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, etfA and gfeE), E. coli (stx) and P. aeruginosa (oprL) 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, etfA, gfeE) genes and 6% possessed two (ace, AS) genes. There were no stx genes detected among all the E. coli isolates. The oprL 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 (gfeE), endocarditis antigen (etfA) 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
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 geE is another zinc metalloprotease encoded genes in the chromosome which involves various hydrolysis processes. Preeth et al. (2012) have proved that etaA 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 mammals and mostly harmless to the hosts. However, small numbers of E. coli namely Shiga toxin-producing E. coli (STEC) may cause 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 causes 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 (Prithviraj 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 oprL gene target is used precisely in the detection of bacteria. The oprL gene, peptidoglycan-associated outer-membrane lipoproteins is involved in protein synthesis and regulation in P. aeruginosa growth (Panmanee et al., 2008). Thus, the detection of oprL 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. faecalis, 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°6’5.9”E), S4: Maludam (01°39’14.1’7”N 111°153.9”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: Sarakei (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).](image)

**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 Nyakundii 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...
using the spread plate method and incubated at 37 ± 1 °C for 24 h.

The collection of the indoor airborne samples procedures was carried out according to Malaysia Veterinary Health. The airborne bacteria were obtained using exposed plate count agar (Scharlau, Spain) in duplicate. The lid of the plates was lifted and exposed in the air for 15 sec inside the swiftlet house. The plates were incubated at 37 ± 1 °C for 24 h in the laboratory. The bacteria colonies were randomly selected and cultured on selective agar (Merck, Germany) and incubated at 37± 1 °C for 24 h.

Identification of airborne Enterococci, E. coli and P. aeruginosa

Ten colonies were randomly selected from each culture plate. The pure isolates were obtained for further identified by using biochemical tests. Isolates that showed Gram positive cocci, catalase-negative and Voges-Proskauer positive were presumed as Enterococcus spp. Isolates that showed Gram negative rod, lactose-positive, oxidase-negative and indole positive were presumed as E. coli. Isolates that showed Gram positive rod, Voges Proskauer-negative, indole-negative, methyl red-negative and catalase-positive were presumed as Pseudomonas spp. Species identification was confirmed by 16S rRNA sequencing.

DNA extraction

The bacteria DNA was extracted using the boiling method described by Sien et al. (2013) with minor modification. A volume of 1.5 mL of 24 h bacteria culture was then transferred into a 2.0 mL microcentrifuge tube and centrifuged at 10,000 rpm for 5 min. The supernatant was discarded. After that, 500 µL of sterile distilled water was added and vortexed to suspend the cell pellet. The microcentrifuge tube was then boiled for 10 min and the supernatant was collected. A total of 2 µL DNA extracted from each sample were then placed directly on the spectrophotometer (NanoDrop, Thermo Scientific) and measures in 230, 260 an 280 nm in order to acquire the DNA purity.

PCR for molecular detection of virulence genes (ace, AS, efaA, gelE) in E. faecalis

The PCR was performed according to the methods described by Dupre (2003). The E. faecalis ATCC 29212 and E. coli ATCC 25922 were used as positive and negative control respectively for the detection of virulence genes. The primers and reaction condition are given in Table 1. The PCR reactions were performed in reaction mixtures containing 2.5 µL of DNA, 1.0 µL each 20 pmol primers (First Base, Malaysia), 0.5 µL of 10 mM of deoxynucleoside triphosphate mix (Promega, USA), 1.5 µL of 25 mM MgCl2 (Promega, USA), 5 µL of 5× 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 shown in Table 1, 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.

Multiplex PCR for molecular detection of Shiga toxin (stx gene) in E. coli

Multiplex-PCR method was adopted (Son et al., 2015) with minor modification. The E. coli O157:H7 (EDL933 strain) used as positive control and E. coli ATCC 25922 used as negative control were included. The primer sequences (stx1, stx2) and reaction condition is given in Table 2. The PCR reactions were performed in reaction mixtures containing 5.0 µL of DNA, 0.5 µL each 20 pmol primers (First Base, Malaysia), 1.0 µL of 10mM of deoxynucleoside triphosphate mix (Promega, USA), 1.5 µL of 25 mM MgCl2 (Promega, USA), 2.5 µL of 5X Buffer solution (Promega, USA), 12.5 µL of distilled water and 0.5 µL of Taq polymerase (Promega, USA). PCR was performed with 30 cycles as follows: initial denaturation at 95 °C for 2 min, denaturation at 95 °C for 1 min, annealing at 95 °C for 1 min, 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.

PCR for molecular detection of oprL gene in P. aeruginosa

PCR analysis was performed for the detection of pathogenic P. aeruginosa as described by Xu et al. (2004) with minor modification. Pseudomonas aeruginosa ATCC 27853 was used as positive control and E. coli ATCC 25922 was used as negative control. The primer sequences for oprL gene are shown in Table 3. The PCR reactions were performed in reaction mixtures containing 5.0 µL of DNA, 1.0 µL each 20 pmol primers (First Base, Malaysia), 1.0 µL of 10mM of deoxynucleoside triphosphate mix (Promega, USA), 2.0 µL of 25 mM MgCl2 (Promega, USA), 3.5 µL of 5X Buffer solution (Promega, USA), 11.0 µL of distilled water and 0.5 µL of Taq polymerase (Promega, USA). PCR was performed with 40 cycles as follows: initial denaturation at 96 °C for 5 min, denaturation at 96 °C for 1 min, annealing for 1 min at the temperature shown in Table 3, 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.
Table 1: Primers sequence for adhesion of collagen (ACE), aggregation substance (AS), endocarditis antigen (efaA), gelatinase (geE).

| Primers name | Primer sequences (5’-3’) | Annealing temperature (°C) |
|--------------|--------------------------|---------------------------|
| ACE-F        | AAAGTGAATTTGATCCACAC     | 56                        |
| ACE-R        | TCTACACATTCCGTTGCG       |                           |
| AS-F         | CCGTAATCAGTCCAGAAACACC   | 54                        |
| AS-R         | TAGCTTTTTTCTATTCTGTGGTT  |                           |
| efaA-F       | CGTGAGAAAGAATGGAGGA      | 56                        |
| efaA-R       | CTTAACAACCGTGGAATG       |                           |
| geE-F        | AGTTCTAGTGCTATTTTTCTCAC  | 56                        |
| geE-R        | CTTTATTCTTTACACGTTTG     |                           |

Table 2: Primer sequence for Shiga toxin 1 (stx1) and Shiga toxin 2 (stx2).

| Primer name | Primer sequence (5’-3’) | Annealing temperature (°C) |
|-------------|-------------------------|---------------------------|
| stx1-F      | ATAAATCGCCATTCTGTGACTAC | 95                        |
| stx1-R      | AGAACGCCACGTGAGATCATC   |                           |
| stx2-F      | TTAACCACACCCACCGGCAGT   | 95                        |
| stx2-R      | GGATATTCTTTACACGTTTG    |                           |

Table 3: Primer sequence for peptidoglycan-associated outer-membrane lipoprotein (oprL) gene.

| Primer name | Primer sequence (5’-3’) | Annealing temperature (°C) |
|-------------|-------------------------|---------------------------|
| oprL-F      | ATGGAATAGCTGAAATTCCAC   | 55                        |
| oprL-R      | CTTCTAGCTGACGGCAGG      |                           |

RESULTS AND DISCUSSION

Prevalence of Enterococcus, E. coli and P. aeruginosa

A total of approximately 50 Enterococci, 30 E. coli and 5 P. aeruginosa were isolated biochemically from the ten swiftlet houses throughout the Borneo. Only 38 Enterococci, 26 E. coli and 2 P. aeruginosa were successfully identified through molecular sequencing analysis (Table 4). There were more fecal bacteria isolated mainly due to the differences in nutrient content available in the samples. The bacterial isolates from the swiftlet faeces were identified as E. gallinarum (51%, n=19), E. faecalis (35%, n=13), E. harae (8%, n=3), E. coli strain 1 (31%, n=8), E. coli strain 2 (35%, n=9), E. coli strain 3 (15%, n=4), E. coli strain 4 (3.8%, n=1), E. coli strain 5 (3.8%, n=1), E. coli 083:H1 (3.8 %, n=1) and P. aeruginosa (100%, n=2). The airborne bacteria isolates from swiftlet houses were identified as E. faecalis (5%, n=2), E. gallinarum (1%, n=1) and E. coli strain 1 (7.6 %, n=2).

The detection of Enterococci and E. coli bacteria was widespread among the wild birds because birds can be the reservoirs for bacterial pathogens growth. No E. faecalis were being isolated from S6 (Betong) and no E. coli being isolated from S2 (Kuching). The absence of these pathogens may due to their geographical location, acidity (pH), moisture content, hygienic condition inside the swiftlet houses and temperature effects during different sampling at the different times of the year which may affect bacterial growth. Woo et al. (2017) has explained that environmental impacts especially geographical location can alter microbial community growth. Similar results were obtained with identification of Enterococcus spp. and E. coli in the Marabou Stock (Leptotilus crumeniferus) and rooks droppings samples (Literák et al., 2007; Nyakundi and Mwangi, 2011). Besides, fecal E. faecalis was also found from the fecal samples obtained from dogs at veterinary hospitals (Ghosh et al., 2012), poultry (Kwon et al., 2012), ducks and wild geese (Han et al., 2011). Our result of this study showed a low prevalence frequency for both E. coli and Enterococcus spp. as pathogen reservoirs in swiftlets. Escherichia coli was widely found in the polluted river water (Tambekar et al., 2008) and pig fecal samples (Meng et al., 2014). Research had proved that E. faecalis and E. coli are naturally present in the animal gastrointestinal tract and able to survive in a harsh bile content environment (Saitoa et al., 2018). Thus, we can assume that Enterococcus spp. and E. coli are common pathogens found in most avian. Only 2 isolates of P. aeruginosa were isolated from S8 (Sarikiel). The study showed that P. aeruginosa is not a common avian pathogen found. Most P. aeruginosa occurs as second invader of skin or nasal infection. Thus, the detection of P. aeruginosa at S8 showing that the pathogens were originated from the skin debris of the birds not from the fecal sample itself.
**RNA sequencing homology search (BLAST) results of the Enterococci, E. coli, and P. aeruginosa isolates.**

| Sites | Source | Bacterial species (Number of isolates) | Size(s) bp | Data base | Homology (%) |
|-------|--------|--------------------------------------|-----------|-----------|--------------|
| S2, S3, S5, S8, S7, S10 | Faeces | Enterococcus qallinacum (19) | 534 | GenBank | 99 |
| S2, S4, S5, S7, S9, S10 | Faeces | Enterococcus faecalis (13) | 1445 | GenBank | 100 |
| S1, S4 | Faeces | Enterococcus harae (3) | 1493 | GenBank | 99 |
| S1, S4 | Air | Enterococcus faecalis (2) | 1445 | GenBank | 100 |
| S5 | Air | Enterococcus qallinacum (1) | 534 | GenBank | 99 |
| S1, S3, S4 | Faeces | Escherichia coli strain 1 (8) | 4887515 | GenBank | 99 |
| S3, S5, S7, S8, S9 | Faeces | Escherichia coli strain 2 (9) | 5038386 | GenBank | 99 |
| S6 | Faeces | Escherichia coli strain 3 (4) | 1537 | GenBank | 99 |
| S3 | Faeces | Escherichia coli strain 4 (1) | 5386223 | GenBank | 99 |
| S3 | Faeces | Escherichia coli strain 5 (1) | 4971461 | GenBank | 99 |
| S3 | Faeces | Escherichia coli 083:H1 (1) | 4747819 | GenBank | 99 |
| S8 | Air | Escherichia coli strain 1 (2) | 5038386 | GenBank | 99 |
| S6, S9 | Faeces | Pseudomonas aeruginosa (2) | 1057 | GenBank | 100 |

**Table 4:** The 16S rRNA sequencing homology search (BLAST) results of the Enterococci, E. coli, and P. aeruginosa isolates.

**Table 5:** Distribution of genotypic virulence determinants among the E. faecalis.

| Isolates (n=15) | Sampling sites | Sources | Detection by PCR* |
|----------------|----------------|---------|-------------------|
|                |                |         | ace AS efaA geE   |
| SWF-SAM-B6     | S1             | Air     | + + + +           |
| SWF-OPP-1D4    | S7             | Faeces  | + + + +           |
| SWF-OPP-2A3    | S7             | Faeces  | + + + +           |
| SWF-OPP-2C10   | S7             | Faeces  | + + + +           |
| SWF-OPP-2D4    | S7             | Faeces  | + + + +           |
| SWF-OPP-2D6    | S7             | Faeces  | + + + +           |
| SWF-NEX-1E8    | S6             | Faeces  | + + - -           |
| SWF-MAL-1B2    | S4             | Faeces  | + + + +           |
| SWF-MAL-1C3    | S4             | Faeces  | + + + +           |
| SWF-MAL-A3     | S4             | Air     | + + + +           |
| SWF-MIRI-2A6   | S10            | Faeces  | + + + +           |
| SWF-MIRI-2A10  | S10            | Faeces  | - + + +           |
| SWF-KCH-2A1    | S2             | Faeces  | - + + +           |
| SWF-SIBU-2C2   | S9             | Faeces  | - + + +           |
| SWF-SPN-2A1    | S5             | Faeces  | - + + +           |

* Bands detected in PCR +: Present; -: absent.

**Virulence genes (ace, AS, efaA, geE) detection in E. faecalis**

The virulence genes presented in the 15 E. faecalis isolates were successfully detected by PCR analysis using the specific primer sequences (Table 1). The distribution of genotypic virulence determinants among the E. faecalis is shown in Table 5 and the agarose gel electrophoresis banding patterns illustrated in Figure 2. There were 67% isolates of E. faecalis detected positive for the four virulence genes. About 27% isolates of E. faecalis possessed three of the virulence genes and 6% isolates of E. faecalis possessed two of the virulence genes. The E. faecalis isolates from S1, S4, S7, S10 confirmed the presence of the above four virulence genes. The result showed that the prevalence of virulence gene was not significantly correlated with the sampling sites. Besides, E. faecalis isolated from S6 (Betong) only revealed the presence of ace and AS genes. Lastly, ace gene was not detected from the E. faecalis isolated from S2, S5, S9, S10.

Most of the E. faecalis isolated carried most virulence genes. The high percentage of virulence genes detection indicated that they were high risk vector in transmission of diseases particularly through the swiftlet birds’ fecal contamination. E. faecalis was also reported as the most...
common pathogens in nosocomial infection, causing fatal outcomes in patients. Cases of *E. faecalis* causing nosocomial infections were increasing especially in Italy thus causing threats to human health. The virulence genes which were expressed on the surface of the membrane (*ace, AS, efaA, geE*) played a vital role in critical prerequisite for *E. faecalis*-induced diseases and their pathogenicity (Ocvirk et al., 2015). The virulence factors of *E. faecalis* enable them to evade the host immune response (Medeiros et al., 2014). The most widespread prevalence gene was *AS* gene (Table 5). A similar result was obtained revealed *Enterococcus* virulence genes especially *AS* gene together with cytolysin activator was found among *E. faecalis* and mostly isolated from beach water, dogs, humans, and birds in Australia (Ferguson et al., 2016). Similar result was also obtained stated that 87.8 % of *ace* gene and 69.4% of *geE* gene were detected clinically. Similar result was proved by Ahmed et al. (2012) that *geE* was the most abundant gene detected among *E. faecalis* from multiple environmental sources which enhance the survivability of *Enterococcus* especially during extra intestinal surroundings (Coque, 2002). The *efaA* gene of *E. faecalis* was detected and associated mainly with infective endocarditis. Similar result was obtained who had isolated *E. faecalis* strains from clinical and food samples that possess *efaA* genes in Brazil (Medeiros et al., 2014). Pathogenic *E. faecalis* had been proved by research findings as a culprit in therapy-resistant endodontic infections. The presence of the virulent strains among *E. faecalis* alone cannot confirm infection occurrence because there may be other mediators of pathogenicity (Kim and Marco, 2013). Pathogenicity of *Enterococcus* is mainly due to the ability of these virulent strains to overgrow in the infection sites and further multiple spreads all over the body. Host factors play a vital role in the ability of *Enterococci* to establish infection (Arias and Murray, 2012). However, the presence of these genes among *E. faecalis* strains from swiftlet warrants further studies to assess potential human health risks.

### Shiga toxin (*stx1* and *stx2*) gene detection in *E. coli*

When the *E. coli* isolates were analysed, the primers targeting the Shiga toxin gene generated bands of *stx1* at size 500 bp and *stx2* at size 250 bp for positive isolates. The agarose gel electrophoresis banding patterns of Shiga toxin detection is shown in Figure 3 and the distribution of genotypic virulence determinants among *E. coli* is shown in Table 6.
virulence study because the genes are responsible in the toxin secretion to disrupt the protein synthesis in their host. The present study was targeted the Shiga toxin genes mainly because all the STEC O157:H7 E. coli consists Stx2 (stx2) and intimin (eae) genes which may link with severe disease (Adamu et al., 2014). Detection of STEC is vital to prevent any STEC infection outbreaks and treatment purposes to decrease further renal damage. The pathogen may spread through reservoir birds to human. Similar result was reported by Chai (2013), none of the E. coli isolated from aquaculture environment showed a positive in Shiga toxin gene detection. On the other hand, Meng et al. (2014) showed that 25% isolates of E. coli isolates from the pig fecal samples were detected with Shiga toxin genes. Based on the results of this study alone, we cannot prove that the E. coli isolate was harmless because there is non-O157 STEC available which cannot be cultured by selective and differential media in the laboratory (Gould, 2012). Apart from that, Gris and et al. (2009) explained that false-negative results may occur during the Shiga toxin detection mainly due to the inhibition of the PCR, sequence variability for the primers and probes, the presence of the toxin gene in quantities less than the detection limit of the assay. Shiga toxins are encoded on a mobile genetic element that can be lost by the bacterial host (Foxman, 2011).

**Table 6**: Distribution of genotypic virulence determinants among the E. coli.

| Isolates       | Sampling sites | Sources | Detection by PCR<sup>a</sup> |
|----------------|----------------|---------|----------------------------|
|                |                |         | stx 1 | stx 2 |
| SWF-SAM-1E4    | S1             | Faeces  | -     | -     |
| SWF-SAM-1E6    | S1             | Faeces  | -     | -     |
| SWF-OPP-2D8    | S7             | Faeces  | -     | -     |
| SWF-NEX-1D2    | S6             | Faeces  | -     | -     |
| SWF-NEX-1D7    | S6             | Faeces  | -     | -     |
| SWF-NEX-1E2    | S6             | Faeces  | -     | -     |
| SWF-MAL-1A9    | S4             | Faeces  | -     | -     |
| SWF-SIBU-2A5   | S9             | Faeces  | -     | -     |
| SWF-SIBU-2D2   | S9             | Faeces  | -     | -     |
| SWF-SPN-2A10   | S5             | Faeces  | -     | -     |
| SWF-RK-2A4     | S8             | Faeces  | -     | -     |
| SWF-RK-2A6     | S8             | Faeces  | -     | -     |
| SWF-RK-2A10    | S8             | Faeces  | -     | -     |
| SWF-RK-B3      | S8             | Air     | -     | -     |
| SWF-RK-B10     | S8             | Air     | -     | -     |
| SWF-SMR-1A1    | S3             | Faeces  | -     | -     |
| SWF-SMR-1A3    | S3             | Faeces  | -     | -     |
| SWF-SMR-1A6    | S3             | Faeces  | -     | -     |
| SWF-SMR-1A10   | S3             | Faeces  | -     | -     |
| SWF-SMR-1C7    | S3             | Faeces  | -     | -     |
| SWF-SMR-1D1    | S3             | Faeces  | -     | -     |
| SWF-SMR-1D6    | S3             | Faeces  | -     | -     |
| SWF-SMR-1D8    | S3             | Faeces  | -     | -     |

<sup>a</sup>Bands detected in PCR + : Present; - : absent.

S1-10: Sampling sites

**OprL gene detection in P. aeruginosa**

The results showed that the oprL gene locus for the P. aeruginosa isolates tested was successfully detected using PCR targeting the specific primer (Table 3). The agarose gel electrophoresis banding patterns of oprL gene detection for P. aeruginosa is shown in Figure 4 and the distribution of genotypic virulence determinants is shown in Table 7.

The result of this study confirmed the presence of oprL gene in both isolates (Table 7) with the expected size of 504 bp is agreed with the research finding reported (Abdullahi et al., 2013). Similar result was reported that oprL gene was successfully detected in most of the P. aeruginosa isolates from the water (Abdullahi et al., 2013). The result showed that pathogenic P. aeruginosa was present in low frequency in the swiftlet houses especially at S6 and S9 is probably due to contamination in the feed or water in the surrounding area and bird skin infection outbreak. Apart from that, the birds flying in and out of the bird houses may introduce more bacterial pathogens from the outside environment into the houses. Current result showed that P. aeruginosa is not a common avian pathogen isolated mostly in birds because they were isolated in low frequency. Our study targeted oprL gene mainly because...
Figure 4: Agarose gel electrophoresis of oprL amplification products from P. aeruginosa isolates. M: 1 kb ladder; C+: Positive control (P. aeruginosa ATCC 27853); 1-2: P. aeruginosa isolates; C-: Negative control (E. coli ATCC 25922).

Table 7: Distribution of genotypic virulence determinants among the P. aeruginosa.

| Isolates       | Sampling sites | Sources | Detection by PCR* |
|----------------|----------------|---------|-------------------|
| SWF-SIBU-1B9   | S9             | Faeces  | +                 |
| SWF-NEX-1C2    | S6             | Faeces  | +                 |

*Bands detected in PCR + : Present
S6, S9: Sampling sites

it is a peptidoglycan associated outer membrane lipoprotein which is sensitive and reliable for detection (Han et al., 2011). Most of the P. aeruginosa causes life-threatening infections and difficult to control clinically. The outer membrane proteins of P. aeruginosa encoded with oprL gene play the main role in the interaction between bacterium with the environment (Jami Al-Ahmadi and Zahmatkesh Roodsari, 2016). Thus, the oprL gene target was used precisely in the detection of them. Pseudomonas aeruginosa produced pyocyanin as their virulence factor which degraded the cell wall membrane of eukaryotic cells, causing lysis and death in patients (Gellatly and Hancock, 2013) and mainly infected the damaged tissues or infected the low immunity patients with symptoms of inflammation and sepsis. Even though there was only a low frequency of P. aeruginosa isolated from swiftlet, precautions should be taken. This bacterium remains a problematic culprit in hospitals, causing virulent and persistent infections despite antibiotic treatment.

CONCLUSION

Potential pathogens (E. faecalis, E. coli, and P. aeruginosa) showed a high prevalence in fecal samples. There were only pathogenic E. facealis detected in airborne samples. 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 which may transmit through the flying swiftlet inside the houses. The prevalence of pathogenic airborne bacteria was depended mainly on the frequency of the birds entering the house, air circulation and the hygiene condition inside the bird houses. Interaction human with swiftlet waste materials may spread pathogenic bacteria potentially creating health hazards in the community. Thus, rules and guidelines on swiftlet farming in Malaysia must be enforced. Swiftlet farming is recommended to build far from the city, away from the people.

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