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Characterization of fluoroquinolone resistance in methicillin-resistant *Staphylococcus aureus* of human and pig origin

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Sir,

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important

multidrug-resistant pathogens responsible for nosocomial and community-associated infections worldwide. On the basis of their origin, MRSA isolates have been categorized as health care-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA), and livestock-associated MRSA (LA-MRSA)[1]. The recently defined LA-MRSA has been isolated in a wide range of food animals, particularly pigs. The prevalence of major MRSA clonal lineages in pigs shows regional variation; for example, clonal complex(CC) 398 is predominant in Europe,while CC9 is the most common clonal lineage in Asia. Several publications have reported carriage and infection of LA-MRSA in humans; in some hospitals located in areas with a high density of livestock farms, LA-MRSA has contributed to a considerable number of cases of human MRSA colonization and infection[2]. LA-MRSA is a growing public health concern because it may transfer to humans through the food chain and/or environment and could potentially become an important source of human MRSA infections.

The fluoroquinolone class of antimicrobial agents is widely used to treat both Gram-positive and Gram-negative bacterial infections. Although several fluoroquinolones have been developed, enrofloxacin is the only one that is exclusively and commonly used in veterinary medicine. Fluoroquinolone resistance in staphylococci is mainly attributed to mutations in the quinolone-resistance determining regions (QRDRs) of genes encoding target enzymes DNA gyrase and DNA topoisomerase [3]. In addition, overexpression of multidrug efflux pumps NorA, NorB, and NorC has an additive effect with QRDR mutations on conferring high-level fluoroquinolone resistance [4].

The present study aimed to compare the clonal lineages as well as fluoroquinolone-resistance phenotype and target gene mutations between MRSA isolates of human and pig origin. We collected 348 nasal swabs between November 2011 and August 2012 from five pig farms and a pig slaughterhouse in Shanghai, two pig farms in Jiangsu province, and a pig farm in Zhejiang province, China. 174 *S. aureus* strains were isolated and 89 of them were identified as MRSA. 120 samples of throat swab and sputum were collected from patients at a hospital in Shanghai between April and May 2012. 52 *S. aureus* strains were isolated and 15 of them were identified as MRSA. The 89 pig-origin and 15 hospital-origin MRSA isolates were included in this study. All these isolates were characterized by multilocus sequence (MLST) and staphylococcal protein A (spa) typing. All 89 pig-origin MRSA isolates were assigned to the ST9-t899 lineage. Among the hospital-origin MRSA isolates, all except one—which belonged to the ST88-t10775 lineage—were identified as belonging to the ST5-t002 lineage. Large-scale surveillance of the prevalent genotypes of human- and pig-origin MRSA isolates, especially in the regions with high density of pig farms, should be conducted to investigate if LA-MRSA invasion and colonization into hospital has already occurred.

Among the 89 pig-origin MRSA isolates, 16 that exhibited enrofloxacin minimum inhibitory concentrations (MICs) ≥1 mg/L were selected for further analysis of fluoroquinolone-resistance phenotype and target gene mutations. In case of human-origin strains, all 15 were included for further analysis. While all 14 ST5-t002 lineage human-origin isolates showed ciprofloxacin resistance (MICs, ≥4 mg/L), the lone ST88-t10775 isolate was

susceptible to ciprofloxacin (MIC, 0.25mg/L) and served as a control. Susceptibility to enrofloxacin, norfloxacin, levofloxacin, ciprofloxacin, and gatifloxacin were determined by the broth microdilution method in accordance with Clinical and Laboratory Standards Institute guidelines[5]. For all 16 pig-origin MRSA isolates, the MICs of each tested fluoroquinolone antibiotic were no greater than two times the resistance breakpoint. In contrast, the majority of human-origin MRSA strains exhibited high-level fluoroquinolone resistance (Table 1).

The QRDRs of the *gyr*A, *gyr*B, *grl*A, and *grl*B genes of all MRSA isolates were amplified and sequenced as described previously[3]. All 16 pig-origin strains exhibited amino-acid mutations both in GrlA (S80F) and GyrA (S84A); strain W111 exhibited two additional mutations—E560H and G571V in GrlB—neither of which has ever been described in fluoroquinolone-resistant staphylococci. Of the 14 hospital-origin isolates, 12 with ciprofloxacin MICs ≥32 mg/L exhibited double amino-acid mutations both in GrlA and GyrA. Two patterns of mutation were observed — S80F/E84K and S84L/S85P in GrlA and GyrA and S80Y/E84K and S84L/E88K in GrlA and GyrA, respectively (Table 1). An additional mutation, A496T in GyrB, was observed in strain H35. The remaining two low-level ciprofloxacin-resistant (MICs, ≤8 mg/L) isolates, H18 and H37, showed single amino-acid mutations both in GrlA(S80Y) and GyrA(S84L). It should be noted that some isolates with the same QRDR mutations exhibited different fluoroquinolone MICs, which might be attributable to differences in expression levels of the multidrug efflux pumps NorA, NorB, and NorC[4]. Further studies should be conducted to confirm this possibility.

In summary, significant differences in predominant clonal types, fluoroquinolone resistance, and QRDR mutation patterns were observed between MRSA isolates of human and pig origin. Further surveillance studies are warranted to investigate if these differences are also present in other regions.

Declarations

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Table 1 MICs of five fluoroquinolones and QRDR mutations inMRSA isolates of human and pig origin

Isolates	Origin and type	MICs of five fluoroquinolones (mg/L)					Mutations in QRDRs of			
		ENR	NOR	LVX	CIP	GAT	GrlA	GyrA	GrlB	GyrB
F39, J21, J8, P24, W1, W220, J13, J18, W77, W101	Pig, ST9-t899	4	32	2	8	1	S80F	S84A	—	—
W111	Pig, ST9-t899	4	32	2	8	1	S80F	S84A	E560H,G571V	—
J3, J19	Pig, ST9-t899	4	16	2	8	0.5	S80F	S84A	—	—
W229	Pig, ST9-t899	2	16	1	4	0.5	S80F	S84A	—	—
J15	Pig, ST9-t899	4	16	2	4	0.5	S80F	S84A	—	—
W109	Pig, ST9-t899	2	8	2	2	0.5	S80F	S84A	—	—
H4, H13, H40	Human, ST5-t002	256	≥512	256	256	64	S80F, E84K	S84L, S85P	—	—
H35	Human, ST5-t002	256	≥512	256	128	32	S80Y, E84K	S84L, E88K	—	A496T
H36	Human, ST5-t002	256	≥512	≥512	256	128	S80F, E84K	S84L,	—	—

H56, H58	Human, ST5-t002	256	≥512	≥512	256	64	S80F, E84K	S85P S84L, S85P	—	—
H16, H17, H33	Human, ST5-t002	256	256	256	128	128	S80Y, E84K	S84L, E88K	—	—
H62	Human, ST5-t002	64	128	256	32	32	S80Y, E84K	S84L, E88K	—	—
H32	Human, ST5-t002	256	128	256	64	64	S80F, E84K	S84L, S85P	—	—
H18	Human, ST5-t002	2	16	2	8	2	S80Y	S84L	—	—
H37	Human, ST5-t002	2	16	1	4	1	S80Y	S84L	—	—
H46	Human, ST88-t10775	0.125	0.5	0.125	0.25	0.125	—	—	—	—

MIC, minimum inhibitory concentration; QRDR, quinolone-resistance determining region; MRSA, methicillin-resistant *Staphylococcus aureus*;ENR, enrofloxacin;NOR, norfloxacin;LVX, levofloxacin;CIP, ciprofloxacin; GAT, gatifloxacin.