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Biorisk Assessment of Antibiotic-Resistant Pathogenic Bacteria Isolated from Swiftlet Houses in Sarawak

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ABSTRACT

The occurrence of antibiotic resistance in pathogens is a growing concern globally. Development of multiple antibiotic-resistant bacteria has overwhelmed new medical advancement and threatens patients with untreatable infections. The qualitative risk assessment study was carried out to investigate the relative effects of the main determinants of antibiotic-resistant pathogenic bacteria and to estimate the risk of the emergence and spread of antibiotic resistance among humans in the swiftlet's faeces and its indoor air to human health. The methodology applied focused mainly on three main sections namely the hazard identification, exposure assessment, and hazard assessment. Sources of data for bio risk assessment include published literature, data from on-going research projects and data collected from the industry. The results showed that the prevalence of isolating Gram-positive bacteria were higher in swiftlet houses. Over half of the pathogenic bacterial isolates were multidrug-resistant to a wide range of commonly used antibiotics such as *Bacillus, Enterococcus*, *Escherichia coli*, *Staphylococcus*, *Lysinibacillus*, *Paenibacillus and Sporosarcina*. 80% of the bacteria isolates showed high MAR index of over 0.2. These emerging pathogenic

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antibiotic resistant bacteria are capable of causing life-threatening infections which pose a health hazard to our biodiversity.

Keywords: Airborne, antibiotic resistant, bacteria, faeces, pathogenic, risk assessment

INTRODUCTION

The increase of human population and uncontrolled development of industrial and agricultural expansion in developing country has led to the occurrence of public health problems such as multi-antibiotic resistant pathogenic bacteria which pose serious health hazards to the human society (Bartoloni et al., 2004). Risk assessment is commonly used to assess the risk in the environment, engineering industry, military industry, food safety and public health services (Lacey, 2011). Risk assessment is defined as the identification of the risks or hazards in certain environment qualitatively and quantitatively. Risk assessment focuses mainly on the quantification on the probability of harmful effect towards individuals or populations from certain human activities (Lerche & Walter, 2006).

Swiftlet industries have sprouted spontaneously across the whole Peninsular Malaysia within a few years due to multi-million dollar potential profit. The waste from the birds especially the faeces is usually used as organic fertilizer in the agricultural industry because of its rich nutrient, water, nitrogen, phosphorus, potassium and minerals content. Most bacteria are able to grow in the bird faeces and the air inside the bird-house. The emergence of antibiotic-resistant bacteria may contaminate vegetables through this bird waste fertilizer. Improper washing of these contaminated vegetables may also be cross-contaminating into our foods in the kitchen, causing harmful diseases to human. Intensive and extensive abuse of antibiotics has caused the formation of an antibiotic-resistant genes pool in the environment. There is a growing concern regarding public health hazards and implications due to the presence of antibiotic residues in the environment and their effects on the development of pathogenic bacterial resistance from these bacteria in the swiftlet faeces. Serious action needs to be taken because there are cases of antibiotic-resistant pathogenic bacteria detected in drinking water (Xi et al., 2009), vegetables, fish (Kabir et al., 2014; Schmidt et al., 2001), healthy adults, food animals, foods and the food industry (Boonyasiri et al., 2014).

Risk assessment is normally focused on all microorganisms isolated from an environment and the results can provide a framework for risk management to minimise the health hazards to human (Michael, 2008). Several pieces of research have demonstrated that cases of increased bacteria resistance to biocide are rising sharply (Langsrud et al., 2003; Walsh et al., 2003). According to the literature, so far no study has been carried out to assess the risk of potential health hazards to human and environment caused by the pathogenic bacteria from the swiftlet houses. The

emergence of the pathogenic antibiotic-resistant bacteria in both humans and food animals is a growing concern to the public health. Therefore, the present study was carried out to investigate the emergence of resistance in any antibiotic-resistant pathogenic bacteria and the subsequent risk to human health relating to specific bacterial isolates from the swiftlets' faeces and indoor air of the swiftlet houses.

MATERIALS AND METHODS

Sampling Sites

Swiftlet houses selected for the present study were located in Kuching (01°32'56.6''N 110°22'27.5''E), Kota Samarahan (01°27'34.2''N 110°27'25.9''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), Betong (01°24'0''N 111°31'0''E), Saratok (01°44'10.32''N 111°21'10.22''E), Sarikei (02°6'3.75''N 111°30'39''E), Sibu (02°19'11.3''N 111°49'50.5''E) and Miri (04°23'39.2''N 113°59'12.2''E).

Sources of Bacterial Isolates

Faecal and Airborne Bacteria Collection. A Total of 1200 faecal and airborne bacteria isolates was selected from a collection of strains obtained from previous studies (Leong et al., 2013a; Leong et al., 2013b). Five faeces samples were collected randomly from the floor of each swiftlet house of the sampling site. The faeces sample was then plated on nutrient agar (Merck, Germany) plates in duplicate and incubated at 37± 1° C for 24 hours. The collection of the indoor airborne samples procedures was carried out according to Department of Veterinary Services (DVS) (2017). 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 seconds inside the swiftlet house. The plates were incubated at 37± 1°C for 24 hours in the laboratory. The bacteria isolates were further identified by using biochemical tests. Species identification was confirmed using 16S rRNA sequencing.

Biorisk Assessment

The biorisk assessment was performed according to the European Agency for Safety and Health at Work (EU-OSHA) (2000), European Parliament and Council directive (EP) (2000), Health Protection Agency (HPA) (2007), Jeenaa et al. (2006) and Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR) (2009) with modification. The methodology of biorisk assessment applied in this study focused mainly on the three main sections namely the hazard identification, exposure assessment, and hazard assessment. Sources of data for biorisk assessment of pathogenic bacterial resistance problem include published literature, data from on-going research projects and data collected by the industry.

Hazard Identification and Exposure Assessment

In relation to the antibiotic-resistant pathogenic bacteria, the microbiological

hazard may be a pathogen with resistance to a particular antimicrobial. Based on the results reported by Leong (2015), the prevalence of each pathogenic antibiotic-resistant bacteria species was determined. Collecting the results from past researches provides an insight into the understanding of the bacterial diversity and how frequently these bacteria are detected in the faecal and airborne samples of the swiftlet houses.

Human Health Risk Assessments

Predisposition of Bacterial Species to Acquire Resistance and Pathogenicity. Bacterial species are categorised according to the assessment procedures described in HPA (2007) and SCENIHR (2009). All the available scientific information and literature evidence of the bacterial collection were assessed according to the following potential risk:

High: *Enterococcus* and *Enterobacteriaceae* bacteria poses high occurrence frequency and specialised mechanisms in horizontal gene transfer. Thus, the probability of pathogenic or resistance gene exchange between related or unrelated species is high among these bacteria genus.

Medium: *Lactococcus* bacteria are susceptible to the intra-generic gene transfer mechanisms. *Lactococcus* poses high-frequency in conjugal transfer of plasmid-encoded pathogenicity and resistance gene. Thus, *Lactococcus* carrying such inherited high-frequency conjugation systems may pose a medium risk to human health.

Low: *Bacillus* bacteria are endospore-forming bacteria which may not have any clear mechanism of conjugation. Horizontal spread of the virulence plasmids is not unusual, thus *Bacillus* still pose a low risk to human health.

Antibiotic Susceptibility Testing and Multiple Antibiotic Resistance (MAR) Indexing of The Isolates. The antibiotic susceptibility testing and MAR indexing were performed according to Leong et al. (2013b). The faecal and airborne bacterial isolates were tested using the disc diffusion method against 23 commonly used antibiotics. The antibiotics impregnated discs and the recommended concentrations for use in this testing were as follows: chloramphenicol (30µg), ampicillin (10µg), tetracycline (30µg), streptomycin (10µg), gentamycin (10µg), erythromycin (15µg), cephalothin (30µg), nitrofurantoin (300µg), tobramycin (10µg), rifampin (5µg), kanamycin (30µg), sulphamethoxazole/ trimethoprim (1.25/ 23.75µg), amikacin (30µg), imipenem (10µg), ceftriaxone (30µg), penicillin G (10U), doxycycline (30µg), ceftazidime (30µg), norflaxacin (10µg), vancomycin (30µg), pipercillin (100µg), ciprofloxacin (5µg) and nalidixic acid (30µg). The MAR index for each bacterium isolated was determined using the method described by Tambekar et al. (2008). A value greater than 0.2 indicated that the culture was MAR and the number of the MAR index for an antibiotic indicated its sensitivity and resistance of certain bacteria to antibiotics. The use of MAR index is to assess the potential health risk.

Pathogenic Gene Detection. Multiplex-PCR was applied in the molecular detection of various pathogenic genes in *E. coli*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Bacillus cereus* to assess their hazardous risk to human health. The *Escherichia coli* O157:H7 (EDL933 strain), *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus cereus* ATCC 11778 and *Escherichia coli* ATCC 25922 were used as positive control respectively for the detection of virulence genes. Specific primer sequences were synthesized in order to identify common pathogenic bacteria. The PCR was performed in the detection of Shiga toxin (*stx* gene) in *E. coli*, *opr*L gene in *P. aeruginosa*, virulence genes (*ace*, *AS*, *efaA*, *gelE*) in *E. faecalis* and enterotoxigenic genes (*hbla*, *entFM*) in *B. cereus*.

Exposure Hazards. The processes that lead to the spread of these pathogenic antibiotics resistant bacteria are described. These bacteria may release, survive and even exposed to the environment, human, animal, plant or other microorganisms, leading to a more serious problem in the future. The exposure assessment identified the reservoirs from which this antibiotic resistance pathogen can emerge. Evaluation of how, and how much, a person, or a population is exposed to the hazard(s) was assessed.

RESULTS

Hazard Identification

The bacteria isolates were confirmed using 16S rRNA sequencing. The agarose gel electrophoresis banding pattern was shown in Figure 1.

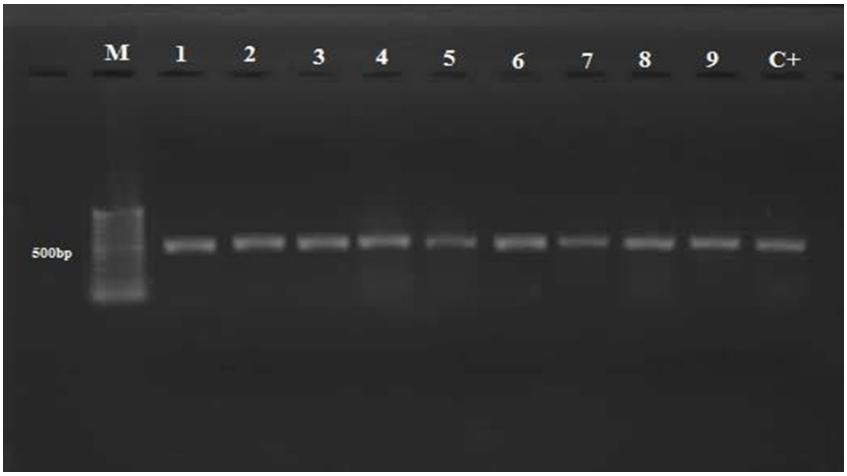

Figure 1. Agarose gel electrophoresis of 16S rRNA sequencing gene amplification products for bacteria isolates. M: 100bp ladder; 1-9: Bacteria isolates; C+: Control

Antibiotic Resistance and MAR Pattern

The results showed pathogenic bacteria isolates such as *Bacillus* spp., *Enterococcus* spp., *E. coli*, *Staphylococcus* spp., *Lysinibacillus* spp., *Paenibacillus* spp. and *Sporosarcina* spp. Had developed antibiotic resistance to a wide range of commonly used antibiotics (Tables 1 and 2). Gram-positive bacteria were the most highly prevalent bacteria found in all the swiftlet houses. 96% and 80% of the Gram-positive faecal and airborne bacteria respectively showed resistance to more than 2 antibiotics tested. *Escherichia coli* were resistant up to 10 types of antibiotics tested with MAR index over 0.47. *Bacillus pumilus* was resistance to all antibiotics tested. MAR indexing of the isolates and elucidation of the resistance patterns revealed that the faecal and indoor airborne bacteria isolates showed high-level MAR index ranged from 0.05 to 1.0.

Pathogenicity

The pathogenicity of the bacteria isolated was discussed in Table 1 and Table 2. Most of the bacteria showed different levels of pathogenicity to human, plants, insects or even animals.

Exposure Assessment

The processes that lead to the acquisition, selection, and spread of the resistant pathogenic bacteria and genes in humans are shown in Table 3. The exposure assessment was assessed for the four main reservoirs (human, plants together with insects, animal and environment) from which antibiotic resistance pathogen could emerge. If these antibiotic-resistant bacteria are exposed, they may cause medium to high risk to human and ecosystem.

DISCUSSION

Bacterial isolates resistant to more than two antibiotics were considered as having multiple antibiotic resistance, accounted for approximately over 80% of the total isolates encountered in the present investigation. Most of these isolates had resistance indices ranging from 0.05 to 1.0. The occurrence of MAR bacteria in the environment is certainly a well-known phenomenon. Many investigators believe that these drug-resistant organisms have become more common recently due to the extensive use of antibiotics in medicine and agriculture throughout the world (Barbosa & Levy, 2000). Apart from variations among the different genera, wide variations in the MAR index and resistance patterns were also noticed within the different strains of the same genera, indicating the diversity of the strains. While maximum variation in the MAR index and resistance patterns were observed among the different strains of *Bacillus* and followed by *Staphylococcus*. This is indicative of a common source of *Staphylococcus* contamination. Studies by Becker et al. (2014) revealed that *Staphylococcus* represented contaminating bacteria.

The results obtained in this study revealed bacteria with multiple antibiotic resistance patterns suggesting the possible horizontal gene transfer among non-related

Table 1

Health risk assessment of the potential antibiotic resistant pathogenic faecal bacteria isolated from the swiftlet houses in Sarawak

Hazard identification (organism)	Health risk assessment			
	Main cause of the event			Risk of acquire resistance and pathogenicity event arising from main cause
	Antibiotic resistance	MAR index	Pathogenicity	
Bacillus amyloliquefaciens	Resistance to 1-2 types of antibiotic tested.	0.05-0.11	Previously reported as non-pathogenic (Priest et al., 1987)	Low
Bacillus botaviensis	Resistance to 6 types of antibiotic tested.	0.32*	Pathogenicity reported as unknown	Low
Bacillus cereus	Resistance to 8-10 types of antibiotic tested.	0.32*-0.47*	Enterotoxigenic genes (*hbla*, *entFM*) detected in most of the faecal and airborne isolates [+] Previously reported as volatile human pathogen (Bottone, 2010)	Low
Bacillus licheniformis	Resistance to 3 types of antibiotic tested.	0.16	Previously reported as food poisoning agent (Turnbull, 1996)	Low
Bacillus pumilus	Resistance to all types of antibiotic tested.	1.00*	Previously reported as pathogen to mango plant (Galal et al., 2006) and human (From et al., 2007)	Low
Bacillus subtilis	Resistance to 1-3 types of antibiotic tested.	0.05-0.16	Previously reported as food poisoning agent (Hoa et al., 2000)	Low
Bacillus thuringiensis	Resistance to 8 types of antibiotic tested.	0.37*	Insect pathogenic gene (*Bt*) detected (Argôlo-Filho & Loguercio, 2013)	Low
Bacillus spp.	Resistance to 1-8 types of antibiotic tested.	0.05-0.42*	Most of Bacillus species were harmless saprophytes (Hoa et al., 2000)	Low
Bacterium culalno	Resistance to 6 types of antibiotic tested.	0.32*	Pathogenicity reported as unknown	Low

Table 1 *(Continue)*

Hazard identification (organism)	Health risk assessment			
	Main cause of the event			Risk of acquire resistance and pathogenicity event arising from main cause
	Antibiotic resistance	MAR index	Pathogenicity	
Corynebacterium spp.	Resistance to 3 types of antibiotic tested.	0.16	Previously reported as non-pathogenic (Burkovski, 2008)	Medium
Enterococcus faecalis	Resistance to 1-6 types of antibiotic tested.	0.09-0.55*	Virulence gens (*ace*, *AS*, *efaA*, *gelE*) was detected +	High
Enterococcus harae	Resistance to 4-8 types of antibiotic tested.	0.09-0.36*	Depressed the young chickens growth and caused diseases in human in previous report (Poyart et al., 2002)	High
Enterococcus gallinarum	Resistance to 1-6 types of antibiotic tested.	0.09-0.55*	Previously reported as non-pathogenic (Gilmore et al., 2002)	High
Escherichia coli	Resistance to 1-10 types of antibiotic tested.	0.05-0.47*	No Shiga toxin (stx1 and stx2) was detected +	High
Klebsiella pneumonia	Resistance to 3 types of antibiotic tested.	0.16	Virulence gene (Gyr-B-2) detected in patients (Foysal et al., 2013)	High
Leucobacter iarius	Resistance to 5 types of antibiotic tested.	0.26*	Previously associated with pathogenic nematodes (Somvanshi et al., 2007)	Low
Lysinibacillus spp.	Resistance to 1-10 types of antibiotic tested.	0.05-0.53*	Previously reported as non-pathogenic	Low
Paenibacillus spp.	Resistance to 2-8 types of antibiotic tested.	0.16-0.42*	Pathogenicity reported as unknown (Ahmed et al., 2007)	Low
Pseudomonas aeruginosa	Resistance to 1 type of antibiotic tested.	0.11	Virulence gene (*oprL*) detected +	High
Sporosarcina aquimarina	Resistance to 2-7 types of antibiotic tested.	0.11-0.37*	Previously reported as non-pathogenic (Janarthine et al., 2012)	Low

Table 1 *(Continue)*

Hazard identification (organism)	Health risk assessment			
	Main cause of the event			Risk of acquire resistance and pathogenicity event arising from main cause
	Antibiotic resistance	MAR index	Pathogenicity	
Sporosarcina spp.	Resistance to 5 types of antibiotic tested.	0.42*	Previously report as non-pathogenic (Janarthine et al., 2012)	Low
Staphylococcus epidermitis	Resistance to 2 types of antibiotic tested.	0.11	Previously reported as non-pathogenic (Fey & Olson, 2010)	Medium
Staphylococcus kloosii	Resistance to 1-5 types of antibiotic tested.	0.05-0.26*	Previously reported as pathogen in human (Peer et al., 2011)	Medium
Staphylococcus lentus	Resistance to 1-5 type of antibiotic tested.	0.05-0.26*	Previously reported as non-pathogenic (Karachalios et al., 2006)	Medium
Staphylococcus nepalensis	Resistance to 2-8 types of antibiotic tested.	0.11-0.37*	Pathogenicity reported as unknown (Nováková et al., 2006)	Medium
Staphylococcus pulvereri	Resistance to 2 types of antibiotic tested.	0.11	Previously reported as pathogen to human and chicken (Zakrzewska-Czerwińska et al., 1995)	Medium
Staphylococcus sciuri	Resistance to 2 types of antibiotic tested.	0.11	Previously reported as highly pathogenic bacteria to the piglets (Chen et al., 2007)	Medium
Staphylococcus spp.	Resistance to 1-5 types of antibiotic tested.	0.05-0.26*	Previously reported as non-pathogenic (Madigan & Martinko, 2005)	Medium

Tested against: C: Chloramphenicol, Amp: Ampicillin, Te: Tetracycline, S: Streptomycin, CN: Gentamycin, E: Erythromycin, KF: Cephalothin, F: Nitrofurantoin, TOB: Tobramycin, RD: Rifampin, K: Kanamycin, SXT: Sulphamethoxazole/ Trimethoprim, AK: Amikacin, IPM: Imipenem, Cro: Ceftriaxone, P: Penicillin G, Do: Doxycycline, Caz: Ceftazidime, Nor: Norflaxacin, VA: Vancomycin, PRL: Pipercillin, CIP: Ciprofloxacin, NA: Nalidixic acid.

* MAR index ≥ 0.2 indicating a high risk of bacterial contamination and pose health hazard to human.
+ Pathogenicity indicated through multiplex-PCR in this study.

Table 2

Health risk assessment of the potential antibiotic resistant pathogenic airborne bacteria isolated from the swiftlet houses in Sarawak

Hazard identification (organism)	Health risk assessment			
	Main cause of the event			Risk of acquire resistance and pathogenicity event arising from main cause
	Antibiotic resistance	MAR index	Pathogenicity	
Bacillus cereus	Resistance to 9 types of antibiotic tested.	0.47*	Enterotoxigenic genes (*hbla*, *entFM*) detected in most of the isolates [+] Previously reported as volatile human pathogen (Bottone, 2010)	Low
Bacillus subtilis	Resistance to 1-3 types of antibiotic tested.	0.05-0.15	Previously reported as food poisoning agent (Turnbull, 1996)	Low
Bacillus spp.	Resistance to 2-7 types of antibiotic tested.	0.11-0.37*	Most of *Bacillus* species were harmless saprophytes (Hoa et al., 2000)	Low
Dermacoccus spp.	Resistance to 2 types of antibiotic tested.	0.11	Pathogenicity reported as unknown (David et al., 2012)	Medium
Enterococcus faecalis	Resistance to 1 type of antibiotic tested.	0.09	Virulence gens (*ace*, *AS*, *efaA*, *gelE*) detected [+]	High
Lysinibacillus fusiformis	Resistance to 5 types of antibiotic tested.	0.26*	Previously reported as unknown (Ahmed et al., 2007)	Low
Lysinibacillus spp.	Resistance to 2-4 types of antibiotic tested.	0.11-0.21*	Previously reported as non-pathogenic	Low
Microbacterium esteraromaticum	Resistance to 2 types of antibiotic tested.	0.11	Pathogenicity reported as unknown (Gneiding et al., 2008)	High
Paenibacillus taiwanensis	Resistance to 1 type of antibiotic tested.	0.05	Previously reported as unknown (Ahmed et al., 2007)	Low
Shingobacterium spp.	Resistance to 4 types of antibiotic tested.	0.21*	Pathogenicity reported as unknown	High

Table 2 *(Continue)*

Hazard identification (organism)	Health risk assessment			
	Main cause of the event			Risk of acquire resistance and pathogenicity event arising from main cause
	Antibiotic resistance	MAR index	Pathogenicity	
Sporosarcina aquimarina	Resistance to 2-19 types of antibiotic tested.	0.11-1.00*	Previously reported as non-pathogenic (Janarthine et al., 2012)	Low
Sporosarcina spp.	Resistance to 1 type of antibiotic tested.	0.05	Previously report as non-pathogenic. (Janarthine et al., 2012)	Low
Staphylococcus spp.	Resistance to 3 types of antibiotic tested.	0.16	Previously reported as non-pathogenic (Madigan & Martinko, 2005)	Medium
Staphylococcus kloosii	Resistance to 3 types of antibiotic tested.	0.16	Previously reported as pathogen in human (Peer et al., 2011)	Medium
Staphylococcus pulvereri	Resistance to 2 types of antibiotic tested.	0.11	Previously reported as pathogen to human and chicken (Zakrzewska-Czerwińska et al., 1995)	Medium

bacterial isolates. MAR index values higher than 0.2 are inferring that they have originated from high-risk sources where antibiotics are often used. MAR index values of less than or equal to 0.2 indicates a strain originated from sources where antibiotics are seldom or never used (Adeleke & Omafuvbe, 2011). This is an indication of a high presence of antibiotics selective pressure, which agrees with the report of Suresh et al. (2000). The bacteria were highly prevalent in developing a high MAR index mainly because of their tendency in accumulating multiple resistances under selection and antibiotic pressure (Berger-Bachi, 2002). This may imply an increase in antibiotic resistance with a higher risk of faecal and airborne bacterial contamination and potentially may pose threat to human health as antibiotic resistance decreases our ability in treating infections and diseases. The MAR index method is a good indicator in the differentiation of bacteria sources by applying antibiotics that are commonly used for human treatments (Osundiya et al., 2013). Tula and Iyoha (2014) discovered that antibiotic resistance increased with the increase of the MAR value. The bacterial isolates from swiftlet houses with the MAR index value of more than 0.2 were mainly from faecal origin. The multiple antibiotic resistance index of the bacterial isolates suggest that they have arisen from sources exposed to high level of antibiotics selective pressure resulting from non-specific, misuse or abuse of antibiotics. Kaneene et al. (2007) had reported that most of the *E. coli* with MAR index over 0.4 were isolated from faecal contaminated water surface. The increasing rate of MAR cases in various sites is spreading fast and poses threat to human health. The drug-resistant bacteria could also act as a reservoir of resistance plasmids which could be freely exchanged with possible pathogens in the intestine.

Indicator organisms, such as commensal *E. coli* and *Enterococcus faecium*, are of interest since they readily develop resistance to antimicrobials (Fair & Tor, 2014) and furthermore, they have the potential to disseminate their resistance genes to other bacteria, including pathogenic bacteria (Beceiro et al., 2013). Indicator bacteria could be transmitted to humans via the food chain in the same way as zoonotic food-borne bacteria, particularly since these bacteria will be present, potentially in high numbers, in most animal species. Hospital patients are especially at risk since *Enterococci* commonly causes hospital-acquired infections (Rice, 2001). The emergence of vancomycin-resistant *E. faecium* (VREf) is of concern since vancomycin is often used in hospitals to treat serious Gram positive bacterial infections (Garcia-Migura et al., 2005), and is chemically related to the growth promoter avoparcin, which was used in Europe since the 1970s until its ban (Acar et al., 2000).

In this study, most of the bacterial isolates showed a high level of antibiotics resistance to the antibiotics tested. This result is in agreement with Muhammad et al. (2010) who reported that the abuse and misuse of antimicrobial agents for growth promotion and prevention of diseases had impressed a

selective pressure that caused the discovery of more resistant bacteria. This is probably true with bacteria associated with faeces collected from swiftlet houses located in urban areas in this study. Hence, the antibiotic selection pressure for resistance by bacteria in birds is high and as a result, their faecal flora contains a high proportion of resistant bacteria. The results of this study on swiftlet indicted the possible biorisk in urban areas. Salehi and Bonab (2006) reported that the resistance of bacteria to the existing antimicrobial agents was widespread and of a great concern to poultry veterinarians. The use of antimicrobial in animal feed can also lead to the selection of antimicrobial resistant zoonotic enteric pathogens, which could be transferred to human through the consumption of contaminated food, or by direct animal contact.

Besides, *Enterococcus* spp. expressed with a high MAR index ≥ 0.2. The pathogenic antibiotic resistant *E. faecalis* isolated in a high frequency of occurrence or prevalence in the swiftlet houses may cause a health hazard to human because *E. faecalis* had been reported as a common pathogen causing infection in nosocomial (Olawale et al., 2011), surgical wounds, blood, urinary tract (Duprè et al., 2003). Although the occurrence of *Enterococcus gallinarum* was low in this study and reported as non-pathogenic by Gilmore et al. (2002), however, with a high MAR index indicating that *E. gallinarum* may become harmful to human. *Enterococcus* spp. has high gene transfer frequency and the gene may be exchanged between related or unrelated species, thus *Enterococcus* spp. may become hazardous to the environment and human health in the future if no prevention was taken.

According to Peer et al. (2011), *Staphylococcus kloosii* has developed linezolid resistance and caused intracranial bleed and sepsis in patients in India. Linezolid was a potent antimicrobial especially against Gram-positive microorganism such as methicillin-resistant coagulase-negative *Staphylococcus*, resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococci* and multidrug-resistant *Streptococcus pneumonia* (Diekema & Jones, 2001). Thus, *S. kloosii* may have the potency to pose a serious threat to humans in the future. *S. pulvereri* was normally isolated from human and diseased chicken carcass, thus it may act as a carrier for spreading the diseases (Zakrzewska-Czerwińska et al., 1995). Furthermore, According to Morita et al. (2014), *P. aeruginosa* has the ability to develop resistance to multiple antimicrobial agents and was able to mutate against antimicrobial agents thus it is considered as a high-risk pathogen. Hancock and Speert (2000) discovered that *P. aeruginosa* had higher intrinsic resistance than other bacteria to most recent antibiotics and decreased the clinical effectiveness. The health hazard existed for several human pathogens and the risk concerns not only the soil bacteria but also the bacteria that colonized various farm animals (*Enterococcus*, *Bacillus*) which are in contact with environmental bacteria (*Pseudomonas*). Consequently, the risk

may spread to food-borne pathogens which are frequently detected in animals and the dissemination of resistance genes may affect *Escherichia* and other bacterial species.

Exposure assessment predicts the outcomes if these antibiotic pathogenic bacteria are exposed (Table 3). The assessment identified various high-risk pathogen infections and high rate of antibiotic resistance transmission. This model believes that the overall risk of the occurrence and spread of the antibiotic resistance pathogen among humans is the highest. The risk of transmission from animal or even environmental reservoirs to humans is high. Infection may be mediated by the high risks of foodborne and waterborne transmission diseases caused by *Bacillus* spp., *Staphylococcus* sp., *Klebsiella* sp., *Enterococcus* sp., *Proteus* sp. and *Pseudomonas* sp. (Table 3). The assessment highlights the hazards, health risks and exposure of bacteria isolated in order to reduce the risk of the emergence and spread of antibiotic resistance. There are limitations in the assessment model because it only pays attention to pathogens of international concern. Besides, the exposure risks of each event stated in Table 3 cannot be generalised to pathogens not included here. This qualitative risk assessment provides early stages of development based on the main microbiological hazard determinant. A more systematic risk model needs to be constructed in the future which be possible to get accurate estimates of transmission risk and to quantitatively measure the effectiveness of interventions on defined outcomes.

Table 3

Exposure hazards assessment of these potential antibiotic resistant pathogen

Event	Exposure hazards on the likelihood of the event happening
Emergence in the human	Harmful to the human, immunodeficiency patients *Bacillus* sp. in the swiftlet faeces may cause food poisoning. *Staphylococcus* sp., *Bacillus* sp., *Klesiella* sp. and *Enterococcus* sp. isolates may cause diseases especially in human. *Proteus* sp. and *Pseudomonas* sp. may cause serious diseases in immunodeficiency patient. Over half of the bacterial isolates developed antibiotic resistance which may be a serious problem nowadays. High potential risk of health hazard to human.
Emergence in the plants and insects	Harmful mainly to the human and plants. Cause diseases among the insects. Some of the *Bacillus* sp. may affect the plant growth. Over half of the bacteria isolates developed antibiotic resistance which may be a serious problem nowadays. Medium potential risk to plants and insects.

Table 3 *(Continue)*

Event	Exposure hazards on the likelihood of the event happening
Emergence in the animals	Harmful to the animals. Lower the poultry production. *Enterococcus* sp. may slow down the poultry growth in farm. Over half of the bacteria isolates developed antibiotic resistance which may cause by illegal use of antibiotics as growth promoter. Food transmission to human may occur if contaminated meat ingested. High potential risk of health hazard to human.
Emergence in the environment	Harmful to the environment if these antibiotic resistance pathogenic gene transfer among the environmental bacteria. Contact with contaminated environment may cause potential health risks to the ecosystem. Medium potential risk to ecosystem.

CONCLUSION

Biorisk assessment of this study revealed that there is a possibility that the pathogenic bacteria may pose public health hazards in the future due to the presence of antibiotic residues in the environment and there is a probability that most pathogenic bacteria that threaten human health may soon be resistant to all known antibiotics. Interaction with human and agricultural waste materials may spread pathogenic bacteria including antibiotic resistant isolates to wildlife, potentially creating an additional environmental reservoir of antibiotic-resistant organisms.

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