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Virulence Genes Detection among the Antibiotic Resistant *Enterococcus faecalis* Isolated from Bird Industry in Borneo

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ABSTRACT

The abuse of antibiotics usage in bird industry has resulted in the emerging antibiotic resistant *Enterococci* worldwide which has posed a threat clinically to human health. The present study was to screen and identify the potential virulence agents in antibiotic resistance *E. faecalis* in bird industry in Borneo. *Enterococcus* bacteria collected from the birds' faeces and indoor air inside ten birdhouses were identified to species level and their antibiotic resistance was checked using antibiotic susceptibility discs. Specific primers using PCR assay were intended for the detection of four potential virulence genes (*ace*, *AS*, *efa*A, *gel*E). Out of the thirty-seven *Enterococci* faecal bacteria, the prevailing bacteria found were *Enterococcus qallinacum* (51%), *Enterococcus faecalis* (35%) and *Enterococcus harae* (8%). The airborne bacteria were reported as *Enterococcus faecalis* (5%) and *Enterococcus qallinacum* (1%). Twenty-seven percent of isolates were reported to have Multiple Antibiotic Resistance (MAR) index ≥ 0.2 with 9 distinct resistance patterns formed. *E. faecalis* showed higher resistance to vancomycin. Virulence genes were successfully reported in the 15 *E. faecalis* isolates. Sixty-seven percent of isolates were detected positive for four virulence genes, 27% possessed three (*AS*, *efa*A, *gel*E) genes and 6% possessed two (*ace, AS*) genes. Antibiotic resistance and virulence genes detection were significantly correlated. These virulence genes or antibiotic resistance genes were important in the pathogenesis of *E. faecalis* infections.

Keywords: Antibiotic resistance, birds, *Enterococcus faecalis*, virulence

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INTRODUCTION

Pathogenic bacteria are defined as the bacteria which cause infection and diseases. Most of the bacteria are useful in various industries and harmless to human, but some bacteria may cause diseases under certain condition. Bacterial pathogens commonly found in wild birds which triggered outbreak were documented as *Enterococcus* spp. (Chung et al., 2018), *Escherichia coli* (Dho-Moulin & Fairbrother, 1999), *Klebsiella* spp. (Giorgio et al., 2018), *Pseudomoas aeruginosa* (Walker et al., 2002) and *Enterobacter* spp. (Giorgio et al., 2018). Bird industries have sprouted spontaneously across the whole Asia countries within a few years due to multimillion-dollar potential profit derived from the commercially high nutritious value-added lucrative bird nest for human.

Enterococci are opportunistic pathogens that cause infections among the patients with impaired immune system (Duprè et al., 2003), cause septicemic disease (Al-Talib et al., 2015) in human. *Enterococcus faecalis*, Group D *Streptococcus* bacteria is a Gram- positive and commensal bacteria normally found inside the human or mammal's gastrointestinal tracts (Ryan & Ray, 2004) and is widely distributed in the water, plants, soil and food by-products (Frazzon et al., 2009). *E. faecalis* as a typical pathogen causing infections in nosocomial (Kayaoglu & Orstavik, 2004), surgical wounds, blood, urinary tract and enterococcal infection (Toledo-Arana et al., 2001). The number of multiple antibiotic resistance cases caused by *E. faecalis* has increased sharply leading to high mortality (Willems et al., 2001). Research done previously discovered that there was close interaction between *Enterococcus* and epithelial cells because of the ability to adhere or form biofilm on epithelial cells (Wells et al., 2000).

As a result of widespread use and misuse of antibiotics in intensive animal rearing and in clinical settings for the treatment of community-acquired infections, the emergence of multidrug-resistant strains such as vancomycin-resistant *Enterococci* has become a major concern worldwide. Antibiotic resistant Enterococci posed the ability to transfer their virulence agents between bacterial strains in the environment or even commensals in gastrointestinal tract.

E. faecalis posed four main virulence genes namely aggregation substance (*AS*), adhesion of collagen (*ace*), gelatinase (*gel*E), endocarditis antigen (*efa*A) which were commonly investigated. The virulence features of *E. faecalis* involved in the adherence process of bacteria to the host cell layers and even to the surrounding surfaces in order to acquire supplements and dodge the host resistant reaction (Medeiros et al., 2014). Previous study explained that *AS* protein expressed on the *E. faecalis* surface played an important role in the adhesion process to the intestinal cells (Suβmuth et al., 2000). Besides, *AS* gene presents in the pheromone responsive plasmid (Wells et al., 2000) acted as facilitators in aggregation and conjugation process between donors and recipient bacteria (Güven & Dag, 2004). The *ace* gene was reported to act as mediator so that *E. faecalis* adhered to collagen

and laminin successfully (Medeiros et al., 2014). Previous study displayed that this *ace* gene protein might be a factor in the transmission of *Enterococci* especially the endocarditis disease (Lebreton et al., 2011). Besides, the gelatinase (*gel*E), zinc metalloprotease which encoded in the chromosomal gene acts as hemoglobin, gelatin, casein, and other bioactive compounds hydrolysis process (Waters et al., 2003). Researchers had proven *E. faecalis* as a culprit in the therapy-resistant endodontic infections which further identified endocarditis antigen (*efa*A) as fundamental harmfulness components related chiefly with infective endocarditis (Preethee et al., 2012). Medeiros et al. (2014) had isolated *E. faecalis* strains found from the clinical and food by-products which had genes that encoded virulence agent in Brazil. Previous studies indicated that the *E. faecalis* isolated from the seashore water and sand posed antibiotic resistant features which might become the potential wellbeing hazard for shoreline goers (Rathnayake et al., 2011). Cases of infection caused by virulent agents presented in antibiotic resistant *Enterococcus* bacteria have been increased tremendously over the past few years. Thus, the aim of this research was to detect and identify the potential virulence agents in antibiotic resistance *E. faecalis* in bird industry in Borneo.

MATERIALS AND METHODS

Location of Study Areas

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

Isolation and Identification of Faecal and Airborne *Enterococci*

Five defecation samples were collected randomly from the floor of each birdhouse in the sampling sites and tested separately using the method as described by Nyakundi & Mwangi (2011). The faecal samples were diluted in ratio 1:9 in sterile 0.85% of saline. The diluted sample was then placed on bile esculin agar (Merck, Germany) in duplicate using the spread plate method and incubated at temperature 37± 1° C for 24 hours. The *Enterococci* bacteria were further identified using biochemical tests. The species identity was confirmed using 16S rRNA sequencing by PCR.

The procedures for the collection of the indoor airborne samples were carried out according to Malaysia Veterinary Health 2017. The airborne bacteria were obtained using exposed plate count agar (Scharlau, Spain) in duplicate. The lid of the plates were lifted and exposed in the air for 15 seconds inside the birdhouse. The plates were then incubated at 37± 1°C for 24 hours. The bacterial colonies were randomly selected and cultured on bile esculin agar (Merck, Germany) and incubated at 37± 1° C for 24 hours.

Antibiotic Susceptibility Test

All the isolates from the birds' defecation and indoor air of the bird-houses was tested for antibiotic susceptibility test. Antibiotic susceptibility test was conducted using disc diffusion standard protocol (CLSI, 2017). Antibiotic discs (Oxoid, England) chosen for the testing represented the commonly utilized clinical antibiotics. *Escherichia coli* ATCC 25922 was applied as positive control. Among the antibiotic discs utilized were Ampicillin (10µg), Chloramphenicol (30µg), Tetracycline (30µg), Erythromycin (15µg), Nitrofurantoin (300µg), Rifampin (5µg), Penicillin G (10U), Doxycycline (30µg), Vancomycin (30µg), Norflaxacin (10µg), Ciprofloxacin (5µg). The overnight bacteria suspensions were used to inoculate the Mueller-Hinton agar (MHA) plate evenly using a sterile cotton swap. The plate was then incubated at 37±1°C for 24 hours. The diameter of inhibition zone was estimated and the perusing was recorded as sensitive (S) or resistance (R).

The Multiple Antibiotic Resistance **(**MAR) index for each identified bacterium from the bird defecation and indoor airborne samples of the birdhouses was determined. MAR indexing of a single bacteria isolate was counted by the formula stated as A/B, where A represented the number of the antibiotics isolate was resistant to and B represented the total antibiotics utilized and exposed in the test. The MAR value greater than 0.2 designated the bacterial isolate was multiple antibiotics resistant and demonstrated its sensitivity and resistance of these bacteria to clinically tested antibiotics.

Bacterial DNA Extraction

Bacterial DNA were extracted using boiling method with minor modification as described by Leong et al. (2013). A volume of 1.5ml bacteria culture grown for 24 hours in nutrient broth (Merck, Germany) was then transferred into a 2.0ml microcentrifuge tube and further centrifuged at 10,000rpm (8944 × *g*) for 5 minutes. 500µl of sterile refined water was added and vortexed in order to suspend the pellet. The microcentrifuge tube was then heated for 10 minutes and immediately moved into the ice for 5 minutes. The final product in the microcentrifuge tube was centrifuged at 10,000rpm (8944 × *g*) for another 10 minutes and the supernatant was stored.

PCR Reaction for Molecular Exposure of Virulence Genes (*ace*, *AS*, *efa*A, *gel*E) in *Enterococcus faecalis*

The PCR was implemented as stated in Duprè et al. (2003). The *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 were applied as positive and negative control, respectively for the detection of virulence genes. Table 1 listed down the primers and reaction conditions. The PCR reactions were conducted in the reaction mixtures containing 1.0 µl each of 20 pmol primers (First Base, Malaysia), 2.5µl of DNA, 0.5 µl of 10mM of deoxynucleoside triphosphate mix (Promega, USA), 5 µl of 5X Buffer solution (Promega, USA), 1.5 µl of 25 mM $MgCl_2$ (Promega, USA), 0.75 µl of Taq polymerase (Promega, USA) and 12.75 µl of sterile refined water. PCR was accomplished with 30 cycles as follows: initial denaturation at 94°C for 2 minutes, denaturation at 94°C for 1 minute, annealing for 1 minute at the temperature as shown in Table 1, primer extension at 72°C, 1 minute and final extension at 72°C, 10 minutes. The PCR products were visualized in 1% agarose gel electrophoresis with ethidium bromide staining for 35 minutes at 80 volt.

Table 1
PCR primers and reaction conditions

Primers name	Primer sequences (5'-3')	Size (bp)	Annealing Temperature (°C)
ACE1	AAAGTAGAATTAGATCCACAC	500	56
ACE2	TCTATCACATTCGGTTGCG		
AS1	CCAGTAATCAGTCCAGAAACAACC	300	54
AS2	TAGCTTTTTTCATTCTTGTGTTTGTT		
*efa*A1	CGTGAGAAAGAAATGGAGGA	1000	56
*efa*A2	CTACTAACACGTCACGAATG		
*gel*E1	AGTTCATGTCTATTTTCTTCAC	750	56
*gel*E2	CTTCATTCTTTACACGTTTG		

RESULTS AND DISCUSSION

Prevalence of *E. faecalis*

A total of 34 faecal and 3 airborne *Enterococcal* bacteria were isolated from the ten birdhouses throughout the Borneo and is shown in Table 2. There were more *Enterococcus* bacteria isolated from faeces than the indoor air inside the birdhouse mainly due to the differences of nutrient content available in the samples. The major components of the faeces are undigested carbohydrate, protein, fat and fibre remainder from the undigested dietary food supply (Rose et al., 2015) which support the bacteria growth. The total *Enterococci* bacterial isolates from the bird faeces were reviewed as *Enterococcus qallinacum* (51%), *Enterococcus faecalis* (35%) and *Enterococcus harae* (8%). The airborne *Enterococci* bacteria isolates of the birdhouses were reported as *Enterococcus faecalis* (5%) and *Enterococcus qallinacum* (1%).

The distribution of *Enterococcus* bacteria was normal among the wild birds because birds can transmit the bacteria by flight travel (Simpson, 2002). *E. qallinacum* can be isolated in most birdhouses except for birdhouses situated in Sibu, Maludam and Kota Samarahan districts. *E. faecalis* can be isolated in most birdhouses except Semarang, Sarikei and Kota Samarahan. Lastly, *E. harae* can only be isolated in birdhouses located in Maludam and Kota Samarahan. This may be due to the geographical location, acidity (pH), moisture content, hygienic condition inside the birdhouses and temperature effects during different sampling at different time of the year which may affect the bacterial growth. The present result was in agreement with Literak et al. (2007) and Nyakundi & Mwangi (2011) that *Enterococcus* spp. were isolated in faeces from the birds such as Marabou Stock (*Leptoptilos crumeniferus*) and rooks. Besides, previous researches had successfully isolated faecal *E. faecalis and E. qallinacum* from dogs at veterinary hospitals (Ghosh et al., 2012), poultry (Kwon et al., 2012), ducks and wild geese (Han et al., 2011). This was not surprising since *Enterococcus* spp. is naturally present in the gastrointestinal tract and able to survive in harsh environment containing bile (Saitoa et al., 2018). Thus, *Enterococcus* species are a common pathogen found in birds.

Table 2
The 16S rRNA sequencing homology search (BLAST) results of the defecation and airborne bacterial isolates from the birdhouses located in Borneo

Birdhouse location	Source	16S rRNA sequencing				
		Bacterial species	Size(s) bp	Number of isolates	Database	Homology (%)
Sepinang	Faeces	*Enterococcus qallinacum*	534	8	GenBank	99
	Faeces	*Enterococcus faecalis*	1445	2	GenBank	99
Semarang	Faeces	*Enterococcus qallinacum*	534	3	GenBank	99
Sarikei	Faeces	*Enterococcus qallinacum*	534	3	GenBank	99
Saratok	Faeces	*Enterococcus qallinacum*	534	2	GenBank	99
	Faeces	*Enterococcus faecalis*	1445	2	GenBank	99
Miri	Faeces	*Enterococcus qallinacum*	534	2	GenBank	99
	Faeces	*Enterococcus faecalis*	1445	1	GenBank	99
Kuching	Faeces	*Enterococcus qallinacum*	534	1	GenBank	99
	Faeces	*Enterococcus faecalis*	1445	3	GenBank	100
Sibu	Faeces	*Enterococcus faecalis*	1445	3	GenBank	99
Maludam	Faeces	*Enterococcus faecalis*	1445	2	GenBank	99
	Faeces	*Enterococcus harae*	1493	1	GenBank	99
Kota Samarahan	Faeces	*Enterococcus harae*	1493	2	GenBank	99
Total faecal bacteria				**34**		
Maludam	Air	*Enterococcus faecalis*	1445	1	GenBank	100
Kota Samarahan	Air	*Enterococcus faecalis*	1445	1	GenBank	100
Sepinang	Air	*Enterococcus qallinacum*	534	1	GenBank	99
Total airborne bacteria				**3**		

Multiple Antibiotic Resistance (MAR) Indexing

The disc diffusion method was then used to affirm the resistant phenotypes of these *Enterococci* bacteria. Strains which are resistant to more than two antibiotics were considered as multiple antibiotic resistance. The MAR indexing and resistance patterns elucidation is shown in Table 3 and illustrated in Figure 1. Table 3 shows the antibiotic resistance pattern and MAR index of the *E. faecalis* isolates. Result revealed that only one *E. faecalis* isolate was reported with MAR at 9 distinct resistance patterns formed. Four *E. faecalis* bacteria had developed resistance to more than two antibiotics and showed an elevated level with MAR index ranged from 0.2 to 0.55.

In this study, almost all of *E. faecalis* bacteria showed antibiotic resistance to at least one commonly applied clinical antibiotic (Table 3). MAR index > 0.2, indicated that they had emerged from high-hazard wellsprings of contamination where antibiotic agents were regularly utilized (Adeleke & Omafuvbe, 2011). This implies that a high nearness of antibiotics selective pressure, which concurs with the report by Suresh et al. (2000). Wang et al. (2017) indicated that *Enterococcus* spp. could exist as ordinary gut flora and they were more exposed to the antibiotics usage and became resistance more readily. The dispersal patterns of antibiotic resistance of *Enterococcus* spp. were done previously in livestock, companion animals (Park, 2013). *Enterococcus* pose the ability to transmit the antibiotic resistance genes to other host especially in gastrointestinal tracts of human or animals. The study by Chung et al. (2014) disclosed that *Enterococcus* were able to spread infection from livestock or companion animals to human.

The *E. faecalis* bacteria with MAR index ≥ 0.2 were isolated from the birdhouses located in Saratok, Miri and Kuching. Antibiotic resistance among the *E. faecalis* and the birdhouse location were significantly correlated ($P = 0.038$, $r = 0.468$, $n = 15$). *E. faecalis* showed a higher resistance to vancomycin, followed by tetracycline, Nitrofurantoin, Rifampin and Ciprofloxacin (Figure 1). *E. faecalis* has the ability to develop multi antibiotic resistance through variety of multiple mechanisms. *E. faecalis* showed the highest resistance to vancomycin. Vancomycin resistant *Enterococci* occurred since 1988, the rates of vancomycin resistance in *E. faecalis* had surpassed 2% (Hidron et al., 2008). Vancomycin is grouped under glycopeptide which adhere to the peptidoglycan precursor terminal d-alanine-d-alanine moiety, restraining the bacterial cross-linking of peptidoglycan chains and inhibiting cell wall composite. The vancomycin resistant *E. faecalis* may change the terminal penta-peptide to d-Ala-d-Lac on the peptidoglycan (Miller et al., 2014) and acquires vancomycin resistance genes (Saitoa et al., 2018). Similar result was obtained by previous researches and cases reviewed by Hayakawa et al. (2013). *E. faecalis* have served as contributors of vancomycin resistance gene groups to the surrounding pathogens. Besides, *E. faecalis* are resistant to doxycylin and tetracylin which are grouped under tetracycline antibiotic (Figure 1). Tetracyclines employ their antimicrobial by attaching to

ribosome and interrupting the docking of the aminoacyl-tRNA. Thus, this study showed that *E. faecalis* were able to efflux the antibiotic and protect their ribosome. This is in agreement with Choi & Woo (2015) who disclosed a high prevalence of tetracycline resistance genes in *E. faecalis* isolated from food products. *E. faecalis* also showed resistance to rifampicin mostly because emergence of transformation in the *rpoB* gene which encoded for the β-subunit of the *Enterococci* DNA-dependent RNA polymerase (Miller et al., 2014). The widespread of rifampicin resistant *Enterococcus* appeared to be over 65% in United States and Europe (Deshpande et al., 2007). These *E. faecalis* acquires various genes encoding antibiotic resistance through transformation or joined with a natural resistance from different antimicrobial determinants. Thus, *E. faecalis* was able to survive in an extreme environment (Arias & Murray, 2012).

Table 3
Antibiotic resistance pattern and MAR index of the E. faecalis from the birdhouses

Isolates	Source	Antibiotic resistance ¶	Pattern	MAR index
SWA-SAM-B6	Air	VA	5	0.09
SWF-OPP-1D4	Faeces	TeRDFPNorCIP	9	0.55
SWF-OPP-2A3	Faces	RD	4	0.09
SWF-OPP-2C10; SWF-OPP-2D4; SWF-OPP-2D6	Faces	CF	5	0.18
SWF-NEX-1E8	Faeces	nil	1	0
SWF-MAL-1B2; SWF-MAL-1C3; SWA-MAL-A3	Faeces, Air	VA	3	0.09
SWF-MIRI-2A6; SWF-MIRI-2A10	Faeces	TeRDEDoVACIP	8	0.55
SWF-KCH-2A1	Faeces	TeDoVACIP	7	0.21
SWF-SIBU-2C2	Faeces	NorVA	6	0.18
SWF-SPN-2A1	Faeces	Nor	2	0.05

¶ Tested against: Te: Tetracycline; Amp: Ampicillin; C: Chloramphenicol; E: Erythromycin; F: Nitrofurantoin; RD: Rifampin; Do: Doxycycline; P: Penicillin G; Nor: Norflaxacin; CIP: Ciprofloxacin; VA: Vancomycin; nil: none

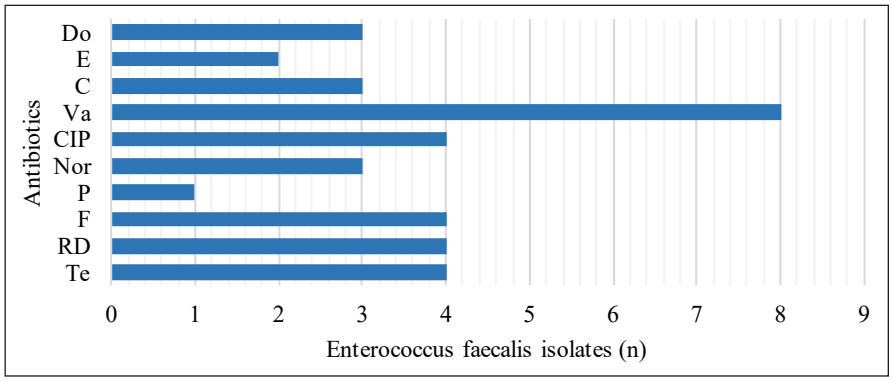

Figure 1. Antibiotic resistance of the *E. faecalis* from the birdhouses. Antibiotic tested: Do: Doxycycline; E: Erythromycin; C: Chloramphenicol; VA: Vancomycin; CIP: Ciprofloxacin; Nor: Norflaxacin; P: Penicillin G; F: Nitrofurantoin; RD: Rifampin; Te: Tetracycline.

Virulence Genes (*AS*, *ace*, *efa*A, *gel*E) Detection in *Enterococcus faecalis*

The virulence genes found in the 15 antibiotic resistant *E. faecalis* isolates were successfully detected by PCR using the specific primer. The dispersal of genotypic virulence agents among the *E. faecalis* is shown in Table 4. Agarose gel electrophoresis banding patterns of virulence genes (*ace*, *AS*, *efaA*, *gelE*) detection for *E. faecalis* is shown in Figure 2. 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 *E. faecalis* isolates from Kota Samarahan, Saratok, Miri and Maludam confirmed the presence of the above four virulence genes. The virulence gene (*AS*, *efaA, gelE*) and birdhouse location were significantly correlated (AS: P = 0.038, r = 0.468, n = 15; *efaA*: P = 0.011, r = 0.557, n = 15; *gelE*: P = 0.011, r = 0.557, n = 15). Besides, *E. faecalis* isolated from Betong only confirmed the presence of *ace* and *AS* genes. Lastly, *ace* gene was unable to discover from the *E. faecalis* isolated from Miri, Kuching, Sibu and Sepinang but *AS*, *efa*A and *gel*E genes were found present. Virulence genes detection were significantly correlated (*ace*: P = 0.039, r = 0.464, n = 15; AS: P = 0.001, r = 1.000, n = 15; *efaA*: P = 0.84, r = 0.001, n = 15; *gelE*: P = 0.84, r = 0.001, n = 15) with antibiotic resistance among the *E. faecalis*.

The high percentage of virulence genes detection implied that *E. faecalis* has a high risk in transmitting the pathogenic diseases through the birds' faecal contamination. *E. faecalis* was also reported as most common pathogens in nosocomial infection, causing fatal outcome in patients. Baldassarri et al. (2001) stated that cases of *E. faecalis* causing nosocomial infections was increasing especially in Italy thus causing threats to human health. The virulence genes which were expressed on the surface of the membrane namely *ace*, *AS*, *efa*A, *gel*E played a vital part in helping the *E. faecalis* adhere to the nosocomial epithelial cell lines and produced biofilms, facilitating diseases (Suβmuth et al., 2000). These virulence factors of *E. faecalis* also involved in the adherence process of bacteria to the host cell layers and to the surrounding surfaces in order to acquire nutrients and dodge the host resistant reaction (Medeiros et al., 2014).

All *E. faecalis* were detected positive with *AS* gene (Table 4). Similar result was obtained by Ferguson et al. (2016) who revealed that *Enterococcus* virulence genes were detected in *E. faecalis* isolated from sea shore water, dogs, birds, and even humans in Australia. The present results showed that more than 70% of the isolates were detected positive with *ace* gene (Table 4). Similar result was also obtained by Wei et al. (2017) where 39.7% of the *E. faecalis* isolated from mineral water and spring water in China were detected with *ace* gene. Besides, the gelatinase (*gel*E) detection was really crucial because they are also a zinc metalloprotease enzyme embedded in the chromosomal gene, functioning as casein, gelatin, other bioactive compounds hydrolysis process in hemoglobin (Waters et al.,

2003). The present results indicated that *gel*E virulence gene were detected among the *E. faecalis* bacteria isolated from the bird faecal samples. The result is in agreement with Al-Talib et al. (2015) who successfully detected 69.4% of *gel*E virulence factor in *E. faecalis* found clinically. Ahmed et al. (2012) proved that virulence genes *gel*E was the greatest gene observed in *E. faecalis* from various environmental surrounding. McBride et al. (2007) suggested that *gel*E was able to enhance the survivability of *Enterococcus* especially during extra intestinal surrounding. Harrington et al. (2004) explained that gelatinase producer *E. faecalis* could be isolated mostly in clinical samples. *The* endocarditis antigen (*efaA*) of *E. faecalis* was identified as one of the crucial harmfulness variables related for the most part with infective endocarditis. Similar result was obtained by Medeiros et al. (2014) who had isolated *E. faecalis* from clinical and food by-products which displayed *efa*A genes in Brazil. The research finding proved *E. faecalis* as culprit in therapy-resistant endodontic infections (Preethee et al., 2012). The occurrence of the virulent strains among these *E. faecalis* alone cannot confirm infection occurrence because there may be other mediators of pathogenicity (Kim & Marco, 2013). Pathogenicity of *Enterococcus* is mainly due to the capability of these virulent bacteria to overgrow in the infection sites which further spread all over the body (Fiore et al., 2019). Host factors display a vast role in the ability of *Enterococci* to establish infection (Arias & Murray, 2012). However, the existing of these genes in *E. faecalis* strains from bird warrants further investigations to survey potential human wellbeing dangers.

Table 4
Dispersal of genotypic virulence determinants agents among the Enterococcus faecalis

Isolates (n=15)	Sources	Detection by PCR†			
		ace	***AS***	***efa*A**	***gel*E**
SWF-SAM-B6	Air	+	+	+	+
SWF-OPP-1D4	Faeces	+	+	+	+
SWF-OPP-2A3	Faeces	+	+	+	+
SWF-OPP-2C10	Faeces	+	+	+	+
SWF-OPP-2D4	Faeces	+	+	+	+
SWF-OPP-2D6	Faeces	+	+	+	+
SWF-NEX-1E8	Faeces	+	+	-	-
SWF-MAL-1B2	Faeces	+	+	+	+
SWF-MAL-1C3	Faeces	+	+	+	+
SWF-MAL-A3	Air	+	+	+	+
SWF-MIRI-2A6	Faeces	+	+	+	+
SWF-MIRI-2A10	Faeces	-	+	+	+
SWF-KCH-2A1	Faeces	-	+	+	+
SWF-SIBU-2C2	Faeces	-	+	+	+
SWF-SPN-2A1	Faeces	-	+	+	+

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

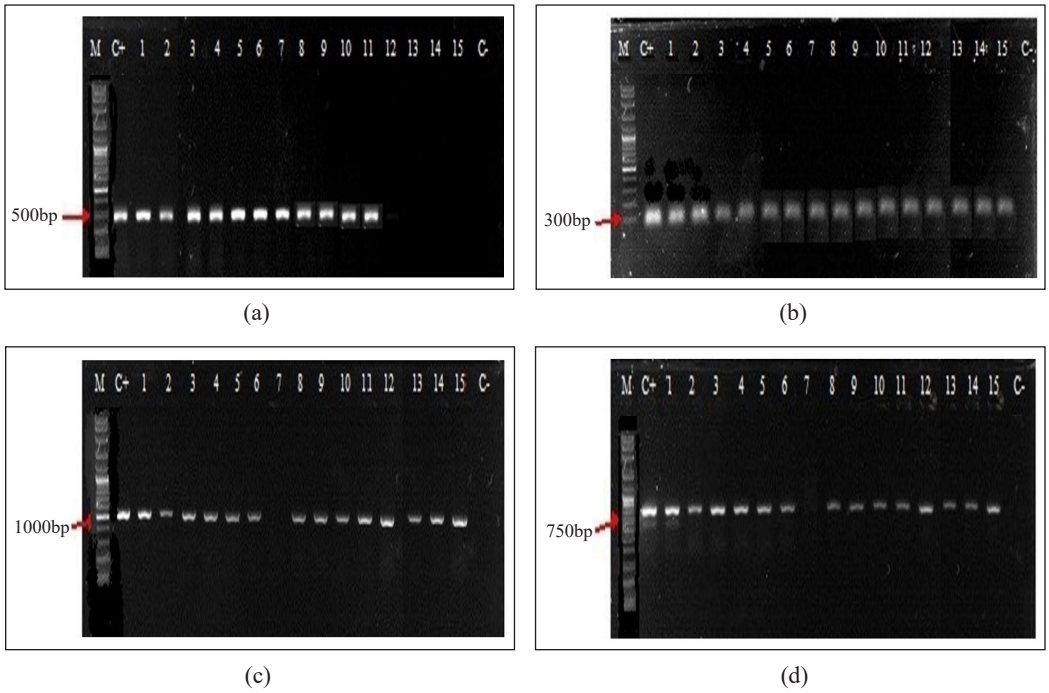

Figure 2. Agarose gel electrophoresis of *ace* (a)*, AS* (b)*, efa*A (c) and *gel*E (d) gene amplification products from *Enterococcus faecalis* isolates. M: 1Kb ladder; C+: Positive control; 1: SWF-SAM-B6; 2: SWF-OPP-1D4; 3: SWF-OPP-2A3; 4: SWF-OPP-2C10; 5: SWF-OPP-2D4; 6: SWF-OPP-2D6; 7: SWF-NEX-1E8; 8: SWF-MAL-1B2; 9: SWF-MAL-1C3; 10: SWF-MAL-A3; 11: SWF-MIRI-2A6; 12: SWF-MIRI-2A10; 13: SWF-KCH-2A1; 14: SWF-SIBU-2C2; 15: SWF-SPN-2A1; C-: Negative control.

CONCLUSIONS

Enteroccus bacteria showed a high prevalence in the faecal samples. The high incidence of virulence genes (*AS*, *ace*, *gel*E, *efa*A) indicates that these genes were widely disseminated among the antibiotic resistance *E. faecalis* found in the birdhouses, suggesting the important issues in the pathogenesis of *E. faecalis* infection which may cause potential health risks to humans.

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