

Supplementary

Molecular and Anti-Microbial Resistance (AMR) Profiling of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from Hospital and Long-Term Care Facilities (LTCF) Environment

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Table S1. Details of samples location, source, types and number.

Sampling Location	Sampling source	Sample types	Number of samples	Total Samples
Changhua city	LTCF environment	Moist samples	45	69
		Arid sample	24	
	LTCF residents	Foley catheter-balloons from LTCF residents	30	63
		Nasogastric tubes from LTCF residents	15	
		Tracheostomy tubes from LTCF residents	18	
	Hospital environment	Outpatient floor	18	32
		Inpatient floor	7	
		Ward (used)	7	
	Changhua city's total samples			164
Chiayi city	LTCF environment	Mild area	26	50
		Severe area	24	
	Hospital environment	Outpatient building 1F	7	32
		Inpatient building 1F	6	
		Ward (vacant)	9	
		Ward (used)	10	
		Total Chiayi city samples		
Total Changhua city & Chiayi city samples			246	

Table S2. MRSA strain identification (ERIC- PCR), *SCCmec* typing and enterotoxin gene detection condition of PCR with primers information.

Target gene	Size	Sequence (5' to 3')	Reaction Materials (Final Volume: 25 µl)	PCR Condition	References
<i>nuc</i> <i>mecA</i>	270 448	nuc-F 5'- GCGATTGAT GGTGATAC- GGTT-3' nuc-R 5'- AGCCAA- GCCTTGAC- GAACTAAAG C-3' mecA-F 5'- CTCAGGTAC TGC- TATCCACC-3' mecA-R 5'- CACTTGG- TATATCTTC ACC-3'	DNA: 100-300 ng Primer: 400 nM nuc FR & mecA FR Master mix: 5 µl	Pre-denatura- tion: 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]
ERIC	----	ERIC1R: 5'- ATGTAA- GCTCCTGGG- GATTCAC-3' ERIC2: 5'- AAGTAAGTG ACTGGGGTG AGCG-3'	DNA: 100-300 ng Primer: 500 nM ERIC1R & ERIC2 Master mix: 5 µ	Denaturation-1: 95°C 5 min Annealing-1: 36°C 1 min Extension-1: 72°C 4 min D.A.E.-1 Cy- cles: 1 cycle Denaturation-2: 95°C 1 min Annealing-2: 36°C 1 min Extension-2: 72°C 4 min D.A.E.-2 Cy- cles: 35 cycles Final extension: 72°C 8 min	[3]
<i>SCCmec</i> I <i>SCCmec</i> II <i>SCCmec</i> II, III <i>SCCmec</i> III <i>SCCmec</i> III <i>SCCmec</i> I, II, IV	495 284 209 243 414 342	CIF2 F2: 5'- TTCGAGTT- GCTGATGAA GAAGG-3' CIF2 R2: 5'- ATTTAC- CACAAGGAC TACCAGC-3' KDP F1: 5'- AATCATCTG CCATT- GGTGATGC- 3' KDP R1: 5'- CGAATGAAG TGAAA- GAAAGTGG- 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-denatura- tion: 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	[4]

		MECI P2: 5'- ATCAA- GACTT- GCATTCAGG C-3' MECI P3: 5'- GCGGTTTCA ATTCACTT- GTC-3' RIF F3: 5'- GTGATTGTT CGAGA- TATGTGG-3' RIF R9: 5'- CGCTTTATCT GTATCTATC GC-3' RIF F10: 5'- TTCTTAAGT ACAC- GCTGAATCG- 3' RIF R13: 5'- GTCACAG- TAATTCCAT CAATGC-3' DCS F2: 5'- CATCC- TATGA- TAGCTT- GGTC-3' DCS R1: 5'- CTAAATCAT AGCCATGAC CG-3'			
SCCmec V	325	Type V-F: 5'-GAACATT- GTTACTTAA ATGAGCG-3' Type V-R: 5'- TGAAAGTT- GTACCCTT- GACACC-3'	DNA: 100-300 ng Primer: 100 nM V-FR Master mix: 5 μl	Pre-denatura- tion: 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 Cy- cles: 10 cycles Denaturation-2: 94°C 45s Annealing-2: 55°C 45s Extension-2: 72°C 1.5 min D.A.E.-2 Cy- cles: 25 cycles Final extension: 72°C 10 min	[5]
PVL	433	PVL-1: 5'- ATCATT- AGGTAAAAT	DNA:100-300 ng Primer: 400 nM FR	Pre-denatura- tion: 94°C 5 min	[6]

		GTCTG- GACATGATC CA-3'	Master mix: 5 μl	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
		entA-F: 5'- TTGGAAC- GGTAAAAAC GAA-3'		
		entA-R: 5'- GAAC- CTTCCCATC AAAAACA-3'		
		entB-F: 5'- TCG- CATCAAAC GACAAACG- 3'		Pre-denatura- tion: 94°C 5 min
		entB-R: 5'- GCAGGTACT CTATAAGTG CC-3'		Denaturation: 94°C 1 min
		entC-F: 5'- GGAGGAA- TAACAAAAAC ATGAAGG-3'		Annealing: 2 min
entA	121	entC-R: 5'- AAAGGCAA- GCAC- CGAAGTAC- 3'	DNA: 100-300 ng Primer: 400 nM Primer FR Master mix: 5 μl	Extension: 72°C 1 min D.A.E. Cycles: 35 cycles Final extension: 72°C 5 min
entB	478	entD-F: 5'- TGGTGGTGA AATAGA- TAGGAC-3'		Annealing- Temp.
entC	459	entD-R: 5'- TGAAGGTGC TCTGTGGA- TAAT-3'		entA: 50°C entB: 55°C entC: 59°C entD: 51°C entE: 55.5°C
entD	384	entE-F: 5'- TGG- TAGCGA- GAAAA- GCGAAG-3'		tsst-1: 54°C eta: 54°C etb: 50.9°C
entE	495	entE-R: 5'- TGTAAG- TAATGCCTT- GCCTGAA-3'		
tsst-1	271	tsst-1-F: 5'- CTGG- TATAGTAG- TGGGTCTG-3'		

[7]

tsst-1-R: 5'-AG-
GTAGTTC-
TATTGGAG-
TAGG-3'
eta-F: 5'-TTT-
GCTTTCTT-
GATT-
GGATTC-3'
eta-R: 5'-
GATGTGTT-
GGTT-
GATTGAC-3'
etb-F: 5'-AC-
GGCTA-
TATACATTC
AATT-3'
etb-R: 5'-
TCCATCGA-
TAATATAC-
CTAA-3'

References

1. Brakstad, O.G.; Aasbakk, K.; Maeland, J.A. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. *Journal of clinical microbiology* **1992**, *30*, 1654-1660.
2. Sakoulas, G.; Gold, H.S.; Venkataraman, L.; DeGirolami, P.C.; Eliopoulos, G.M.; Qian, Q. Methicillin-resistant *Staphylococcus aureus*: comparison of susceptibility testing methods and analysis of mecA-positive susceptible strains. *Journal of clinical microbiology* **2001**, *39*, 3946-3951.
3. Ye, Y.; Jiang, Y.; Fan, T.; Jiang, Q.; Cheng, Y.; Lu, J.; Lin, L. Resistance characterization, virulence factors, and ERIC-PCR fingerprinting of *Aeromonas veronii* strains isolated from diseased *Trionyx sinensis*. *Foodborne pathogens and disease* **2012**, *9*, 1053-1055.
4. Oliveira, D.C.; Lencastre, H.d. Multiplex PCR strategy for rapid identification of structural types and variants of the mec element in methicillin-resistant *Staphylococcus aureus*. *Antimicrobial agents and chemotherapy* **2002**, *46*, 2155-2161.
5. Zhang, K.; McClure, J.-A.; Elsayed, S.; Louie, T.; Conly, J.M. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*. *Journal of clinical microbiology* **2005**, *43*, 5026-5033.
6. Asghar, A.H. Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated from tertiary care hospitals. *Pakistan journal of medical sciences* **2014**, *30*, 698.
7. Fooladi, A.A.I.; Ashrafi, E.; Tazandareh, S.G.; Koosha, R.Z.; Rad, H.S.; Amin, M.; Soori, M.; Larki, R.A.; Choopani, A.; Hosseini, H.M. The distribution of pathogenic and toxigenic genes among MRSA and MSSA clinical isolates. *Microb Pathogenesis* **2015**, *81*, 60-66.