaxis. In this study, we sought to understand the molecular basis for fluoroquinolone resistance in VGS causing bacteraemia in cancer patients by assigning 115 VGS bloodstream isolates to specific species using multilocus sequence analysis (MLSA), by sequencing the quinolone resistance-determining regions (QRDRs) of *gyrA*, *gyrB*, *parC* and *parE*, and by testing strain susceptibility to various fluoroquinolones. Non-susceptibility to one or more fluoroquinolones was observed for 78% of isolates, however only 68.7% of patients were receiving fluoroquinolone prophylaxis. All but one of the determinative QRDR polymorphisms occurred in GyrA or ParC, yet the pattern of determinative QRDR polymorphisms was significantly associated with the fluoroquinolone prophylaxis received. By combining MLSA and QRDR data, multiple patients infected with genetically indistinguishable fluoroquinolone-resistant *Streptococcus mitis* or *Streptococcus oralis* strains were discovered. Together these data delineate the molecular mechanisms of fluoroquinolone resistance in VGS isolates causing bacteraemia and suggest possible transmission of fluoroquinolone-resistant *S. mitis* and *S. oralis* isolates among cancer patients.

1. Introduction

Infection is a leading cause of morbidity and mortality in neutropenic cancer patients, who are often given prophylactic antimicrobials in an attempt to decrease infection rates. Fluoroquinolones are the major class of antibiotics used for infection prophylaxis, particularly levofloxacin and ciprofloxacin [1]. However, widespread use of fluoroquinolone prophylaxis has led to increasing emergence of antimicrobial-resistant organisms [1]. Viridans group streptococci (VGS) are a large category of genetically heterogeneous streptococci that often cause serious infections in cancer patients, in part because of their propensity to develop fluoroquinolone resistance [1].

Although VGS encompass at least 15 distinct species capable of causing disease in humans, commonly used phenotypic and genotypic techniques often fail to accurately classify VGS strains to species level [2]. Multilocus sequence analysis (MLSA) was recently developed and is now considered a new 'gold standard' for VGS species identification as it has been the most successful approach to date at resolving species clusters for this taxonomically challenging group of organisms [2].

Previous studies of fluoroquinolone resistance in VGS have employed methodologies known to be problematic for proper VGS species identification [3,4]. Therefore, data that include accurate species identification when considering the specific genetic mechanisms underlying fluoroquinolone resistance and its epidemiology in particular VGS species are needed. In order to address this, we

* Corresponding author at: Department of Infectious Diseases and Department of Genomic Medicine, MD Anderson Cancer Center, Unit 1460, 1515 Holcombe Boulevard, Houston, TX 77030, USA. Tel.: +1 713 792 3629; fax: +1 713 792 5381.
E-mail addresses: sshelburne@mdanderson.org, samuel.shelburne@gmail.com (S.A. Shelburne).

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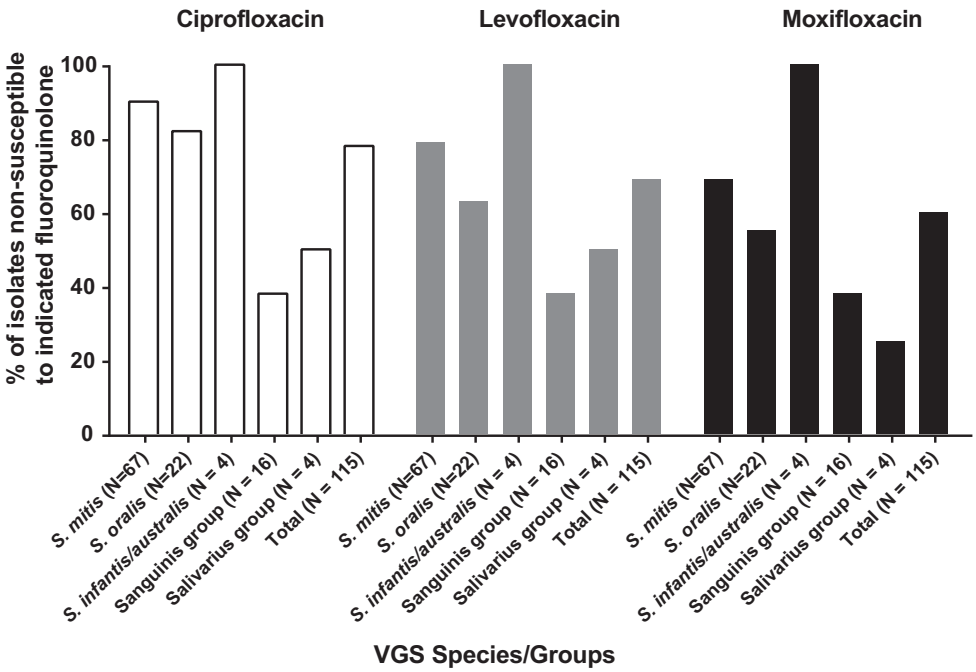

Fig. 1. Fluoroquinolone susceptibilities among the different viridans group streptococci (VGS) species causing bacteraemia in cancer patients. The percentage of strains that are non-susceptible to ciprofloxacin, levofloxacin and moxifloxacin is designated by species, with some VGS isolates placed into groups for ease of depiction. The number of isolates per species/group is indicated at the bottom of the graph. The two Anginosus group strains, which were both fully susceptible to all tested fluoroquinolones, are not included for space purposes.

took advantage of a large sample of VGS bacteraemia isolates and patient-specific clinical data and combined these resources with the improved speciation technique of MLSA.

2. Materials and methods

2.1. Bacterial strains, culture conditions, minimum inhibitory concentrations (MICs) and species determination

VGS bloodstream isolates were collected from unique patients at MD Anderson Cancer Center hospital (Houston, TX) from 15 April 2011 to 31 December 2012. Clinical and microbiological data, including receipt of fluoroquinolones, were abstracted from electronic medical records using a standardised data collection form. Initial identification of VGS strains was performed using standard microbiological techniques. Strains were grown in Todd–Hewitt broth with 0.2% yeast extract (Becton, Dickinson, and Co., Sparks, MD) at 37 °C with 5% CO_2. The MIC of various fluoroquinolones was determined by Etest (bioMérieux, Marcy l'Étoile, France). MICs were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria as follows: for ciprofloxacin and levofloxacin, sensitive ≤2 μg/mL, intermediate 4 μg/mL and resistant ≥8 μg/mL; and for moxifloxacin, sensitive ≤1 μg/mL, intermediate 2 μg/mL and resistant ≥4 μg/mL. VGS species determination and phylogenetic analyses were performed using MLSA as previously described [2].

2.2. Sequencing of gyrA, gyrB, parC and parE quinolone resistance-determining regions (QRDRs)

Amplification and sequencing of *gyrA*, *gyrB*, *parC* and *parE* loci in each of the VGS strains was done using previously published primers and protocols [3]. Delineation of the QRDRs corresponded to the following amino acid residues from *Escherichia coli*: 46–172 for GyrA; 371–512 for GyrB; 35–157 for ParC; and 398–483 for ParE [5]. QRDRs were compared with the consensus sequence for *Streptococcus pneumoniae* (National Center for Biotechnology Information) given that a consensus sequence, or susceptible allele, has not been established for each VGS species. Polymorphisms in the VGS QRDRs were considered determinative if the polymorphisms had been previously described as contributing to fluoroquinolone resistance, or if the polymorphism was present in non-susceptible VGS strains with no other polymorphisms known to contribute to fluoroquinolone resistance [1,3,6–8]. Polymorphisms not meeting these criteria were considered non-determinative.

2.3. Statistical analysis

The relationship between QRDR polymorphisms and type of fluoroquinolone prophylaxis was determined using χ^2 analysis. The statistical significance of the differences between the rates of fluoroquinolone non-susceptibility among distinct VGS species/groups was analysed by Fisher's exact test. All tests of significance were two-sided, and statistical significance was defined at $P \leq 0.05$. Statistical analysis was performed using IBM SPSS Statistics for Windows v.19.0 (IBM Corp., Armonk, NY).

3. Results and discussion

3.1. Clinical characteristics of infected patients and fluoroquinolone susceptibility of viridans group streptococci isolates

During the study period, 115 unique patients with VGS bacteraemia were identified; 93 (80.9%) were neutropenic and 79 (68.7%) were receiving fluoroquinolone prophylaxis at the time of infection onset. Of the 79 patients receiving fluoroquinolone prophylaxis, 57 (72%) were receiving levofloxacin, 17 (22%) ciprofloxacin and 5 (6%) moxifloxacin (Supplementary Table S1). Rates of VGS non-susceptibility to each of the tested fluoroquinolones were as follows: ciprofloxacin, 78% [MIC_{50} (MIC required to inhibit 50% of the isolates) ≥32 μg/mL; MIC_{90} (MIC