P1096

Incidence of Fluoroquinolone-Resistant Beta-Haemolytic Streptococci in North America and Europe: Report from the SENTRY Antimicrobial Surveillance Program, 1997-2002

DJ BIEDENBACH, M TOLEMAN, T WALSH, RN JONES
The JONES Group/JMI Laboratories, North Liberty, IA, USA and BCARE, Bristol, UK

ECCMID 2004
The JONES Group/JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background: Recent publications have reported rare cases of beta-haemolytic streptococci (BHS) with resistance (R) to fluoroquinolones (FQ). These pathogens can cause invasive disease and have generally remained susceptible (S) to the FQ class. This multi-center investigation was initiated to determine the rate of FQ-R and the responsible quinolone resistance-determining region (QRDR) mutations among BHS.

Methods: The SENTRY Program has tested FQ against BHS in North America (NA) and Europe (EU) since 1997. This study used NCCLS broth microdilution and Etest methods to determine S to ciprofloxacin (CIP), gatifloxacin (GAT), levofloxacin (LEV), garenoxacin (GAR), gemifloxacin (GEM) and moxifloxacin (MOX). Nineteen BHS isolates from NA and EU had CIP MIC results >2 mg/L. Vitek and API 20 strep as well as conventional methods and colony morphology were used to confirm identification. Eleven strains were available for molecular analysis using PCR to determine mutations in the QRDR. Primers were designed to amplify the QRDR of gyrase and topoisomerase genes (*gyrA, gyrB, parC, parE*) from *S. pyogenes* (BSA) and *S. agalactiae* (BSB) against respective genes found in the GenBank database. PCR products were sequenced on both strands by the dideoxy-chain termination method and analysis performed using laser gene DNA Star software.

Results: The rate of FQ-R BHS was 0.36% (EU) and 0.46% (NA) during the study period with the highest rate in 2002 (1.7%), a concern! These isolates included BSA (8), BSB (8) and *S. dysgalactiae* (BSC/G; 3). The MIC_{90} for the FQs (mg/L) showed highest potency for: GEM (2) > GAR (4) > MOX (8) > GAT (16) > LEV (>4) > CIP (>32). All strains had significant mutations in either *parC* (positions 79 or 83) and/or *gyrA* (positions 81 or 85). Two BSA strains with lower level R to CIP (4 mg/L) had only *parC* mutations (Ser79 to Phe). All isolates of BHS with high-level R (>32 mg/L) to CIP had *gyrA* mutations and often *parC* mutations. Numerous other mutations in the QRDR region were found including *gyrB* and *parE,* although their significance remains unknown.

Conclusions: The increasing rate of FQ-R streptococci including *S. pneumoniae*, viridans group streptococci and more recently reported, BHS, is becoming a clinical concern due to the morbidity and mortality caused by these pathogens. Strains of BHS with high-level R to FQ have point mutations common to other streptococci in *gyrA* and *parC* and were most prevalent in 2002.

INTRODUCTION

Recent antimicrobial agents in the fluoroquinolone class have retained excellent potency against streptococci since they were introduced and increasingly used in clinical practice starting in the late 1980s. However, there have been reports of streptococcal strains with elevated MIC values to fluoroquinolones and there has been a noticeable increase in the resistance rate to this class particularly among strains of viridans group streptococci and *S. pneumoniae* in longitudinal surveillance programs. Resistance to fluoroquinolones have been mainly caused by target site alterations in the fluoroquinolone-resistance determining region (QRDR) and/or drug efflux. Recent reports of *Streptococcus agalactiae* (a ß-haemolytic Lancefield group B streptococcus) and *S. pyogenes* (a ß-haemolytic Lancefield group A streptococcus) with high-level resistance to fluoroquinolones suggest that resistance is analogous to the reported mechanisms for *S. pneumoniae* and viridans group streptococci. Specific reports documenting fluoroquinolone-resistant strains of *S. dysgalactiae* subspecies *equisimilis* (Lancefield groups C and G) have been limited and a literature search showed no evidence of studies having evaluated the target site alterations of the QRDR for this species.

INTRODUCTION (Continued)

The SENTRY Antimicrobial Surveillance Program has monitored the antimicrobial susceptibility profile of ß-haemolytic streptococci since 1997. Numerous fluoroquinolones have been tested including those with increased Gram-positive potency. This study will evaluate the reference MIC values that were obtained for older and newer generation fluoroquinolones tested against ß-haemolytic streptococci. Target site alterations for available strains of ß-haemolytic streptococci, including group C and G, that had elevated fluoroquinolone MICs were also evaluated in this study.

MATERIALS AND METHODS

Bacterial Identification and Susceptibility Test Methods. Between 1997 and 2002, the SENTRY Antimicrobial Surveillance Program collected and tested 4,487 isolates of ß-haemolytic streptococci from North American (2,827 strains) and European (1,660 strains) medical centers. A direct comparison of 3,344 strains of ß-haemolytic streptococci were assessed using NCCLS antimicrobial susceptibility testing methods against four fluoroquinolones including ciprofloxacin, gatifloxacin, levofloxacin and garenoxacin. Etest (AB BIODISK, Solna, Sweden) was used to test the susceptibility of the four mentioned fluoroquinolones plus gemifloxacin and moxifloxacin against strains with ciprofloxacin MIC results of > 2 mg/L. Isolates were identified by the local laboratory and confirmed by the reference laboratory (JMI Labs, North Liberty, IA, USA) using colony morphology and haemolysis characteristics. Conventional methods including PYR, bacitracin and CAMP tests were also used as needed. Vitek and API 20 strep (bioMerieux, Hazelwood, MO, USA) were used to confirm the identification of isolates with elevated ciprofloxacin MIC results (19 strains).

Primer Design. Primers used to amplify the expected QRDR of gyrase and topoisomerase genes from *S. pyogenes* and *S. agalactiae* were designed from sequences of the respective genes found in the GenBank database (Table 1). These primers were also used to amplify the respective genetic regions of the other ß-streptococci. Sequences that could not be amplified using these primers were amplified with “degenerate” primers designed using an alignment of all available streptococcal gyrase and topoisomerase genes found in the databases.

PCR Conditions. PCR was performed using AB-gene Expand Hi-Fidelity master mix containing a mix of PFU-non-proofreading TAQ polymerases and dNTPs (ABGENEhouse, Surrey, UK). Primers were used at 10 pico-molar concentrations and 1µl of bacterial culture at density OD 1 at 600nm was used as template. Cycling parameters were: 95ºC for five minutes followed by 30 cycles of 95ºC for one minute, annealing at 45ºC for one minute and extension 68ºC for one minute and ending with a five minute incubation at 68ºC. PCR products were visualized by electrophoresis on 0.8% agarose gels in Tris Boric Acid/EDTA buffer (pH 7.0) and stained with 1% ethidium bromide. PCR products were sequenced on both strands by the dideoxy-chain termination method with a Perkin Elmer Biosystems 377 DNA sequencer. Sequence analysis was performed using the Lasergene DNASTAR software package.

Amino acid positions were numbered according to the gene in the databases displaying most identity. For the ß-haemolytic streptococci whose species identity was not determined: 1) All *parC* genes were most similar to *parC* from *S. pneumoniae* and were numbered accordingly; 2) all *gyrA* genes were most homologous to *S. agalactiae*; 3) Strains 30-7056, 15-2668 and 13-14536 *parE* genes were most similar to *S. pyogenes parC* and their *gyrB* genes were most similar to *S. agalactiae gyrB*; 4) Strains 51-628 and 84-16073 *parE* were most similar to *S .pneumoniae parE*; 5) Strain 51-628 *gyrB* was most similar to *S. pneumoniae gyrB*; and 6) Strain 84-16073 was closest to *S. pyogenes gyrB*.

RESULTS

Table 1. Primers designed to amplify quinolone-resistance determining region of gyrase and topoisomerase genes.[a]

Primer	Sequence	Target gene/ accession number	Expected size of product
BSAGYRAF	AGTGTCATTGTGGCAAGAGC	*gyrA* AB101455	500 bp
BSAGRYAR	CAGCGTCAATAGATTCTGCC	*S. pyogenes*	
BSAGYRBF	ATTGGGCAACTCAGAAGTGG	*gyrB* AE014146	600 bp
BSAGYRBR	TCACTACCGACCTTAACACC	*S. pyogenes*	
BSAPARCF	CCGTAAATCAGCCAAATCAG	*parC* AE006540	520 bp
BSAPARCR	CACGTCCCTTACCTGTTTCG	*S. pyogenes*	
BSAPAREF	CGAAAAGCGCGTGATGACTC	*parE* AE006540	576 bp
BSAPARER	CGCAGATCTTCCAATTCACC	*S. pyogenes*	
BSBGYRAF	CGAGTTTTATCGATTACGCC	*gyrA* NC004116	511 bp
BSBGYRAR	ATCACCAAGGCACCAGTAGG	*S. agalactiae*	
BSBGYRBF	CTGCTTCCAAAACAGGTCGC	*gyrB* NC004116	644 bp
BSBGYRBR	GGAGAAGATGTTCGTGAAGG	*S. agalactiae*	
BSBPARCF	AAGGGATTTCGCAAATCTGC	*parC* NC004116	494 bp
BSBPARCR	TCCTTGAATGATAGCGCCAG	*S. agalactiae*	
BSBPAREF	CGTAAGGCAATAAAAGCACG	*parE* NC004116	547 bp
BSBPARER	CTATATCCGTCCAAGCATAC	*S. agalactiae*	
STREPGYRAF	ATYGAYGCBATGAGTG	*gyrA* degenerate	~350 bp
STREPGYRAR	ATRCGYGCYTCDGTATAACG	*Streptococcus*	
STREPGYRBF	TTRAYACCATARATWGGYGG	*gyrB* degenerate	~650 bp
STREPGYRBR	CCMAATCCDCARTTTGAAG	*Streptococcus*	
STREPPARCF	TAYATYATTCARGAMCGGGC	*parC* degenerate	~500 bp
STREPPARCR	TCWGMYARATTRTGYGGTGG	*Streptococcus*	
STREPPAREF	TGAAYTSTATYTRGTYGARGG	*parE* degenerate	~550 bp
STREPPARCR	TGRTYVGCATTCATYTCMCC	*Streptococcus*	

a. Sequences were from the GenBank database.

Table 2. Comparative potencies of four fluoroquinolones tested against 3,344 strains of ß-haemolytic streptococci.

Organism (no. tested)/ Antimicrobial agent	MIC (mg/L) 50%	90%	Range
Streptococcus group A (1,568)			
Ciprofloxacin	0.5	1	≤0.25->2
Levofloxacin	0.5	1	≤0.03->4
Gatifloxacin	0.25	0.25	≤0.03->4
Garenoxacin	0.06	0.12	≤0.03-2
Streptococcus group B (1,295)			
Ciprofloxacin	0.5	1	≤0.25->2
Levofloxacin	0.5	1	0.06->4
Gatifloxacin	0.25	0.25	0.06->4
Garenoxacin	0.06	0.12	≤0.03-2
Streptococcus group C, G (449)			
Ciprofloxacin	0.5	1	≤0.25->2
Levofloxacin	0.5	0.5	0.06->4
Gatifloxacin	0.25	0.25	≤0.03-4
Garenoxacin	0.06	0.12	≤0.03-1
Streptococcus group F (32)			
Ciprofloxacin	0.5	1	≤0.25-1
Levofloxacin	0.5	0.5	≤0.03-0.5
Gatifloxacin	0.12	0.25	≤0.03-0.25
Garenoxacin	0.03	0.06	≤0.03-0.06

- The overall rate of resistance to fluoroquinolones (ciprofloxacin MIC, > 2 mg/L) in North America and Europe during 1997 to 2002 was very low at 0.46% and 0.36%, respectively.
- The potencies of newer fluoroquinolones, garenoxacin (MIC_{90}, 0.06 - 0.12 mg/L) and gatifloxacin (MIC_{90}, 0.25 mg/L) was superior to levofloxacin (MIC_{90}, 0.5 - 1 mg/L) and ciprofloxacin (MIC_{90}, 1 mg/L) against all serogroups of ß-haemolytic streptococci (Table 2).
- Among the 19 strains of ß-haemolytic streptococci with ciprofloxacin MIC values of > 2 mg/L, the rank order of quinolone potency was typically gemifloxacin > garenoxacin > moxifloxacin >gatifloxacin > levofloxacin (Table 3).
- Amino acid substitutions among ß-haemolytic streptococci included the common point mutations found in *S. pneumoniae* and viridans group streptococci for *gyrA* (Ser81 and Glu85) and *parC* (Ser79, Asn91 and Asp83).
- Previously undescribed point mutations in *gyrA* (Asp80Ala and Ala115Pro) and *parC* (Ala121Val) were detected.
- Group C and G ß-haemolytic streptococci had similar amino acid susbstitutions as those observed in group A and B. However, all three strains had different amino acids at positions 56 in *gyrA* and positions 56 and 58 in *parC*.

CONCLUSIONS

- Fluoroquinolone resistance among ß-haemolytic streptococci remains a rare occurrence in North America and Europe during 1997 - 2002 multicenter surveillance.
- Newer generation fluoroquinolones have enhanced activity against ß-haemolytic streptococci.
- Amino acid substitutions in the QRDR of ß-haemolytic streptococci appear similar to those found in other streptococcal species.
- More fluoroquinolone-resistant ß-haemolytic streptococci were discovered in the last year of the study (2002).

SELECTED REFERENCES

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. *Antimicrobial Agents and Chemotherapy* 42:2792-2798.

Janoir C, Zeller V, Kitzis M-D, Moreau NJ, Gutmann L. (1996). High-level fluoroquinolone resistance in *Streptococcus pneumoniae* requires mutations in *parC* and *gyrA*. *Antimicrobial Agents and Chemotherapy* 40:2760-2764.

Kawamura Y, Fujiwara H, Mishima N, Tanaka Y, Tanimoto AS, Ikawa S, Itoh Y, Ezaki T. (2003). First *Streptococcus agalactiae* isolates highly resistant to quinolones, with point mutations in *gyrA* and *parC*. *Antimicrobial Agents and Chemotherapy* 47:3605-3609.

Yan SS, Fox ML, Holland SM, Stock F, Gill VJ, Fedorko DP. (2000). Resistance to multiple fluoroquinolones in a clinical isolate of *Streptococcus pyogenes*: Identification of *gyrA* and *parC* and specification of point mutations associated with resistance. *Antimicrobial Agents and Chemotherapy* 44:3196-3198.

Table 3. Distribution of fluoroquinolone MICs for 19 strains of ß-haemolytic streptococci with ciprofloxacin MIC values ≥4 mg/L and the amino acid substitutions responsible for elevated fluoroquinolone MIC results.

Organism/isolate #	Year	MIC (mg/L)[a] CIP	LEV	GAT	MOX	GAR	GEM	Amino acid substitution *gyrA*	*parC*
Group A streptococci									
71-3319D	1998	>2	1	0.5	NT[b]	NT	NT	NT	NT
65-3302A	2000	4	2	0.5	0.25	0.25	0.12	None	Ser79Phe, Ala121Val[c]
89-7025A	2000	>32	>4	4	2	1	0.5	Asp80Ala[c]	Ser79Phe, Asn91Asp
89-7974A	2000	4	2	1	0.5	1	0.25	None	Ser79Phe
88-11944A	2002	>32	>4	32	8	4	2	Ser81Phe	ND[d]
32-122A	2002	8	2	1	0.5	0.25	0.25	NT	NT
51-1556D	2002	8	2	1	0.5	0.25	0.25	NT	NT
12-3081A	2002	8	2	1	1	0.5	0.25	NT	NT
Group B streptococci									
14-1608D	1997	>32	>4	4	4	2	2	Glu85Lys	Asp83Asn
66-1784A	1998	>2	2	0.5	NT	NT	NT	NT	NT
14-3753A	1998	>32	>4	4	4	2	2	Glu85Lys	Asp83Asn
51-628H	2001	>32	>4	4	2	0.5	0.5	Ser81Tyr, Ala115Pro[c]	ND
19-756A	2002	>32	>4	>32	8	4	4	NT	NT
82-1658A	2002	4	2	1	0.5	0.25	0.25	NT	NT
106-4910A	2002	>32	4	4	4	1	2	NT	NT
21-15207A	2002	>32	>4	8	4	1	0.5	Glu85Lys	None
Group C, G streptococci									
15-2668A	2000	>32	>4	4	2	1	0.25	Ser81Phe, Thr56Met[c]	Asp56Glu[c], Ser58Gly[c]
30-7056A	2001	>32	>4	16	4	2	2	Glu85Lys, Thr56Met[c]	Asn91Asp, Asp56Glu[c], Ser58Gly[c]
13-14536A	2002	>32	4	2	2	0.5	0.5	Ser81Phe, Thr56Met[c]	Asp83Tyr, Asp56Glu[c]

a. CIP = ciprofloxacin; LEV = levofloxacin; GAT = gatifloxacin; MOX = moxifloxacin; GAR = garenoxacin; and GEM = gemifloxacin.
b. NT = not tested.
c. Mutation not previously reported.
d. ND = not determined.