

One Health approach for investigation of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) in broiler chickens and their environments throughout the chicken meat production system

Gi Yong Lee¹, Haeng Ho Lee¹, Hong Sik Eom¹, Soo-Jin Yang¹

¹School of Bioresources and Bioscience, Chung-Ang University, Anseong 17546, Korea



ABSTRACT

Occurrence of **LA-MRSA** has recently been reported in human patients via direct or indirect contact with infected animals. MRSA has frequently been isolated from mucosal surfaces of humans and animals, foods of animal origin, and farm environment, causing a significant threat to public health. Thus, a holistic **One Health approach** involving farm workers, animals, and the environment is necessary to investigate prevalence and transmission of LA-MRSA among different sectors. The current investigation was designed to assess the nationwide prevalence of LA-MRSA and LA-MSSA in the **chicken meat production system** involving broiler chickens, chicken meat, workers, and environment of the facilities in farms, slaughterhouses, and retail markets.

A total of **200 *S. aureus* (43 MRSA and 157 MSSA)** strains were isolated and examined for their genotypic characteristics and antimicrobial resistance (AMR) phenotypes. Multilocus sequence type (MLST) analysis revealed that the most prevalent clone was clonal complex (CC) 5 (n=76, 38%), followed by CC398 (n=56, 28%), CC8 (n=34, 17%), CC1 (n=24, 12%) and CC30 (n=2, 1%). While only two sequence types (STs), **ST692** and **ST188** (CC385 and CC1), were confirmed in MRSA strains, various STs were observed in MSSA strains. ST692 and ST188 MRSA strains displayed higher levels of AMR and multidrug resistance phenotypes than MSSA strains. **Of note, all of the ST692 and ST188 MRSA strains exhibited quinolone resistance through the mutations in *gyrA* (S84L) and *griA* (S80F).** In addition to the AMR, 60% of *S. aureus* strains (n = 120), particularly all of ST188 *S. aureus* (n = 10), showed resistance to zinc chloride.

MATERIALS AND METHODS

- A total of **200 *S. aureus* strains** were isolated from samples collected from chicken farms (n=960), slaughterhouses (n=309), and retail markets (n=107).
- *S. aureus* was identified by using both Vitek 2 system (BioMérieux, France) and 16S rRNA sequencing (Bionics, Korea).
- **Antimicrobial and zinc chloride susceptibility tests** were performed using the disc diffusion methods according to the 2017 CLSI guidelines. The antimicrobial agents used were ampicillin (AMP), cefoxitin (CEF), chloramphenicol (CHL), clindamycin (CLI), erythromycin (ERY), fusidic acid (FUS), gentamycin (GEN), mupirocin (MUP), penicillin (PEN), rifampicin (RIF), sulfamethoxazole-trimethoprim (SXT), quinupristin-dalfopristin (SYN), tetracycline (TET). In addition, resistance to fluoroquinolones (ciprofloxacin, enrofloxacin, and levofloxacin) were determined for all *S. aureus* isolates. The minimum inhibitory concentrations (MICs) to zinc chloride were determined by the agar dilution assay.
- **Genetic diversities** of *S. aureus* isolates were examined by standard molecular typing methods such as MLST, *spa*, and *agr*. The clonal relationships among all of *S. aureus* strains were analyzed using eBURST V3 (<http://eburst.mlst.net>).
- Types of **SCCmec** were determined on all *mecA*-positive *S. aureus* strains by multiplex PCR analysis.

RESULTS

Prevalence of MRSA and MSSA in the chicken meat production system in Korea

	Farms (n = 960)			Slaughterhouses (n = 309)			Retail markets (n = 107)		
	Chickens (n = 820)	Workers (n = 69)	Environ. (n = 71)	Carcasses (n = 240)	Workers (n = 39)	Environ. (n = 30)	Meats (n = 100)	Workers (n = 3)	Environ. (n = 4)
MRSA	37 (4.5%)	0	3 (4.2%)	1 (0.4%)	0	0	5 (5%)	0	0
MSSA	51 (6.2%)	2 (2.9%)	4 (5.6%)	33 (13.8%)	4 (10.3%)	4 (13.3%)	59 (59%)	0	0

Genetic characterization of *S. aureus* strains revealed high prevalence of ST692 and ST188 among LA-MRSA strains

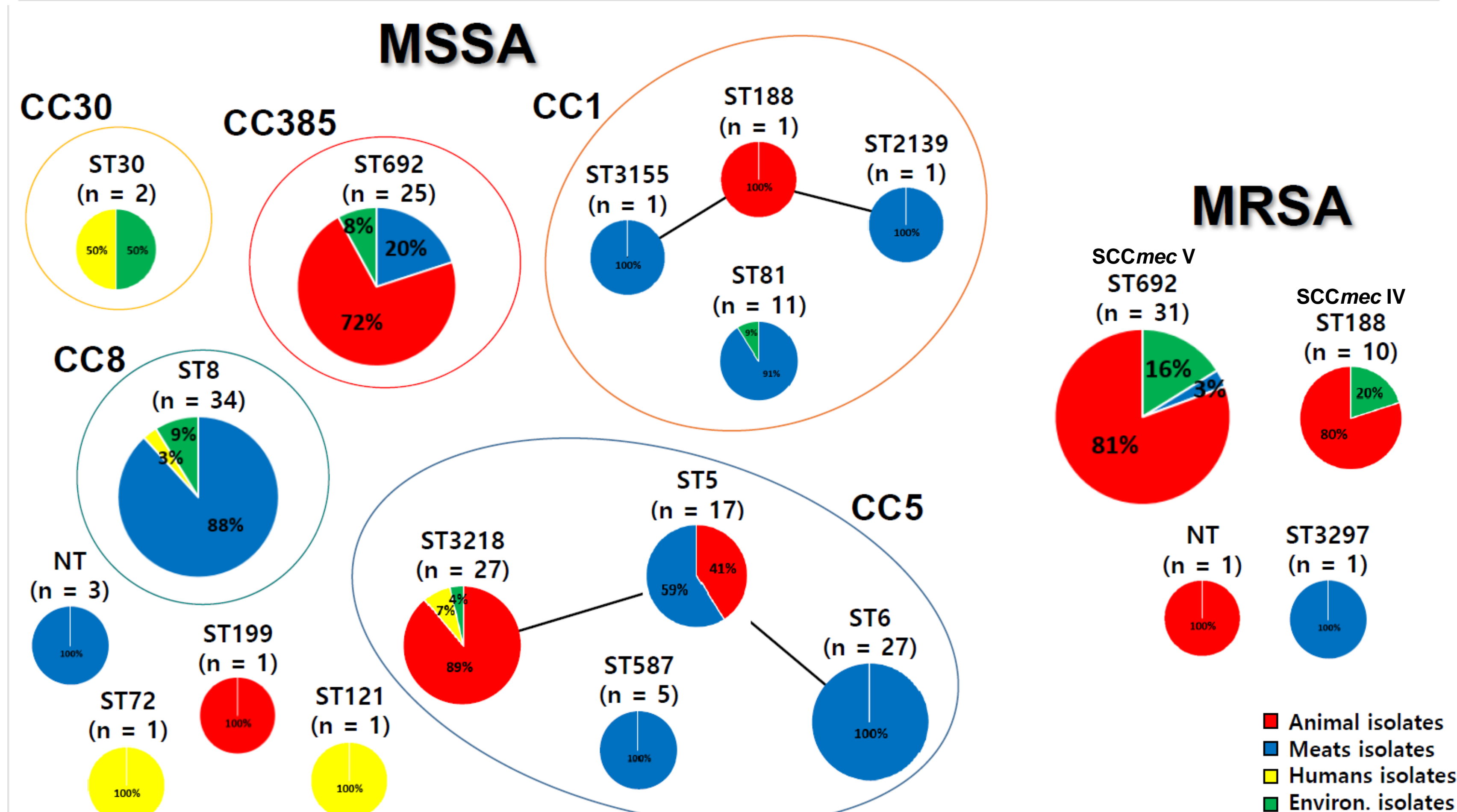


Fig 1. Clonal distribution of *S. aureus* strains in chicken farms, slaughterhouses, retail markets. Sequence type (ST) of the lineages based on MLST forming the connections between clonal complex (CC) types; CC1 (ST81, ST188, ST2139, and ST3155), CC5 (ST5, ST6, ST587, and ST3218), CC8 (ST8), CC385 (ST692), and CC30 (ST30). NT, non-typeable.

LA-MRSA strains tended to have higher level of antimicrobial resistance phenotypes than LA-MSSA strains

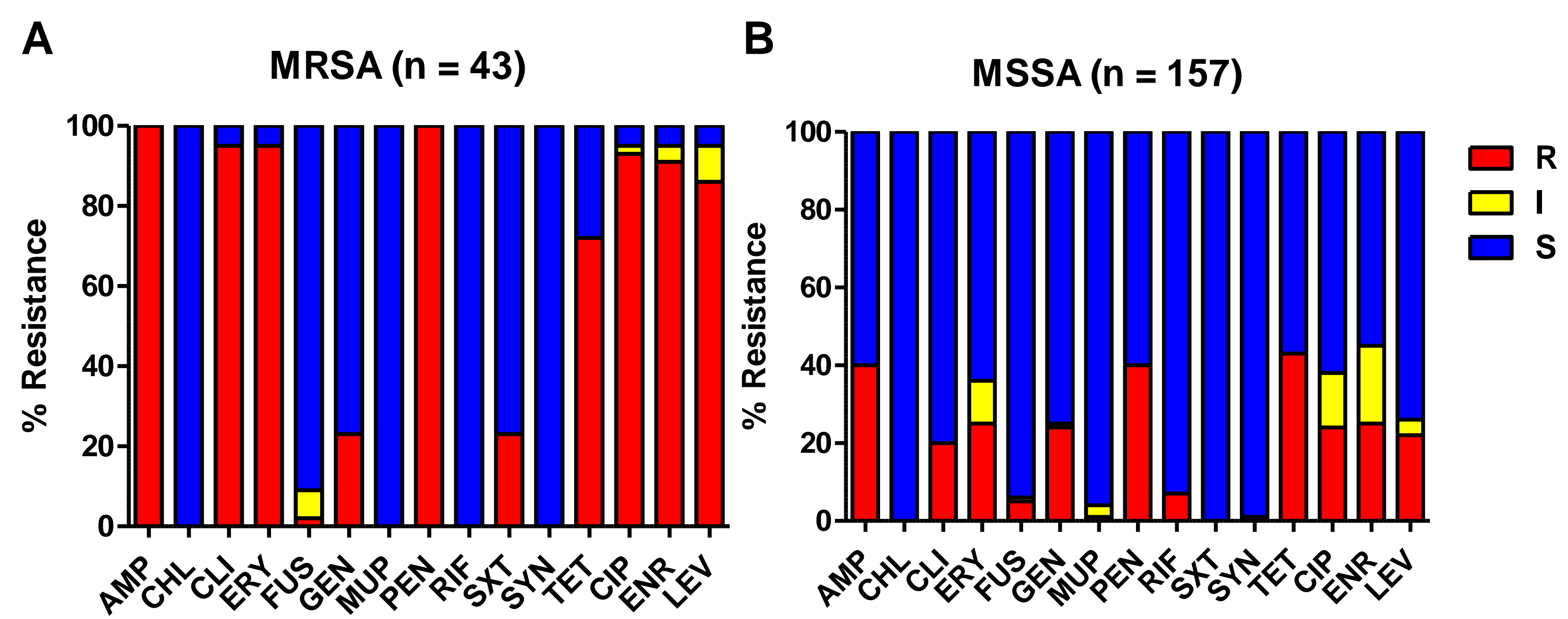
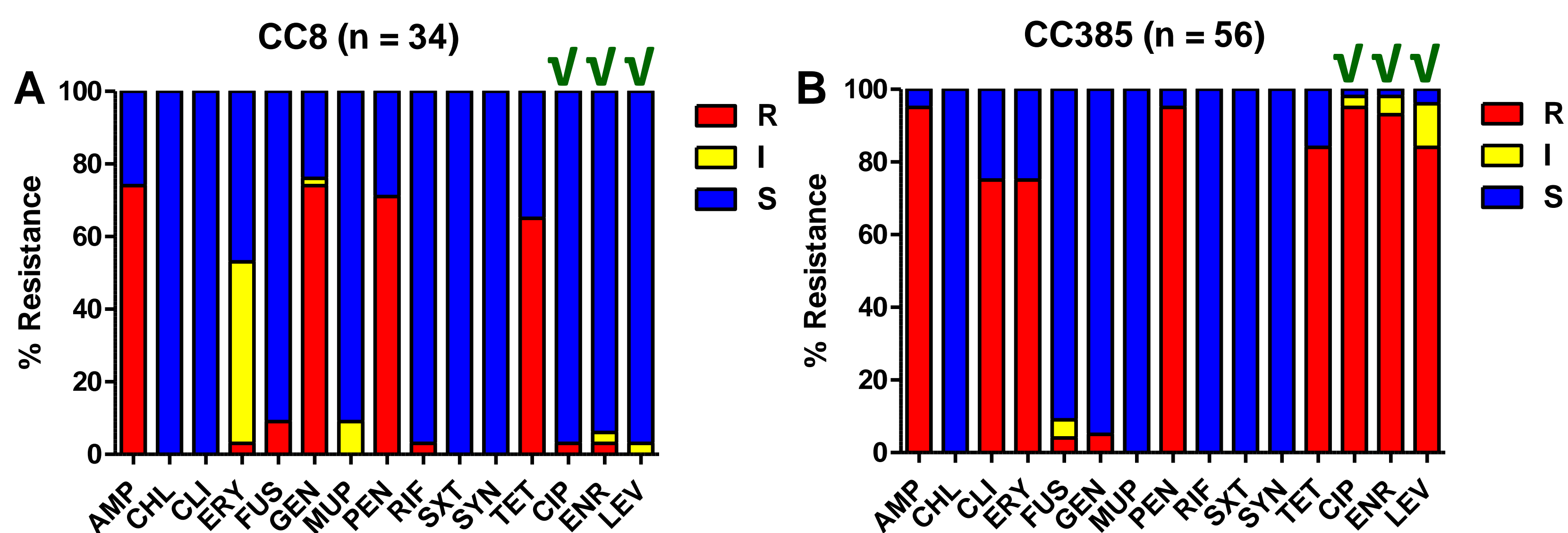


Fig 2. Antimicrobial resistance profiles of MRSA (A) and MSSA (B) strains.

CC385 LA-MRSA strains exhibited highest level of antimicrobial resistance, especially quinolone resistance



All quinolone-resistant *S. aureus* strains possessed dual point mutations in both *gyrA* (S84L) and *griA* (S80F) genes

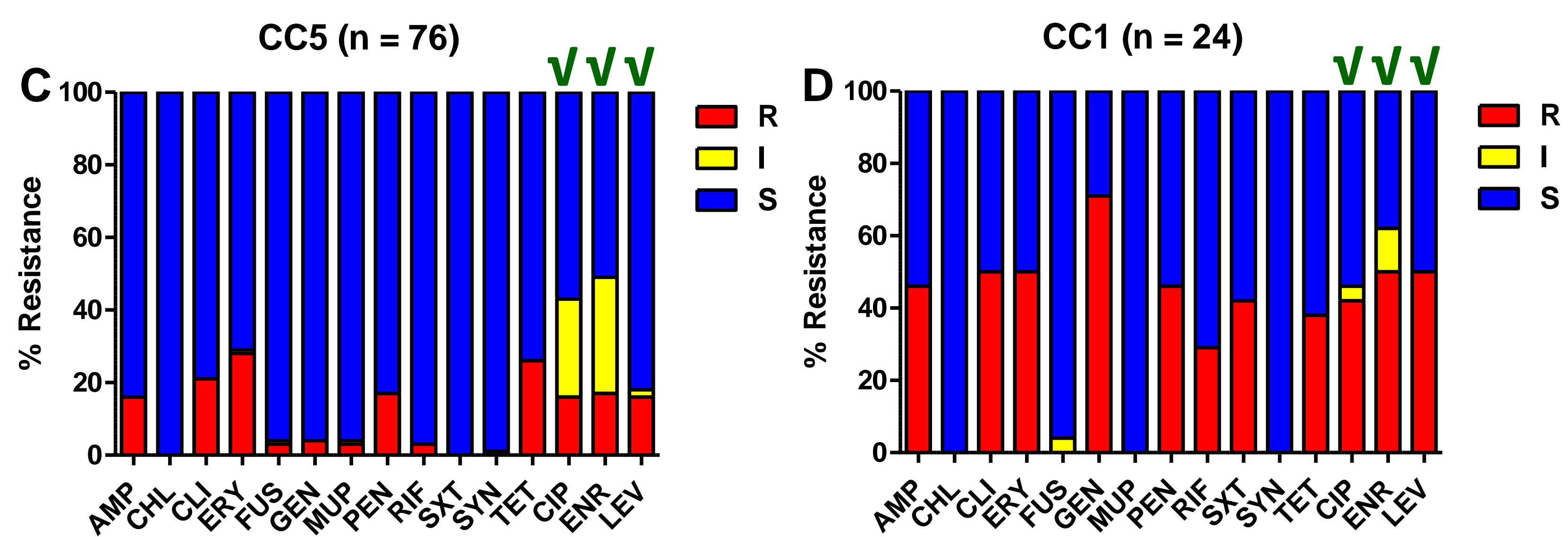


Fig 3. Antimicrobial resistance profiles of *S. aureus* strains in chicken farms, slaughterhouses, retail markets. This figure shows antimicrobial resistance of four major CC types in chicken meat production system in Korea; CC8 (A), CC385 (B), CC5 (C), and CC1 (D). Three marks indicate fluoroquinolones used including ciprofloxacin (CIP), enrofloxacin (ENR), and levofloxacin (LEV).

ST188 *S. aureus* strains belonging to CC1 showed resistance to zinc chloride

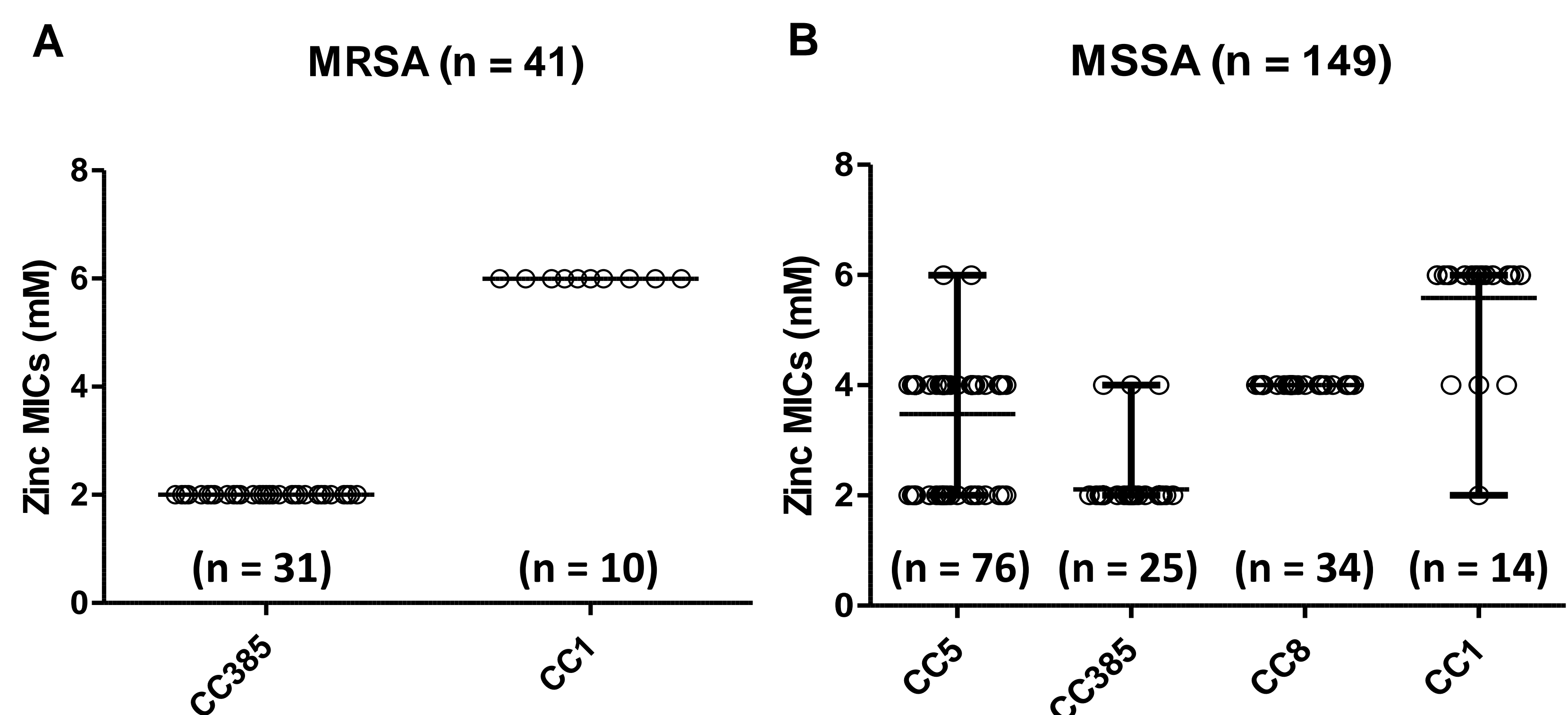


Fig 4. Zinc chloride MICs of *S. aureus* strains. Zinc chloride MICs of > 2 mM indicate zinc resistance phenotypes as previously described¹. MIC, minimum inhibitory concentration.

¹Aarestup FM et al. Susceptibility of different bacterial species isolated from food animals to copper sulphate, zinc chloride, and antimicrobial substances used for disinfection. *Vet Microbiol*, 2004.

CONCLUSIONS

- This study is the first to implement One Health approach to investigate prevalence, genetic diversity, and antimicrobial resistance of MRSA & MSSA in chicken meat production system in Korea.
- AMR, especially quinolone resistance, and zinc resistance may have played a significant role in the high prevalence and persistence of ST692 and ST188 LA-MRSA clonal lineages in broiler farms in Korea.

ACKNOWLEDGEMENTS

- This work was carried out by the support of Research of Korea Centers for Disease Control and Prevention (Project No. 2017NER54060 and 2020ER540500 to S.J.Y)