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First Molecular Characterization of Fluoroquinolone Resistance in *Aerococcus* spp.[▽]

Aerococcus urinae and *Aerococcus sanguinicola* are emerging Gram-positive pathogens responsible for urinary tract infections, especially in elderly patients (2). Although they seem to be intrinsically susceptible to fluoroquinolones (3, 8, 10), acquired fluoroquinolone resistance has not been yet reported. Resistance to fluoroquinolones in Gram-positive bacteria is mainly due to point mutations in the quinolone-resistance determining regions (QRDRs) of the GyrA and GyrB subunits of the DNA gyrase and QRDRs of ParC and ParE subunits of the topoisomerase IV (4). Decreased accumulation of fluoroquinolones is a second resistance mechanism that is mediated by the overexpression of efflux pump systems (4). Since QRDR sequences of *A. urinae* and *A. sanguinicola* are not available, the aim of this study was to elucidate the mechanisms associated with the fluoroquinolone resistance.

Nineteen *A. urinae* and 8 *A. sanguinicola* urinary isolates, previously identified by 16S rRNA sequencing, were studied (2). The MICs of ofloxacin, ciprofloxacin, levofloxacin, and moxifloxacin were established using the Etest method (AB Biodisk, Solna, Sweden) on Mueller-Hinton agar supplemented by 5% horse blood. The MICs of ciprofloxacin were also determined in the presence of reserpin, an efflux pump inhibitor, incorporated in the medium (10 μg/ml) (11). Against *A. urinae* isolates, moxifloxacin (MIC_{50}, 0.12 μg/ml) was 4- and 16-fold more active than ciprofloxacin/levofloxacin (MIC_{50}, 0.5 μg/ml) and ofloxacin (MIC_{50}, 2 μg/ml), respectively (Table 1). Against *A. sanguinicola* isolates, moxifloxacin (MIC_{50}, 0.25 μg/ml) was also 4- and 16-fold more active than ciprofloxacin/levofloxacin (MIC_{50}, 1 μg/ml) and ofloxacin (MIC_{50}, 4 μg/ml), respectively (Table 1). The potent activity of moxifloxacin against *Aerococcus* spp. is concordant with data previously reported (8). Finally, active efflux did not seem to play a major role in fluoroquinolone resistance in aerococci, since the MICs of ciprofloxacin were similar in the absence or presence of reserpin (Table 1).

Following the use of degenerate primers, the DNA fragments corresponding to QRDRs of GyrA, GyrB, ParC, and ParE were amplified using standard PCR conditions with novel specific primers (Table 2). The sequences of GyrA, GyrB, ParC, and ParE of *A. urinae* were 100%, 98%, 88%, and 93% identical to those of *A. sanguinicola*, respectively. In *A. urinae*, a serine residue and a glutamate residue were found at positions 84 and 88 (corresponding to 83 and 87 in *Escherichia coli* numbering) in GyrA and also at positions 79 and 83 (corresponding to 80 and 84 in *E. coli* numbering) in ParC, as described in *Enterococcus faecalis* (9). The unique difference with

TABLE 1. Susceptibility to fluoroquinolones and mutations in QRDRs of *gyrA*, *gyrB*, *parC*, and *parE* genes of *A. urinae* and *A. sanguinicola*

Organism (no. of strains)	MIC (μg/ml) of[a]:					QRDR mutation(s)			
	OFX	CIP	CIP+R	LVX	MXF	*gyrA*	*gyrB*	*parC*	*parE*
A. urinae strains (19)									
HM 335	≥32	≥32	≥32	≥32	≥32	S84L		S79R	
HM 365	2	0.5	0.5	0.25	0.12				
HM 384	0.5	0.12	0.12	0.12	0.12				
HM 525	0.5	0.25	0.25	0.12	0.06				
HM 580	1	0.25	0.25	0.25	0.03				
HM 693	2	0.5	0.5	0.5	0.25				
HM 704	4	1	1	1	0.25				
HM 713	2	0.5	0.25	0.5	0.12				
HM 743	2	0.5	0.25	0.5	0.12				
HM 827	1	0.25	0.25	0.25	0.06				
HM 834	≥32	≥32	≥32	≥32	2			E83K	
HM 867	2	0.5	0.5	0.5	0.12				
HM 915	0.5	0.12	0.12	0.12	0.03				
HM 963	2	0.5	0.25	0.5	0.12				
HM 980	1	0.25	0.25	0.25	0.12				
HM 1010	0.5	0.25	0.25	0.25	0.06				
HM 1062	2	1	1	0.5	0.12				
HM 1107	2	0.5	0.25	0.5	0.06				
HM 1230	2	1	1	0.5	0.25				
A. sanguinicola strains (8)									
HM 826	≥32	8	8	8	1			D78N, S79T	
HM 833	4	1	0.5	1	0.5				
HM 946	1	0.5	0.5	0.5	0.25				
HM958	≥32	16	16	8	1			D83G	
HM 962	4	1	1	1	0.25				
HM 1014	2	1	1	1	0.25				
HM 1036	4	2	1	1	0.5				
HM 1273	1	1	0.5	1	0.25				

[a] OFX, ofloxacin; CIP, ciprofloxacin; CIP+R, ciprofloxacin plus reserpin (10 μg/ml), LVX, levofloxacin; MXF, moxifloxacin.

TABLE 2. Deoxynucleotide primers used in this study

Species	Primer[a]	Sequence (5′–3′)	Gene	Annealing temp (°C)	Product size (bp)
A. urinae	gyrA-uri-F	TCT CAA ACC CGT CCA CCG	*gyrA*	55	232
	gyrA-uri-R	GGC TTG GTC CCC GTC GA			
	gyrB-uri-F	TTG AAG GGC AAA CCA AGA TG	*gyrB*	52	486
	gyrB-uri-R	TCA CCA ATT TAT GGT AAC GG			
	parC-uri-F	TAT ATT ATT CAA GAA CGC GC	*parC*	50	333
	parC-uri-R	GTC CTT AAG TAG TTC ATC GG			
	parE-uri-F[b]	ATT TGA AGG TCA AAC CAA GG	*parE*	50	504
	parE-uri-R[b]	GCA TCG GTC ATG ATG ATC AC			
A. sanguinicola					
	gyrA-san-F	GGG ATG AAA CCT GTC CAC CG	*gyrA*	55	244
	gyrA-san-R	GCT AAG CGG CAG CCT GGT C			
	gyrB-san-F	CAG ACC CAC AGT TTG AAG G	*gyrB*	52	521
	gyrB-san-R	ATC GAC ATC GGC ATC AGT C			
	parC-san-F	TAC ATT ATT CAA GAA CGG GC	*parC*	52	389
	parC-san-R	GGT TCT TCC TCA GTA TCA TC			

[a] F, sense primer; R, antisense primer.
[b] Amplification of the *parE* gene from *A. sanguinicola* was also performed using primers parE-uri-F and parE-uri-R.

A. sanguinicola was the presence of an aspartate residue at position 83 in ParC, as described in *Streptococcus pneumoniae* (9). Whereas all susceptible strains possessed no mutation, at least one mutation was found in *gyrA* and/or *parC* in all four resistant strains (Table 1). Except for S79T, similar amino acid changes in hot spot positions of ParC have been identified in other Gram-positive bacteria; these are S79R in *E. faecalis* and *Enterococcus faecium*, E83K in *E. faecium* and *Staphylococcus aureus*, and D78N and D83G in *S. pneumoniae* (1, 5, 7, 9). Concerning GyrA, an identical mutation (S84L) has also been identified in *S. aureus*, *E. faecium*, and *Streptococcus agalactiae* (6, 9). These findings suggest that topoisomerase IV seems to be the primary target of fluoroquinolones in *Aerococcus* spp., as previously described in other Gram-positive bacteria (4, 9).

Nucleotide sequence accession numbers. The QRDR sequences of the *gyrA*, *gyrB*, *parC*, and *parE* genes of *A. urinae* HM 384 (susceptible strain) have been submitted to GenBank under accession no. HM744700, HM744701, HM744702, and HM744703, respectively, as have those of *A. sanguinicola* HM 1273 (susceptible strain), under accession no. HM744704, HM744705, HM744706, and HM744707, respectively.

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