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Genotypic profiles of virulent genes detected among the *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* isolated from swiftlets in Borneo

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ABSTRACT

Aims: The occurrence of multiple pathogenic *Enterococcus faecalis*, *Escherichia coli*, and *Pseudomonas aeruginosa* are important nosocomial and hazardous infection clinically challenge worldwide. Thus, the aim of this study was to screen for the virulent genes profiles to ascertain their prevalence in swiftlets in Borneo.
Methodology and results: The *Enterococci*, *E. coli* and *P. aeruginosa* bacteria were isolated from the swiftlets' faeces and air inside swiftlet houses, which located in the Southern, Central and Northern regions of Borneo. The isolates were identified to the species level by 16S rRNA sequencing assay. Specific primers were designed for detection of the potential virulence genes in *E. faecalis* (*ace*, *AS*, *efa*A and *gel*E), *E. coli* (*stx*) and *P. aeruginosa* (*opr*L) by PCR assay. A total of 38 *Enterococci*, 26 of *E. coli* and 2 of *P. aeruginosa* fecal and airborne bacteria were identified. Sixty-seven percent of *E. faecalis* isolates were detected positive for four virulence genes, 27% possessed three (*AS*, *efa*A, *gel*E) genes and 6% possessed two (*ace*, *AS*) genes. There were no *stx* genes detected among all the *E. coli* isolates. The *opr*L gene was detected in all the *P. aeruginosa* isolates.
Conclusion, significance and impact of study: Virulence genes are important in the pathogenesis of both clinical and avian infections which considered to be a serious public health threat. The high incidence of virulence genes detection in *E. faecalis* and *P. aeruginosa* indicates these genes were widely disseminated among the bacteria found in swiftlet houses, suggesting the important issues in the pathogenesis of infections and diseases which may cause potential health risks to humans.

Keywords: Virulent genes, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, swiftlets

INTRODUCTION

Pathogenic bacteria are defined as bacteria which caused infection and diseases. Most pathogenic bacteria are useful in various industries and harmless to humans, but some bacteria may cause diseases under certain conditions. Pathogens commonly found in wild birds that caused an outbreak were *Enterococcus* spp., *Escherichia coli*, *Klebsiella* spp. (Radimersky *et al*., 2010), *Pseudomonas aeruginosa* and *Enterobacter* spp. (Benskin *et al*., 2009).

Enterococci are facultative anaerobic Gram-positive diplococci bacteria. *Enterococcus faecalis* and *Enterococcus faecium* are the common commensal bacteria normally found inside the mammal's gastrointestinal tracts (Lebreton *et al*., 2014) and they are widely distributed in soil, water, plants, and even food products (Frazzon *et al*., 2009). *Enterococci* are opportunistic pathogens found in wild birds which may cause septicemic disease in a patient with the low immune system (Al-Talib *et al*., 2015). Multiple antibiotic resistance cases of *E. faecalis* have been reported in most 80-90% infections related to nosocomial (Fisher and Phillips, 2009), surgical wounds, blood and urinary tract which led to a high mortality rate. Research did previously discover that the pathogenicity of *E. faecalis* is closely linked to epithelial cells with the four main virulence genes, namely: aggregation substance (*AS*), adhesion of collagen (*ace*), gelatinase (*gel*E), endocarditis antigen (*efa*A) which were commonly investigated although the mechanism was not well known. *E. faecalis* are able to adhere to the host cell films and ecological surfaces in order to acquire nutrients needed and to evade the host immune response (Medeiros *et al*., 2014). The *AS* gene expressed is responsible in the sex pheromone-responsive plasmid (Tremblay and Archambault, 2013) in

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the aggregation and conjugation process between donors and recipients' bacteria. The *ace* gene is reported to act as a mediator in collagen and laminin adherence (Medeiros *et al*., 2014), contributing to the pathogenesis of endocarditis disease (Lebreton *et al*., 2011). In addition, the *gel*E is another zinc metalloprotease encoded genes in the chromosome which involves various hydrolysis processes. Preethee *et al*. (2012) have proved that *efa*A gene in *E. faecalis* is responsible for causing the failure of therapy in treating the resistant endodontic infections.

Escherichia coli as a Gram-negative rod-shaped bacterium under *Enterobacteriaceae* family is a normal flora found in the intestinal tract of mammalians and mostly harmless to the hosts. However, small numbers of *E. coli* namely Shiga toxin-producing *E. coli* (STEC 0157: H7) pose hazardous diseases called hemorrhagic colitis (HC) with the symptom of bloody diarrhea, acute abdominal cramps, vomiting, and large bowel inflammation (Andrew and Growther, 2011). These pathogenic *E. coli* are worldwide zoonotic pathogens that caused extra-intestinal diseases in birds especially chickens, turkeys (Schouler *et al*., 2012), cattle and ruminant hosts (Ferens and Hovde, 2011). *Escherichia coli* are classified based on their virulence factors. The STEC which produces harmful toxin named Shiga toxin 1 and Shiga toxin 2, causing infection by disrupting the host protein synthesis process (Chai, 2013). STEC affected mostly children, inducing HC followed by hemolytic uremic syndrome. Transmission of disease to people occurs via the ingestion of under-processed contaminated food and improper hygienic farm management.

Pseudomonas aeruginosa is another opportunistic pathogen, mostly isolated from soil, aquaculture environment, skin and even man-made environments for the purpose of development. *Pseudomonas aeruginosa* commonly caused diseases to animals and humans (Klockgether and Tümmler, 2017). However, *P. aeruginosa* is a common avian pathogen isolated mostly in birds and may infect the damaged avian tissues or low immunity patients with symptoms of inflammation and sepsis (Kalle *et al*., 2012). The bacteria colonized and multiplied in critical human organs such as urinary tract, kidneys, lungs, leading to high mortality. *Pseudomonas aeruginosa* poses virulence factor which is able to degrade the cell wall membrane of eukaryotic (Prithiviraj *et al*., 2005). *Pseudomonas aeruginosa* was the main pathogen causing high mortality and spoilage in fish and fish products (Abdullahi *et al*., 2013). Apart from that, *P. aeruginosa* is considered a high-risk pathogen because of their presence of outstanding intrinsic antibiotic resistance ability thus further decreased the clinical effectiveness (Cabot *et al*., 2016). Most of the *P. aeruginosa* are harmful and the *opr*L gene target is used precisely in the detection of bacteria. The *opr*L gene, peptidoglycan-associated outer-membrane lipoprotein is involved in protein synthesis and regulation in *P. aeruginosa* growth (Panmanee *et al*., 2008). Thus, the detection of *opr*L gene is vital in detection of its pathogenicity.

Nowadays, most of the swiftlet houses are built in the urban area, thus the unhygienic mismanaged waste and pollution may have cultivated more potential pathogens over the years. A study regarding the pathogenicity caused by these bacteria from the swiftlet industries is yet to be discovered. In this study, the virulent genes profiles of bacteria species including *E. facealis*, *E. coli*, and *P. aeruginosa* were studied in order to ascertain their prevalence in swiftlets in Borneo.

MATERIALS AND METHODS

Location of study areas

Sampling of the swiftlet bird fecal and airborne bacteria was carried out from March 2015 till September 2016 from the ten swiftlet houses located in the Southern, Central and Northern regions of Borneo (Figure 1). The sampling sites that were chosen for the Southern Sarawak were S1: Kota Samarahan (01°27'34.2''N 110°27'25.9''E), S2: Kuching (01°32'56.6''N 110°22'27.5''E), S3: Semarang (01°40'40.0''N 111°6'5.92''E), S4: Maludam (01°39'14.17''N 111°1'53.9''E), S5: Sepinang (01°40'11.8''N 111°7'5.9''E) and S6: Betong (01°24'0''N 111°31'0''E). The sampling sites chosen for the Central Sarawak were S7: Saratok (01°44'10.32''N 111°21'10.22''E), S8: Sarikei (02°6'3.75''N 111°30'39''E) and S9: Sibu (02°19'11.3''N 111°49'50.5''E). The sampling site chosen for the Northern Sarawak was S10: Miri (04°23'39.2''N 113°59'12.2''E).

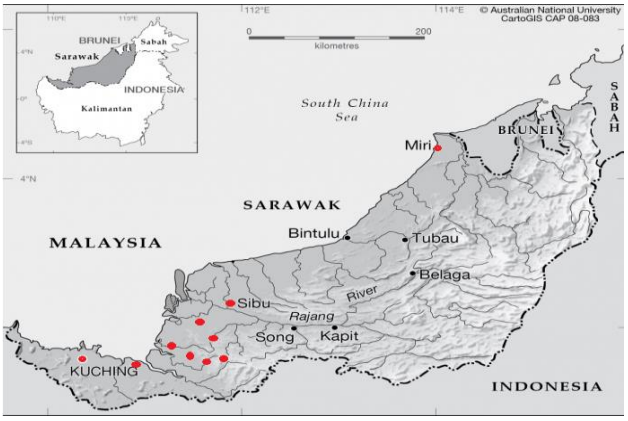

Figure 1: Location of study areas: Sarawak, Borneo (shown in red dots).

Isolation of feacal and airborne *Enterococci*, *E. coli* and *P. aeruginosa*

Five faeces samples were collected randomly from the floor of each swiftlet house of the sampling site as described by Nyakundi and Mwangi (2011). Each collected fecal sample was diluted in ratio 1:9 in sterile 0.85% saline solution. The diluted sample was then cultured on bile esculin agar, MacConkey agar and Pseudomonas agar (Merck, Germany), plates in duplicate

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