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Letter to the Editor

Antibiotic Resistance and Virulence Genes Detection among the *Enterococcus faecalis* Isolated from Swiftlet Industry in Borneo

Sui Sien LEONG [1], Samuel LIHAN [2], Hwa Chuan CHIA [2]

1. *Department of Animal Sciences and Fishery, Faculty of Agricultural and Food Sciences, Universiti Putra Malaysia, Sarawak, Malaysia*
2. *Department of Molecular Biology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, Sarawak, Malaysia*

***Corresponding Author:** Email: leongsuisien@upm.edu.my

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Dear Editor-in-Chief

Enterococcus faecalis is a Gram-positive and commensal bacteria normally found inside the human or mammal's gastrointestinal tracts and is widely distributed in the soil, water, plants and food products (1). *E. faecalis* has been reported as a common pathogen usually found in birds and caused 80% to 90% of *Enterococcal* infection in nosocomial, surgical wounds, blood, and urinary tract in human (2). The number of multiple antibiotic resistance cases caused by *E. faecalis* has increased sharply leading to high mortality. *E. faecalis* posed four main virulence genes namely aggregation substance (*AS*), adhesion of collagen (*ace*), gelatinase (*gel*E), endocarditis antigen (*efa*A). The virulence factors of *E. faecalis* adhered to the host cell membranes and to the environmental surfaces to obtain nutrients and evade the host immune response (3). *AS* gene acted as facilitators in aggregation and conjugation process between donors and recipient bacteria (2). The *ace* gene act as mediator so that *E. faecalis* adhered to collagen and laminin successfully (3) may also contribute to the pathogenesis of *Enterococci* especially endocarditis disease. The gelatinase (*gel*E) encoded in the chromosomal gene which acts as gelatin, casein, hemoglobin and other bioactive compounds. *E. faecalis* as culprit in the therapy-resistant endodontic infections identified endocarditis antigen (*efa*A) as one of the vital virulence factors associated mainly with infective endocarditis (4). *E. faecalis* strain possess genes that encode virulence factors in Brazil. Cases of infection caused by pathogenic *Enterococcus* bacteria poses antibiotic-resistant genes isolated from the beach water and sand have been tremendously increased over the past few years may pose potential health risk for beach goers. The objective of this study was to screen the potential virulence factors in antibiotic resistance *E. faecalis* and to ascertain their prevalence in swiftlet industry in Borneo.

Samples of the swiftlet faecal and airborne bacteria were collected from Mar 2015 to Sep 2016 from the ten swiftlet houses located in the Southern, Central and Northern regions of Borneo. The faecal and airborne *Enterococci* bacteria isolates were identified to the species level, and their antibiotic resistance was determined using antibiotic susceptibility discs. Specific primers were designed for detection of four potential virulence genes (*ace*, *AS*, *efa*A and *gel*E) by multiplex PCR assay. The primers and reaction condition are follows: ACE1 5-AAAGTAGAATTAGATCCACAC-3, ACE2 5-TCTATCACATTCGGTTGCG-3 (56 °C); AS15-CCAGTAATCAGTCCAGAAACAACC-

3,AS25-GCTTTTTTCATTCTTGTGTTTGTT-3 (54 °C); *efa*A1 5- CGTGAGAAAGAAATGGAGGA-3, *efa*A2 5-CTACTAACACGTCACGAATG-3 (56 °C); *gel*E1 5-AGTTCATGTCTATTTTCTTCAC-3, *gel*E2 5- CTTCATTCTTTACACGTTTG-3 (56 °C). The PCR reactions were performed in reaction mixtures containing 2.5µl of DNA, 1.0 µl each 20 pmol primers (First Base, Malaysia), 0.5 µl of 10mM of deoxynucleoside triphosphate mix (Promega, USA), 1.5 µl of 25 mM $MgCl_2$ (Promega, USA), 5 µl of 5X Buffer solution (Promega, USA), 12.75 µl of distilled water and 0.75 µl of Taq polymerase (Promega, USA). PCR was performed with 30 cycles as follows: initial denaturation at 94 °C for 2 min, denaturation at 94 °C for 1 min, annealing for 1 min at the temperature mentioned, primer extension at 72 °C for 1 min and final extension at 72 °C for 10 min. The PCR products were visualized with ethidium bromide staining after 1% agarose gel electrophoresis for 35 min at 80 volts.

Overall, 34 faecal and 3 airborne *Enterococcal* bacteria isolates were identified as *E. qallinacum* (51%), *E. faecalis* (35%) and *E. harae* (8%). The airborne bacteria isolates were identified as *E. faecalis* (5%) and *E. qallinacum* (1%). The agarose gel electrophoresis banding patterns of virulence genes detection for *E. faecalis* is shown in Fig. 1.

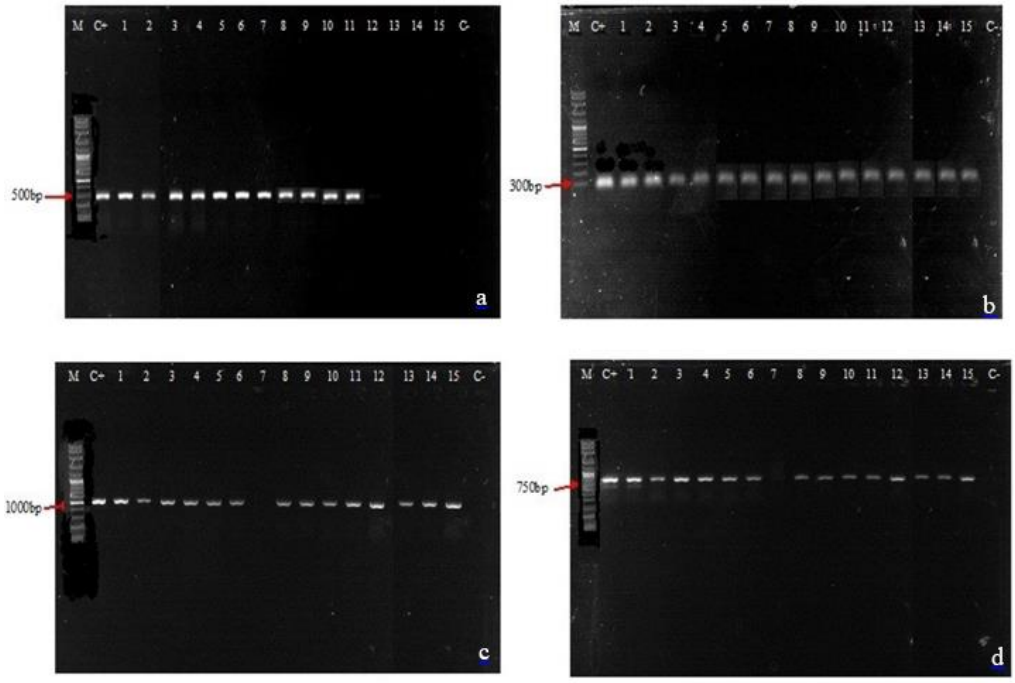

Fig. 1: Agarose gel electrophoresis of *ace* (a), *AS* (b), *efa*A (c) and *gel*E (d) gene amplification products from *Enterococcus faecalis* isolates. M: 1Kb ladder; C+: Positive control; 1-15: *Enterococcus* isolates; C-: Negative control

There were 10 out of 15 (67%) isolates of *E. faecalis* detected positive for the four virulence genes. Four out of fifteen (27%) isolates of *E. faecalis* possessed three of the virulence genes and one out of fifteen (6%) isolates of *E. faecalis* possessed two of the virulence genes. The disc diffusion method was used to confirm the resistant phenotypes of these isolates. Results revealed that 27% of the isolates were MAR with 8 different resistance patterns from the faecal and airborne bacterial isolates. Four *E. faecalis* bacteria showed multiple resistance with high MAR index (0.2 to 0.55).

Enteroccus bacteria showed a high prevalence in faecal isolates and high incidence of virulence genes were widely disseminated among the anti-

biotic resistance *E. faecalis*, suggesting the importance in the pathogenesis of *E. faecalis* infections which may cause potential health risks to humans

Conflict of interest

None conflict.

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