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Introduction: Enterococci of animal origin have potential risks of transferring their antimicrobial resistance genes to humans via food-chain. Linezolid is one of the only few antimicrobial agents currently available for treatment of multidrug-resistant Gram-positive bacteria including MRSA and VRE.

Materials and Methods: Altogether 7,720 *Enterococcus faecalis* and 3,939 *E. faecium* isolated from food animals and animal carcasses during 2003-2014 in Korea were investigated to determine if linezolid resistant(LR) enterococci are present. Antimicrobial susceptibility testing was performed by broth dilution method according to CLSI guidelines. Genes that confer transferable resistance to oxazolidinones and phenicols were examined by PCR and sequencing. Domain V region of 23S rRNA gene, ribosomal protein L3 gene and ribosomal protein L4 gene were amplified and sequenced to detect the presence of mutations associated with linezolid resistance. The transferability of plasmid carrying *optrA* and *fexA* genes was determined by filter-mating. Molecular typing of LR *E. faecalis* and *E. faecium* isolates was done by pulsed field gel electrophoresis (PFGE).

Results: Twelve (0.16%) *E. faecalis* and 27 (0.69%) *E. faecium* were found to be resistant to linezolid (≥8μg/ml), respectively. Most of the LR isolates were also resistant to chloramphenicol (97.44%) and florfenicol (92.31%). Molecular analysis showed no mutations in the 23S ribosomal RNA and in the ribosomal protein L3. The *optrA* gene was found in 89.74% of the LR *Enterococcus*, 12 *E. faecalis* and 23 *E. faecium* isolates. Among them, 30 *optrA*-positive isolates co-carried phenicol exporter gene *fexA*. Seven LR *E. faecium* isolates had Asn130Lys mutations in the ribosomal protein L4, of which six isolates also carried *optrA* gene. None of the isolates carried the mutliresistance gene *cfr*. Transfer of *optrA* gene was observed in 16 out of the 35 *optrA*-positive isolates by conjugation. Pulsed-field gel electrophoresis demonstrated that the vast majority of *Enterococcus* strains carrying *optrA* gene were genetically heterogeneous. Multi-locus sequence typing revealed eight novel Sequence types among *E. faecalis* and *E. faecium* strains.

Conclusions: To our knowledge, this is the first report of *optrA* gene in isolates from cattle and animal carcasses. This study also reports the *optrA* gene for the first time in Korea indicating the emergence of plasmid transferable *optrA* gene in a new geographical setting. Active surveillance of *optrA* in enterococci of both human andanimal origin is urgently warranted.

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Cloning and expression of F4-F18-LT fusion protein using transformed *Escherichia coli*

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Introduction: Postweaning diarrhoea (PWD) is mainly caused by enterotoxigenic *Escherichia coli* (ETEC) expressing F4 and F18 fimbriae and causes ongoing economic losses in the affected farm worldwide. It is imperative that vaccine for PWD contain F18 protein to protect the attachment of ETEC in the gut. However, F18 is poorly expressed in the artificial media in vitro. To overcome the obstacle, in current study, F4-F18-LT fusion protein was cloned, expressed and evaluated in the artificial media.

Materials and Methods: To construct the F4-F18-LT fusion protein, antigenic motif of FaeG, FedF and LT was modified and amplified using *E. coli* KEFS1057 (F4:LT:STb) and KEFS218 (F18:LT:STa:STX2e). To set up the plasmid, restriction site at the end of 5' end (*EcoR*) and 3' end (*Xho* I) was inserted to pJ201 (DNA 2.0, USA). After treatment of the restriction enzyme, the plasmid was transformed in to pET28-MBP (maltose-binding protein) by heat shock. The F4-F18-LT fusion protein was induced by lactose in the induction medium at 25 and 37℃, respectively. The cell harvested was sonicated and centrifuged at 12,000 rpm. The supernatant was purified using MBP-trapped column (GE, USA) and applied to 10% SDS-PAGE. After electrophoresis, each protein was visualized by western blot using mono and polyclonal antibodies.

Results: F4-F18-LT fusion sequence was evaluated by sequencing. F4 and F18 protein was highly expressed in insoluble form at 37℃ while soluble protein was more expressed in 25℃ condition. LT was confirmed by western blot. Each protein was highly produced during logarithmic phase.

Conclusions: In present study, F4-F18-LT fusion protein was produced by transformed vector system and confirmed by western blot. It suggests that the fusion protein can be used for vaccine candidate. The fusion protein will be applied to mass production system and animal experiment to see if the protein shows immunity.

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P-111

Antimicrobial resistance and detection of integrative and conjugative element- associated genes in *Pasteurella multocida* isolated from swine

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Introduction: *Pasteurella multocida* is an important cause of pneumonia and atrophic rhinitis in pigs and is responsible for significant losses on farms worldwide. Antimicrobials are used for prevention and treatment of respiratory disease by *P. multocida*. Misuse or overuse of antimicrobials in pigs can select for antimicrobial resistance. Aims of this study were to investigate antimicrobial resistance profile and to detect an Integrative and conjugative (ICE) element in *P. multocida* isolated from pigs.

Materials and Methods: A total of 599 *P. multocida* were isolated from swine pneumonic lung lesions and nasal cavity during 2010-2015 in Korea. Antimicrobial susceptibility was assessed by minimum inhibitory concentrations (MICs) for 17 antimicrobial agents by broth microdilution method in microtiter (Trek Diagnostics Inc., BOPO6, UK) according to Clinical Laboratory StandardsInstitute guidelines. PCR was used to screen for macrolide resistance genedeterminants and integrative conjugation element (ICE)-associated genes. Representative PCR products were sequenced to confirm the accuracy of amplified fragments.

Results: The most prevalent phenotypes detected were resistance to oxytetracycline (74.79%), followed by sulphadimethoxine(59.4%), and tiamulin (51.4%). While less than 5% of isolates were resistance to tilmicosin, tulathromycin, and ceftiofur. The MIC_{50} and MIC_{90} of the *P. multocida* isolates were also remained the same or tended to decrease. No macrolide resistant genes, *erm (42), mph (E),* and *msr (E)* weredetected in 29 macrolide resistant *P.multocida* isolates. ICE-associated genes, hypothetical protein, tyrosine recombinase-1 family protein, and transposase *tnpA* was observed in a multiple resistant *P. multocida* isolate. Sequencing results revealed that ICE-associated genes of this study were identical with those from *P. multocida* 36950 genome.

Conclusions: In Korea, *P. multocida* organisms have frequently been isolated from pigs, andthey represent a significant cause of territorial outbreak of respiratory infections. Although resistance rate was not high in most tested antimicrobials in this study, continued surveillance of susceptibility patterns of clinical isolates of this pathogen is essential. Furthermore, detection of ICE-associated genes in this study is new information that may affect strategies on antimicrobial use for treatment respiratory disease in swine industry.

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T-cell responses in porcine peripheral blood mononuclear cells infected with North American porcine reproductive and respiratory syndrome virus

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Introduction: A reproductive failure in sows and respiratory distresses in all ages of groups of pigs is characteristic of porcine reproductive and respiratory syndrome (PRRS). The disease first appeared in North America in 1987, and then in Europe in 1990. Since the emergence of North American (NA) genotype PRRSV in the early 1990 in South Korea, EU genotype PRRSV was first detected in 2005. The rapid generation of genetic variation in porcine reproductive and respiratory syndrome virus (PRRSV) makes it difficult to characterize the porcine T-cell response to this virus. This study shows that serum neutralizing antibody titer among viruses is related to certain cytokines. We evaluated the dynamic fluctuations of porcine T-cell responses in peripheral blood mononuclear cells (PBMCs) infected with NA PRRSV(strain 42) followed by inoculating PRRSVs with high-(strains 383 and LMY;Group 1) and/or low-potency (strain 415 and 380; Group 2) neutralizing antibodies for strain 42.

Materials and Methods: We evaluated the PBMCs infected with NA PRRSV (strain 42) followed by inoculating PRRSVs with high- (Group 1)and/or low-potency (Group 2) neutralizing antibodies for strain 42. The PBMCs were inoculated into MARC-145 cells and strain 42 of culture supernatant was extracted and applied to the reverse transcription (RT)-polymerase chain reaction (PCR). The confirmation of the virus was done through sequence analysis of ORF5 gene. The relative numbers of T cells were determined two, six, and eight hours post-inoculation (hpi) by flow cytometry. The secretion of cytokines (IL-2, IL-6, and IL-10) was examined eight, twenty-four, fourty-eight hpi by enzyme-linked immunosorbent assay (ELISA).

Results: The rapid generation of genetic variation in PRRSV makes it difficult to characterize the porcine T-cell response to this virus. We evaluated the dynamic fluctuations of porcine T-cell responses in PBMCs infected with North American PRRSV (strain 42) followed by inoculating PRRSVs with high-(strains 383 and LMY; Group 1) and/or low-potency(strain 415 and 380; Group 2) neutralizing antibodies for strain 42. The relative numbers of T cells were determined two, six, and eight hpi by flow cytometry and cytokines (IL-2, IL-6, and IL-10)were examined by ELISA. Populations of $CD4^+$, $CD4^-$, and $CD8^-$ cells were not significantly different upon infection with Group 1 and 2 viruses in PBMCs. However,