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Introduction: Enterococci are gram-positive, facultative anaerobic and catalase-negative oval cocci that are present as the part of the commensal organism of the digestive tracts of animals and humans. Recently enterococci are being recognized as leading nosocomial pathogens. Their abilities to acquire resistance against numerous antimicrobials and harbour putative virulence traits are considered as main reasons for their opportunistic infections. However, their prevalence and characteristics in horses have been less reported. In the present study, we investigated the occurrence of antimicrobial resistances and virulence factors among enterococci collected from horses in Korea, during 2013.

Materials and Methods: A total of 3,078 swab samples were obtained from 2 national race parks and 14 private riding clubs in Korea. Horse samples were isolated from skins, nasal cavities, feces, feed boxes, water buckets, and beddings. *Enterococcus* spp. were speciated using species-specific polymerase chain reaction (PCR) and VITEK Ⅱ. Antimicrobial susceptibility tests were performed for eleven antimicrobials by the disk diffusion method according to the Clinical and Laboratory Standards Institute(CLSI) guidelines. In addition, the antimicrobial resistance and virulence associated genes were detected by PCR using specific primers. The biofilm formation ability was evaluated and PFGE was performed to analyze for clonal relatedness among the isolates.

Results: Overall, 264 *Enterococcus* spp. (8.6%) were isolated among the horse associated swap samples and 6 groups of enterococcal species were identified. *E. faecalis* (50.0%) represented the dominant species, followed by *E. faecium* (22.3%). It was found that 14.0% enterococci isolates exhibited resistance toat least two antimicrobials. The highest rate of antimicrobial resistance was detected from *E. faecalis* isolates toquinupristin/dalfopristin (107/132, 81.1%). All isolates were susceptible to vancomycin, teicoplanin and linezolid. Among the 48 isolates resistant to tetracycline, *tetM* and *tetL* genes were detected in 87.8% and28.6% of the isolates, respectively. The *gelE* gene was detected from 131 (49.6%) enterococcal isolates. In contrast, *esp* and *hyl* genes were not detected. Using the crystal violet method, 50.8% of isolates were identified as biofilm formers. The PFGE results revealed that horse isolates were closely related to horse-associated environmental isolates in the same places.

Conclusions: Most *Enterococcus spp.* isolates from horses had not developed resistance to several antimicrobials and VRE was not detected. However, continuous monitoring was needed to prevent transmission to human by direct contact, since the spread of *Enterococcus spp.* between horses and environments was possible and half of enterococcal isolates had biofilm formation ability.

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P-015

Characterization of *Clostridium perfringens* and Bacteriophages Isolated from Chicken and Animal Application

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Introduction: *Clostridium perfringens* produces diverse virulent toxins that causenecrotic enteritis in poultry, resulting in a great negative impact on the poultry industry. To reduce the number of this bacterium, bacteriophages have been studied for industrial application as a control agent.

Materials and Methods: We isolated 70 *C. perfringens* from chicken with necrotic enteritis. The isolates were characterized phenotypically and geneticallyby disk diffusion, toxin gene polymerase chain reaction (PCR) and pulsed-field gel electrophoresis (PFGE). Also, 12 bacteriophages were obtained from chicken feces, and the host spectrum was researched by spot and standard plaque assay. The genetic diverse of bacteriophages were determined with Random Amplified Polymorphic DNA (RAPD) method. Day-old commercial broiler chicken were used to examine the effectivess of a bacteriophage (SJ Φ21) in treatment.

Results: The isolated *C. perfringens* were resistant to more than 5 antibiotics. *C. perfringens* isolates were classified into 13 groups in PFGE patterns. When the genetic correlation among the isolates was close, their antimicrobial resistance and toxin gene patterns were related closely. All bacteriophages totally differ in RAPD patterns, which indicates that phages were distinguished each other in genetic scope. Most of phages can lysis more than four representative isolates and had wide host spectrum. Over one Log CFU/g reduction was observed in the animal application experiment.

Conclusions: There have been the needs to study for alternative control agent which supersede with antimicrobials. We recommend a cocktail of bacteriophages as a sanitization agent in chicken laying field.

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P-016

Genetic Analysis of the Spike Gene of a Porcine Epidemic Diarrhea Virus (PEDV) Strain Isolated from Gyeonggi Province in 2014

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Introduction: Porcine epidemic diarrhea virus (PEDV) is an etiological agent causing acute diarrhea, dehydration and high mortality in sucking piglets. PED has been reported sporadically since explosive outbreaks in Korea in the 1990's, but recently recurs as more severe cases despite of vaccination in national wide. Thus, there is a strong controversy on the protective efficacy of commercial vaccines available on the market, speculating that newly emerging virus strains might be evolved over a period of time. In this light, new vaccine candidates capable of dealing with virus strains prevalent in swine farms are actively being searched. Here we report that a field strain was newly isolated in 2014 and its spike gene was genetically analyzed.

Materials and Methods: Virus isolation was performed on Vero cells with the small intestine of a piglet affected with severe diarrhea. When cytopathic effect obviously appears, the virus was purified by a serial plaque assay and then subjected to immunofluorescence assay with PEDV- specific monoclonal antibody. After amplification of the purified virus, viral RNA was extracted from culture supernatant using the RNeasy Mini kit (Qiagen). PEDV-specific spike gene was amplified by PCR using the One-Step RT-PCR kit (Invitrogen). The sequence of amplified DNA products was confirmed in a both-direction sequencing method. The full-length spike gene of the isolate was analyzed using a DNAMAN program (Lynnon Biosoft) and neighborhood-joining tree was generated using a Clustral X program.

Results: A field strain, named KBNP-PPC410, was isolated from a diseased piglet submitted from a swine farm located on the province of Gyeonggi in 2014. It was confirmed by PEDV-specific PCR, and immunofluorescence assay. Homology analysis with the full-length spike gene sequence revealed that KBNP-PPC410 showed a similarity of 94.0%, 96.5% to SM98, CV777, respectively, at the nucleotide. Also, the similarity of 95.5%, 96.5% to SM98, CV777, respectively, was found at amino acid. However, three distinct insertion sequences, including a neutralizing epitope on the spike gene were detected compared to that of a vaccine strain SM98.

Conclusions: KBNP-PPC410 was isolated as a field strain currently circulating in swine farms in Korea and classified into a similar group of a prototype CV777. Nevertheless, it would be worth of investigating the biological significance of such insertions leading to genetic differences between KBNP-PPC410 and SM99 strains.

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P-017

Evaluation of Nine Germicides against the Pathogen of Avian Botulism

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Introduction: Recently, avian botulism has emerged as a problem in Korea. The pathogens of all avian botulism cases in Korea since 2012 have been *Clostridium botulinum* toxin type C/D. But, there is no article on effective germicide of this pathogen. So weconducted the experiment to find effective germicide killing spores of *C. botulinum* toxin type C/D.

Materials and Methods: Nine kinds of germicides (3 oxidants, 1 acidulant, 4 aldehydes, 1 extra germicide)distributed in Korea were selected for this study. To evaluate the sporicidal activity of these nine kinds of germicides, dilution-neutralization method was used. 100㎕ of germ suspension was added in 400㎕ of each germicides. Neutralizing broth has been applied after 30m each. And each sample is spread on McClung Toabe Agar after 10-fold serial dilution. Colony Forming Unit (CFU) has been checked after anaerobic incubation for 24 hours in 37℃. Spore-killing ability of germicides had been expressed as Inactivation Factor (IF), calculated by $\log_{10}$ (CFU decrease). And germicides which were represented more than 4 IF have been judged as 'effective'.

Results: Only 'Willow vet HALASOL (Sodiumhypochlorite, Yuhan Corporation)' represented more than 4 IF.

Conclusions: Based on this result, we would recommend HALASOL to farm affected by avian botulism.