antibiotics

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Article

Role of Bioaerosols on the Short-Distance Transmission of Multidrug-Resistant Methicillin-Resistant *Staphylococcus aureus* (MRSA) in a Chicken Farm Environment

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Supplementary Materials

Table S1. The details of bioaerosol sampling of chicken farm environment.

Sampling point	Global positioning system (GPS)	Samples type	Air sampling duration	Air flow speed	Total Volume of air sampling
1st chicken shed	23°58'73.80″ N, 120°49'09.34″ E	Bioaerosol	10 min	28.3 (L/min)	283 Litter
2nd chicken shed	23°58'78.32″ N, 120°49'07.84″ E	Bioaerosol	10 min	28.3 (L/min)	283 Litter
Exposure square	23°58'75.76″ N, 120°49'07.95″ E	Bioaerosol	10 min	28.3 (L/min)	283 Litter
3m Downwind	23°58'76.18″ N, 120°49'08.88″ E	Bioaerosol	10 min	28.3 (L/min)	283 Litter
5m Downwind	23°58'76.31″ N, 120°49'10.00″ E	Bioaerosol	10 min	28.3 (L/min)	283 Litter
20m Downwind	23°58'63.17″ N, 120°49'11.04″ E	Bioaerosol	10 min	28.3 (L/min)	283 Litter
50m Downwind	23°58'58.66″ N, 120°49'16.07″ E	Bioaerosol	10 min	28.3 (L/min)	283 Litter
50m Upwind	23°58'78.25″ N, 120°48'93.12″ E	Bioaerosol	10 min	28.3 (L/min)	283 Litter

Table S2. MRSA strain identification, *Spa* typing, *SCCmec* typing, and virulence factors detecting genes primers list and PCR conditions.

Target gene	Size	Sequence (5′ to 3′)	Reaction Materials Final Volume: 25 μl	PCR Condition	Reference
nuc *mecA*	270 448	nuc-F 5′-GCGATTGATGGTGATACGGTT-3′ nuc-R 5′-AGCCAAGCCTTGACGAACTAAAGC-3′ mecA-F 5′-CTCAGGTACTGCTATCCACC-3′ mecA-R 5′-CACTTGGTATATCTTCACC-3′	DNA: 100-300 ng Primer: 400 nM nuc FR & mecA FR Master mix: 5 μl	Pre-denaturation: 95°C 5 min Denaturation: 94°C 60s Annealing: 55°C 60s Extension: 72°C 60s D.A.E. Cycles: 30 cycles Final extension: 72°C 10 min	[1,2]

Spa	270	Spa-1113F: 5′- TAAAGACGATCCTTCGGTGAGC -3′ Spa-1514R: 5′- CAGCAGTAGTGCCGTTTGCTT -3′	DNA: 100-300 ng Primer: 200 nM Spa FR Master mix: 5 µl	Pre-denaturation: 80°C 5 min Denaturation: 94°C 45s Annealing: 60°C 45s Extension: 72°C 90s D.A.E. Cycles: 35 cycles Final extension: 72°C 10 min	https://spaserver.ridom.de/
SCCmec I SCCmec II SCCmec II, III SCCmec III SCCmec III SCCmec I, II, IV	495 284 209 243 414 342	CIF2 F2: 5′-TTCGAGTTGCTGATGAAGAAGG-3′ CIF2 R2: 5′-ATTTACCACAAGGACTACCAGC-3′ KDP F1: 5′-AATCATCTGCCATTGGTGATGC-3′ KDP R1: 5′-CGAATGAAGTGAAAGAAAGTGG-3′ MECI P2: 5′-ATCAAGACTTGCATTCAGGC-3′ MECI P3: 5′-GCGGTTTCAATTCACTTGTC-3′ RIF F3: 5′-GTGATTGTTCGAGATATGTGG-3′ RIF R9: 5′-CGCTTTATCTGTATCTATCGC-3′ RIF F10: 5′-TTCTTAAGTACACGCTGAATCG-3′ RIF R13: 5′-GTCACAGTAATTCCATCAATGC-3′ DCS F2: 5′-CATCCTATGATAGCTTGGTC-3′ DCS R1: 5′-CTAAATCATAGCCATGACCG-3′	DNA: 100-300 ng Primer: 400 nM CIF-FR, 200 nM KDP-FR, 200 nM RIFF3R9 400 nM MECI-FR, 400 nM RIFF10R13, 800 nM DCS-FR Master mix: 5 µl	Pre-denaturation: 94°C 4 min Denaturation: 94°C 30s Annealing: 53°C 30s Extension: 72°C 1 min D.A.E. Cycles: 30 cycles Final extension: 72°C 4 min	[3]
SCCmec V	325	Type V-F: 5′-GAACATTGTTACTTAAATGAGCG-3' Type V-R: 5′-TGAAAGTTGTACCCTTGACACC-3'	DNA: 100-300 ng Primer: 100 nM V-FR Master mix: 5 µl	Pre-denaturation: 94°C 5 min Denaturation-1: 94°C 45s Annealing-1: 65°C 45s Extension-1: 72°C 1.5 min D.A.E.-1 Cycles: 10 cycles Denaturation-2: 94°C 45s Annealing-2: 55°C 45s Extension-2: 72°C 1.5 min D.A.E.-2 Cycles: 25 cycles Final extension: 72°C 10 min	[4]
SCCmec VI	134	ccrB4-F: 5′-CGAAGTATAGACACTGGAGCGATA-3′ ccrB4-R: 5′-GCGACTCTCTTGGCGTTTA-3′	DNA: 100-300 ng Primer: 100 nM FR, Master mix: 5 µl	Pre-denaturation: 95°C 10 min Denaturation: 95°C 30s Annealing: 50°C 30s Extension: 72°C 30s D.A.E. Cycles: 40 cycles Final extension: 72°C 5 min	[5]

SCCmec VII SCCmec VIII	473 138	Type VII F: 5′-GTGACGTTGATATTGCAGTGGT-3′ Type VII R: 5′-TGAAGAAGTTTGTTCCGCGT-3′ Type VIII F: 5′-AGCGACGATGAACAACACCGCTACTTACTCAA-3′ Type VIII R: 5′-TTGGTTGAGAATGAGAACAGTGGTAAGATC-3′	DNA: 100-300 ng Primer: 400 nM FR Master mix: 5 µl	Pre-denaturation: 95°C 2 min Denaturation: 95°C 30s Annealing: 54°C 1 min Extension: 72°C 1 min 20s D.A.E. Cycles: 35 cycles Final extension: 72°C 7 min	[6]
PVL	433	PVL-1: 5′-ATCATTAGGTAAAATGTCTGGACATGATCCA-3' PVL-2: 5′-GCATCAAGTGTATTGGATAGCAAAAGC-3′	DNA: 100-300 ng Primer: 400 nM FR Master mix: 5 µl	Pre-denaturation: 94°C 5 min Denaturation: 94°C 40s Annealing: 53°C 40s Extension: 72°C 1 min D.A.E. Cycles: 35 cycles Final extension: 72°C 10 min	[7]
entA *entB* *entC* *entD* *entE* *tsst-1* *eta* *etb*	121 478 459 384 495 271 464 200	entA-F: 5′-TTGGAACGGTTAAAACGAA-3′ entA-R: 5′-GAACCTTCCCATCAAAAACA-3′ entB-F: 5′-TCGCATCAAACTGACAAACG-3′ entB-R: 5′-GCAGGTACTCTATAAGTGCC-3′ entC-F: 5′-GGAGGAATAACAAAACATGAAGG-3′ entC-R: 5′-AAAGGCAAGCACCGAAGTAC-3′ entD-F: 5′-TGGTGGTGAAATAGATAGGAC-3′ entD-R: 5′-TGAAGGTGCTCTGTGGATAAT-3′ entE-F: 5′-TGGTAGCGAGAAAAGCGAAG-3′ entE-R: 5′-TGTAAATAATGCCTTGCCTGAA-3′ tsst-1-F: 5′-CTGGTATAGTAGTGGGTCTG-3′ tsst-1-R: 5′-AGGTAGTTCTATTGGAGTAGG-3′ eta-F: 5′-TTTGCTTTCTTGATTTGGATTC-3′ eta-R: 5′-GATGTGTTCGGTTTGATTGAC-3′ etb-F: 5′-ACGGCTATATACATTCAATT-3′ etb-R: 5′-TCCATCGATAATATACCTAA-3′	DNA: 100-300 ng Primer: 400 nM Primer FR Master mix: 5 µl	Pre-denaturation: 94°C 5 min Denaturation: 94°C 1 min Annealing: 2 min Extension: 72°C 1 min D.A.E. Cycles: 35 cycles Final extension: 72°C 5 min AnnealingTemp. *entA*: 50°C *entB*: 55°C *entC*: 59°C *entD*: 51°C *entE*: 55.5°C *tsst-1*: 54°C *eta*: 54°C *etb*: 50.9°C	[8]

Table S3. Pearson correlation coefficient (r) value and level of significant for environmental parameters and total bacteria count.

	Correlations test				
	Ammonia	**Methylamine**	**Sampling Distance**	**Wind speed (m/s)**	**Total bacteria count**
Ammonia	1.000	0.961**	-0.092	-0.447	0.865**
Methylamine	0.961**	1.000	-0.089	-0.443	0.940**
Sampling Distance	-0.092	-0.089	1.000	-0.490	-0.048
Wind speed (m/s)	-0.447	-0.443	-0.490	1.000	-0.476
Total bacteria count	0.865**	0.940**	-0.048	-0.476	1.000
**. Correlation is significant at the 0.01 level (2-tailed).					

Table S4. Standard score of Zone of inhibition diameter measurement in disk diffusion method to determine the microbial resistance property.

Antibiotic name	Zone of inhibition diameter measurement value (mm)		
	Resistant	Intermediate	Susceptible
Chloramphenicol	≦12	13-17	≧18
Ciprofloxacin	≦15	16-20	≧21
Clindamycin	≦14	15-20	≧21
Erythromycin	≦13	14-22	≧23
Gentamicin	≦12	13-14	≧15
Rifampicin	≦16	17-19	≧20
Tetracycline	≦14	15-18	≧19
Sulfamethoxazole-Trimethoprim	≦10	11-15	≧16
A reference documents CLSI M100-S27 which is modified in 2017 has been used to determine the score of Zone of inhibition diameter measurement [9]			

Table S5. The results of Chi-square test between sampling point with MRSA strain characterization

Chi-Square test			
		Sampling point	
Variables	Degree of freedom (df)	Pearson Chi-Square	Asymptotic Significance (2-sided)
SCCmec typing	7	23.000[a]	0.002
MRSA grouping	7	23.000[a]	0.002
Spa type	7	23.000[a]	0.002
Virulence factors	21	53.959[b]	0.000
Multiple drug resistance	14	26.680[c]	0.021
a. 16 cells (100.0%) have expected count less than 5. The minimum expected count is .09.			
b. 32 cells (100.0%) have expected count less than 5. The minimum expected count is .09.			
c. 24 cells (100.0%) have expected count less than 5. The minimum expected count is .04.			

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