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Fluoroquinolone-Resistant *Haemophilus parasuis* Isolates Exhibit More Putative Virulence Factors than Their Susceptible Counterparts

Qiang Zhang, Jiantao Liu, Shuxian Yan, Yujie Yang, Anding Zhang, Meilin Jin

Division of Animal Infectious Disease, State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, China

The prevalence of 23 putative virulence factors among fluoroquinolone-susceptible and -resistant *Haemophilus parasuis* isolates was analyzed. Putative hemolysin precursor, fimbrial assembly chaperone, and type I site-specific restriction modification system R subunit genes were more prevalent among fluoroquinolone-resistant *H. parasuis* isolates than among fluoroquinolone-susceptible *H. parasuis* isolates. Fluoroquinolone resistance may be associated with an increase in the presence of some virulence factors.

To date, many mechanisms of fluoroquinolone resistance have been identified. These mechanisms include mutations in the topoisomerase II and IV genes, overexpression of efflux pumps, and decreases in cell wall permeability. However, these mechanisms only partly explain fluoroquinolone resistance. The reason is that the acquisition of resistance may be directly associated with phenotypic changes in bacteria, such as a change in the virulence factors (1).

To add to the body of knowledge concerning fluoroquinolone resistance, the correlation between fluoroquinolone resistance and putative virulence factors in *Haemophilus parasuis* was investigated in this study. In our previous study, 138 *H. parasuis* isolates were collected in the period of 2002 to 2009 in seven different Chinese provinces. In addition, the susceptibility of these isolates to enrofloxacin and levofloxacin was determined using the Etest (AB Biodisk, Solna, Sweden). The results indicated that 83 (60.1%) of the isolates were enrofloxacin resistant and that 8 (5.8%) of the isolates were levofloxacin resistant (2).

The virulence factors of *H. parasuis* have not been unequivocally defined, but many putative virulence-associated genes have been identified and tested (3). A total of 23 putative virulence factors were examined by PCR, like in previous studies (4, 5). The primers and reaction conditions are listed in Table SA1 in the supplemental material. The prevalence of 23 putative virulence factors was 100% in the *H. parasuis* SH0165 strain, which was used as the positive control. The distribution of the putative virulence factors according to the fluoroquinolone resistance phenotype in 138 *H. parasuis* isolates is described in Table 1. Statistical analyses were performed using Fisher's exact or chi-square tests, and those virulence factors that yielded a *P* value of less than 0.05 were presented. The prevalences of *hhdA* (putative hemolysin precursor), *fimB* (fimbrial assembly chaperone), and *hsdR* (type I site-specific restriction-modification system, R subunit) in the enrofloxacin-resistant isolates were 77.1%, 75.9%, and 48.2%, respectively. Compared with the enrofloxacin-susceptible isolates (29.1%, 20%, and 14.5%), these prevalences were all statistically significant (all *P* values were <0.001). A similar finding was obtained for the levofloxacin-resistant and -susceptible isolates (all *P* values were <0.05). The analyses demonstrated that both enrofloxacin resistance and levofloxacin resistance are associated with a significantly increased prevalence of *hhdA*, *fimB*, and *hsdR* (Table 1). The results suggest that fluoroquinolone resistance may be directly associated with additional putative virulence factors in *H. parasuis*.

Some reports provided evidence that an inverse relationship between quinolone resistance and virulence occurred in uropathogenic *Escherichia coli* strains from phylogenetic group B2 (6–8), but it was possible that this phenomenon may be special among strains of the B2 phylogenetic group (9). Our finding proved that fluoroquinolone resistance may be associated with an increase in the presence of some virulence factors in *H. parasuis*, and it suggested that the relationship of resistance and virulence in *H. parasuis* is the opposite of the inverse relationship in *E. coli*. Although this hypothesis that fluoroquinolone resistance is relevant to the prevalence of certain virulence factors is already known, its mechanism remains unclear (10).

H. parasuis is a common epiphyte of the upper respiratory tract of pigs. The strains that exhibit more virulence factors may have been more strongly influenced by antibiotics in their evolutionary history, because pigs that were infected with these strains were more easily found and treated using a high dose of drug. Therefore, a higher incidence of quinolone resistance was observed in those strains that exhibited more virulence factors than among those strains that exhibited fewer virulence factors (11).

In conclusion, this study provides the first observation that fluoroquinolone resistance could be directly associated with a higher prevalence of putative virulence factors (*hhdA*, *fimB*, and *hsdR*) in *H. parasuis* rather than a lower prevalence. And this finding enriches the body of knowledge concerning fluoroquinolone resistance.

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Address correspondence to Meilin Jin, jinmeilin@mail.hzau.edu.cn.
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TABLE 1 Distribution of putative virulence factors according to the fluoroquinolone resistance phenotype in 138 *H. parasuis* isolates

Putative virulence factor[a]	No. (%) of isolates[b]					
	Enrofloxacin			Levofloxacin		
	Susceptible ($n = 55$)	Resistant ($n = 83$)	*P* value	Susceptible ($n = 130$)	Resistant ($n = 8$)	*P* value
hhdA	16 (29.1)	64 (77.1)	<0.001	72 (55.4)	8 (100.0)	0.021
fimB	11 (20.0)	63 (75.9)	<0.001	68 (52.3)	8 (100.0)	0.008
hsdR	8 (14.5)	40 (48.2)	<0.001	42 (32.3)	6 (75.0)	0.021
aidA	15 (27.3)	29 (34.9)	0.359	42 (32.3)	2 (25.0)	0.968
wza	50 (90.9)	68 (81.9)	0.216	112 (86.2)	6 (75.0)	0.327
fhuA	55 (100.0)	81 (97.6)	0.517	128 (98.5)	8 (100.0)	1
hhdB	53 (96.4)	83 (100.0)	0.306	128 (98.5)	8 (100.0)	1
dsbA	55 (100.0)	80 (96.4)	0.276	127 (97.7)	8 (100.0)	1
groel	55 (100.0)	80 (96.4)	0.276	127 (97.7)	8 (100.0)	1
hkte	55 (100.0)	81 (97.6)	0.517	128 (98.5)	8 (100.0)	1
cirA	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
pilA	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
pilB	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
tolC	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
dsbc	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
omp2	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
omp5	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
sodC	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
dohhoA	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
cdtA	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
cdtB	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
cdtC	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—
cdt gene cluster	55 (100.0)	83 (100.0)	—	130 (100.0)	8 (100.0)	—

[a] These putative virulence factors were reported in previous studies: *hhdA*, putative hemolysin precursor; *fimB*, fimbrial assembly chaperone; *hsdR*, type I site-specific restriction-modification system, R (restriction) subunit; *aidA*, putative pertactin family virulence factor; *aidA*, adhesin; *wza*, polysaccharide export protein; *fhuA*, ferrichrome receptor precursor protein; *hhdB*, heme-hemopexin-binding protein B; *dsbA* and *dsbc*, thiol:disulfide interchange proteins DsbA and DsbC, respectively; *groel*, chaperonin GroEL (HSP60 family); *hkte*, catalase; *cirA*, iron-regulated outer membrane protein; *pilA*, pilus A; *pilB*, TFP pilus assembly pathway, ATPase PilB; *tolC*, RND efflux system outer membrane lipoprotein; *omp2* and *omp5*, outer membrane proteins 2 and 5, respectively; *sodC*, superoxide dismutase; *dohhoA*, periplasmic serine protease DohhoA-like precursor; *cdtA*, *cdtB*, *cdtC*, and *cdt* gene cluster, cytolethal distending toxins A, B, C, and holotoxin, respectively.

[b] —, the *P* value cannot be analyzed using Fisher's exact or chi-square tests.

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