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LOGISTIC REGRESSION MODEL FOR PREDICTING MICROBIAL GROWTH AND ANTIBIOTIC RESISTANCE OCCURRENCE IN SWIFTLET (*Aerodramus Fuciphagus*) FAECES

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Abstract: This study proposes a logistic model of the environmental factors which may affect bacterial growth and antibiotic resistance in the swiftlet industry. The highest total mean faecal bacterial (FB) colonies counts (11.86±3.11 $\log_{10}$ cfu/ g) were collected from Kota Samarahan in Sarawak, Malaysia, and the lowest (6.71±1.09 $\log_{10}$ cfu/g) from Sibu in both rainy and dry season from March 2016 till September 2017. FB isolates were highly resistant against penicillin G (42.20±18.35%). *Enterobacter* and *Enterococcal* bacteria were resistant to streptomycin (40.00±51.64%) and vancomycin (77.50±41.58%). The model indicated that the bacteria could grow well under conditions of higher faecal acidity (pH 8.27), dry season, higher mean daily temperature (33.83°C) and faecal moisture content (41.24%) of swiftlet houses built in an urban area with significant regression ($P<0.0005$, $N=100$). The probability of the development of antibiotic resistance (%) increased 0.50 times if the faecal acidity increased by one unit with significant contribution to the prediction ($P = 0.012$). Understanding how these microbial species react to environmental parameters according to this model, allowed us to estimate their interaction outcomes and growth, especially in an urban environment, which may pose a health hazard to people.

Keywords: Antibiotic resistance, bird, faeces, microbial growth, model.

Introduction

The swiftlet nest industry in Sarawak, Malaysia, has been growing tremendously over the past few years. A swiftlet house contains a large number of birds, resulting in a high density of nests and faeces. Many bacteria are able to grow in swiftlets' faeces (Nyakundi & Mwangi, 2011). Swiftlet houses built in an urban area cause noise and faeces pollution, with birds flying around, especially near food stalls. The concentration of bacteria in a swiftlet house, especially in the faeces, may contaminate the environment, causing harmful diseases to humans and also to the swiftlet bird itself.

Microbial growth, especially bacteria, is influenced by physical or chemical conditions of the environment. Some bacteria are unable to grow if environmental conditions change but some bacteria can tolerate diverse conditions. Bacterial multiplication depends strongly on the suitability of environmental factors and the availability of metabolic energy sources (Egli, 2015). Environmental conditions strongly affect microorganism viability, differentiation, growth and reproduction. There are intrinsic and extrinsic factors that influence microbial growth (Wolf-Hall & Nganje, 2017). Intrinsic factors refer to the characteristics of the media, such as moisture content, acidity and nutrient content, while the extrinsic factors the environment surrounding the media, such as temperature and time. Each bacterial species has a particular environmental range in which they grow optimally and cause infection (Porter, 2013). The close relationship between these factors provide us with a better understanding of how the environment can affect bacterial growth.

Water is vital and plays an important role in microbial multiplication. Bacteria cannot grow without water, except spore-forming bacteria which can survive for years without water (Stevenson *et al.*, 2015). The moisture

content is useful to determine the type of microorganism growing in a media and the rate of growth (Borowik & Wyszkowska, 2016). Research has shown that the viability of most indoor microorganism communities are affected strongly by the dynamic interactions of the surrounding external environment (NA Engineering, 2015).

Besides moisture content, bacteria are sensitive towards acidity. Bacteria are very sensitive towards the hydrogen ion because it affects enzyme production, metabolic process and can denature their shape. Most bacteria grow best at neutral pH of 7.2 -7.4. According to McEgan *et al.* (2013), acidity weakens the growth of faecal coliforms and *Salmonella* sp. Temperature is also one of the most important factors affecting bacterial growth. The log phase of bacterial growth is proportional to the temperature in the environment. Most microorganisms grow best from 20 to 40°C, and bacterial growth doubles after a long period of low temperature storage. De Silvestri *et al.* (2018) demonstrated that temperature affects the growth of Aeromonas *hydrophila, Listeria monocytogenes DSM-20600,* and *Yersinia enterocolitica*. Sinton *et al.* (2007) also mentioned that temperature strongly influences the growth of pathogenic bacteria in bovine faecal samples and *Enterococci* grow best in high temperatures. Temperature is crucial to the regulation and expression of virulence genes in pathogens (Guijarro *et al.*, 2015).

Time is also one of the major factors affecting bacterial growth. Janice *et al.* (2005) found that environmental factors analysis, such as moisture, light intensity, gases, chemicals and temperature play an important role in preventing microbial infection of harvested cereal grain and bacterial infection in the tomato plant. Apart from environmental factors, carefully assessing other external factors of the swiftlet houses is also crucial. Information regarding the effect of environmental factors on the bacteria in swiftlet houses is rather limited. Hence, this study was undertaken to propose a logistic model of the environmental factors, such as the location of the site, the age of the swiftlet house and bird nests production rate, which may affect the bacterial growth and antibiotic resistance of the bacteria isolated from the swiftlets' faeces.

Materials and Methods

Locations of the Study Areas

Sampling of the faecal bacteria samples was carried out in both rainy and dry seasons from March 2016 till September 2017 from the swiftlet houses located in Sarawak (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), 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).

Fecal Bacteria (FB) Count

Microbial colony count was done to determine the number of microorganisms in the collected swiftlet faecal samples. The plate counting was performed on TSA plates for swiftlet faeces, as described by Leong *et al.* (2013). The plates that contained FB colonies in the range of 30-300 were selected for counting. The result of the FB colony count was expressed as the number of colony forming unit (CFU) and calculated as equation [1].

$$\text{CFU} = (\text{number of colony} \times \text{dilution factor}) \div (\text{volume culture plate}) \quad [1]$$

Antibiotic Susceptibility Test

Antibiotic susceptibility test was conducted using disc diffusion method as described by the National Committee for Clinical Laboratory Standards (NCCLS) 2016. All the bacteria isolates from the swiftlet faeces were tested for antibiotic susceptibility. Antibiotic discs (Oxoid, England) chosen for testing represent agents historically or currently used in clinical

practice. *Escherichia coli* ATCC 25922 was used as positive control. Among the antibiotic discs used in this testing were chloramphenicol (30 µg), ampicillin (10 µg), tetracycline (30 µg), streptomycin (10 µg), gentamycin (10 µg), erythromycin (15 µg), cephalothin (30 µg), nitrofurantoin (300 µg), tobramycin (10 µg), rifampicin (5 µg), kanamycin (30 µg), sulphamethoxazole/ trimethoprim (1.25/ 23.75 µg), amikacin (30 µg), imipenem (10 µg), ceftriaxone (30 µg), penicillin G (10 U), doxycycline (30 µg), ceftazidime (30 µg), norflaxacin (10 µg), vancomycin (30 µg), piperacillin (100 µg), Ciprofloxacin (5 µg) and Nalidixic acid (30 µg). The bacteria cultures were prepared by growing the bacteria in Luria broth (Scharlau, Spain) at 37±1°C for 24 hours in the laboratory. Then, the bacteria suspensions were used to inoculate the Mueller-Hinton agar (MHA) plate evenly using sterile cotton swabs. A pair of sterile forceps was used to pick up the antibiotic disc and placed on the surface of the MHA agar plate. The plate was incubated at 37±1°C for 24 hours. The diameter of the inhibition zone was measured and the reading was recorded as sensitive (S) or resistant (R) based on WHO Drug Information and NCCLS.

Intrinsic and Extrinsic Factors Analysis

Season of the Faecal Samplings

The season of the sample collection was recorded, as described by Williams *et al.* (2013). In Sarawak the rainy season commences in October and ends in March, while the dry season starts in April and ends in September. The first sampling of the faecal and airborne bacteria samples was carried out during the rainy and dry season from 21st March 2015 till 27th February 2016, while the second sampling was from 24th April 2015 till 15th September 2016, from the swiftlet houses located in the southern, central and northern regions of Sarawak, Malaysia.

Acidity (pH) Measurement of the Faecal Samples

The pH of the swiftlet faecal samples was measured as described by Akinori *et al.* (2001) with minor modification. The fresh faecal samples were diluted with distilled water (5:1 ratio) and then shaken for 2-3 minutes, and allowed to settle for about 10 minutes. The pH value was measured by direct insertion of stainless steel pH probes of an IQ150 pH meter system (IQ Scientific Instruments, Inc., Carlsbad, CA, USA) into the homogenized faeces. Calibration of the pH meter was done before measurement.

Moisture Content of the Swiftlet Faeces

*T*he moisture content of all fresh faecal samples was measured. The 50ml falcon tubes before and after being filled with the faecal sample were weighed. Then, the faecal samples in the falcon tubes were dried in an oven at 60 ± 1°C for 48 hours. After drying, the falcon tube together with the swiftlet faecal samples was weighed again. Calculation was done with the formula provided by Nishimuta *et al.* (2006).

Mean Daily Temperature (MDT) of the Sampling Sites

The MDT of the sampling areas were collected from the Malaysian Meteorological Department. The standard measurement of the MDT was calculated using the average temperature between the minimum and maximum temperature observed at 3 or 4 fixed times.

Survey on the Location and Age of Swiftlet Houses and Birds' Nests Production Rate

The information regarding the location and age of the swiftlet houses and bird nests production rate for the different bird houses was collected. The survey of the 10 swiftlet houses in the southern, central and northern regions in Sarawak, found that 80% of the bird nests production rate was recorded as high (more than 2kg per harvest) and 20% as low (less than 2kg per harvest) respectively. The information was provided by the owner of the swiftlet house.

Statistical Analysis

Data were analysed by logistic and multiple

regression analyses. All differences between the mean were compared using Duncan multiple range test (DMR) after a significant F-test at P< 0.05, P<0.01 and P< 0.001.

The study was conducted to test the following hypotheses:

1. The FB colony count is affected by the mean daily temperature, season, faecal acidity (pH) and moisture content.
2. Faecal acidity (pH) increases the FB resistance to antibiotics.

Variables measured are as follows:

Dependent:

FB colony count: Entered as value measure
Antibiotic resistance: Low (<50%) = 0, High (>50%) = 1

Independent:

Faecal acidity (pH): Entered as value measure
Faecal moisture content: Entered as number of %
MDT (°C): Entered as number
Sampling season: Dry = 0, Wet = 1
Site location: Rural = 0, Urban = 1
Age of the swiftlet house: Entered as number of years
Bird nest production rate: Low (<2.0 kg per harvest) = 0, High (> 2.0 kg per harvest) = 1

Results and Discussion

Microbial Colony Count

The FB colonies count (F=8.265, $\log_{10}$ cfu/g) were significantly (P<0.05) affected by and between the sampling sites (Table 1). The highest total mean of the bacterial colony count of 11.86±3.11 $\log_{10}$ cfu/g was found in Kota Samarahan mainly because it is situated on the west coast of Sarawak, which has nutrient-rich water considering the shallow coastal depth and the abundance of sunlight in this area (Conventry, 2001). Thus, western Sarawak is more conducive to bacterial growth. Miri is in northern Sarawak, and has a higher temperature than the other regions (Malaysia Meteorological Department, 2017). The total mean FB colony counts were found to be the lowest in Sibu (6.71±1.09 $\log_{10}$ cfu/g) probably because of the relatively high rainfall with low temperature in central Sarawak. Temperature can affect the growth of various bacterial species and the growth rate of microorganisms doubled as the temperature increases by one unit, as described by Huete-Stauffer *et al.* (2015).

Intrinsic and Extrinsic Factors Analysis

The total mean acidity (pH) of the faecal samples were significantly different (F=3.617, P<0.05) from one sampling site to another and were in the range of pH 6.69 to pH 8.27 (Table 1). The highest total mean acidity (pH) of the faecal samples was pH 8.27 ± 0.87, which was collected in Kota Samarahan and the lowest at pH 6.69 ± 0.83 was recorded in Sibu. Thus, it showed that the faecal acidity (pH) varied widely according to the sites and the time of collection. According to Adrian and Daniella (2010), most of the birds' excretion was a mixture of dropping plus urine and uric acid, thus the birds' faeces were slightly acidic. Uric acid is the nitrogen catabolism end product in birds. Besides, the faeces' composition and quantity were affected strongly by the type of food the birds ingested (Michael & Anthony, 2015), thus resulting in different faecal acidity in different sites and at time of sampling. Most of the bacteria reacted sensitively to acidity (Konstantinos *et al.*, 2016), thus this may determine which microorganism would grow faster. According to Eleri *et al.* (2015), the optimum pH level for most of the bacteria, such as *Staphylococcus* sp, *Bacilus cereus* and *Escherichia coli* is pH 6.5 to pH 7. Even though most of the bacteria grow best in neutral pH values around pH 6.5 to 7.0, some bacteria may tolerate pH as low as 1.0 or as high as 8.0 (Isnawati & Trimulyono, 2018). Bacteria reacted sensitively towards the hydrogen ion in their growing environment because the pH affects bacteria enzyme production, metabolic process and denatured their shapes.

Table 1 shows that the faecal samples contained 18.10% to 41.24% of total mean

Table 1: The total mean bacterial colony count, pH, moisture content and daily temperature of the swiftlet faecal samples collected

Site	Total mean bacterial colony count (log_{10} cfu/g)	Total mean pH	Total mean moisture content (%)	Total mean daily temperature (°C)
Kota Samarahan	11.86 ± 3.11[a]	8.27 ± 0.87[a]	30.33 ± 4.48[ab]	31.67 ± 1.86[a]
Saratok	7.48 ± 1.55[bc]	7.48 ± 0.76[ab]	41.24 ± 16.58[b]	31.67 ± 1.86[a]
Betong	6.98 ± 1.13[b]	7.37 ± 0.96[ab]	25.80 ± 14.27[a]	32.00 ± 1.90[a]
Maludam	7.85 ± 0.53[bc]	7.28 ± 0.68[ab]	21.11 ± 7.13[a]	33.67 ± 0.52[a]
Miri	8.77 ± 0.25[c]	7.13 ± 0.75[b]	22.90 ± 5.15[a]	30.67 ± 0.82[ab]
Kuching	8.24 ± 0.53[bc]	6.92 ± 0.53[b]	23.97 ± 7.02[a]	30.50 ± 0.55[ab]
Semarang	8.08 ± 1.06[bc]	7.04 ± 0.76[b]	18.10 ± 7.16[a]	33.83 ± 0.41[a]
Sepinang	7.71 ± 1.28[bc]	7.31 ± 0.67[ab]	24.63 ± 9.84[a]	31.83 ± 2.04[a]
Sarikei	7.28 ± 1.40[bc]	7.62 ± 0.78[ab]	26.99 ± 5.24[a]	27.33 ± 4.76[b]
Sibu	6.71 ± 1.09[b]	6.69 ± 0.83[b]	19.05 ± 4.55[a]	27.17 ± 4.96[b]

abc Means with different superscript letters in a column are significantly different at 5% level.

moisture content in this study. The total mean moisture content of the faecal samples was significantly different ($F=5.427$, $P<0.05$) between the sampling sites. The faecal samples collected from Saratok had the highest total mean moisture content of 41.24 ± 16.58%, while the samples collected from Semarang had the lowest total mean moisture content of 18.10 ± 7.16%. The mean moisture content of faecal samples collected from Saratok was significantly different from all the other sampling sites. Moisture content is defined as the quantity of water contained in a material. Water plays an important role in microbial multiplication. The result of this study is in agreement with the research done by Stevenson *et al.* (2015) where it was explained that microorganisms need water to grow and multiply. According to Tang & Chen (2017), the moisture content was affected strongly by the surrounding temperature. Therefore, bacteria would grow slowly in high-temperature dry surroundings because of the low moisture content.

In this study, the total MDT was in the range of 27.17°C to 33.83°C and significantly different ($F=19.841$, $P<0.05$) from one sampling site to another (Table 1). The highest total MDT (33.83 ± 0.41°C) was collected in Semarang and the lowest (27.17 ± 4.96°C) was recorded in Sibu. The total MDT in Sarikei and Sibu were not significantly different from Miri, and Kuching but were significantly different from the other sampling sites. The high temperature in Semarang may result in the lowest moisture content of the samples collected there. According to Tang & Chen (2017), the temperature affects the moisture content during storage. Besides, Angelovič *et al.* (2015) reported that high surrounding temperature could decrease the moisture content of the maize grains stored in a closed area.

It was observed that the higher MDT in the dry season could increase the FB count. This result is in agreement with the findings reported by Barry *et al.* (2013) that the growth rate of microorganisms doubled as the temperature increased by one unit and Huang *et al.* (2011), who stated that temperature was one of the most important factors affecting bacterial growth. The higher temperature increases the enzyme activity in the bacteria cell, thus increasing the multiplication rate. Mesophilic bacteria are the common bacteria isolates which grow well around 30°C to 37°C. A similar result

had been reported by NA Engineering (2015), who collected the daily temperature data from the weather stations in order to analyse the temperature effect on microbial growth.

The other external factors that may influence the microbial growth are shown in Table 2. It showed that 55% and 45% of the swiftlet samples were collected during the dry and rainy seasons, respectively. Swiftlet houses built between 2.5 years and 8 years are mostly located in the rural area and the urban area and they accounted for 60% and 40%, respectively. These extrinsic (external) factors may influence microbial growth in this study. It was very important to carefully assess these factors with respect to the swiftlet houses because they provide an explanation about the swiftlet birds' habitat condition, their adaptation to the climate and house operation, which may influence bacterial growth.

Antibiotic Susceptibility Test

The percentage of antibiotic resistance for the 23 types of antibiotics among the 1,000 FB isolates are shown in Table 3 and illustrated in Figure 1-2. The highest degree of antibiotic resistance among faecal bacteria isolates was detected against penicillin G (42.20±18.35%), followed by ampicillin (36.50±19.49%), ceftazidime (18.10±19.06%), cephalothin (15.80±18.08%), rifampicin (15.70±7.38%), sulphamethoxazole/ trimethoprim (13.60±18.22%), ceftriaxone (11.50±18.31%), gentamycin (9.10±10.83%), doxycycline (8.30±10.26%), tetracycline (7.40±7.34%), erythromycin (7.10±10.18%), nitrofurantoin (4.10±6.06%), chloramphenicol (2.20±3.74%), kanamycin (2.10±3.84%) and least resistant to tobramycin (1.80±3.61%), ciprofloxacin (1.50±2.46%), norflaxacin (1.20±2.49%), amikacin (1.10±1.91%)and imipenem (0.40±1.26%). The *Enterobacter* and *Enterococcus* bacteria isolates were resistant to streptomycin (40.00±51.64%) and vancomycin (77.50±41.58%).

Table 2: The other factors: age of the swiftlet houses, bird nests production rate and season of sampling that may influence the microbial growth

Sampling Sites	Season		Location	Age of Swiftlet Houses	Bird Nests Production Rate per Harvest
	S1	S2			
Kota Samarahan	Wet	Wet	Rural	2.5	High
Saratok	Dry	Wet	Rural	4	High
Betong	Dry	Wet	Rural	5	High
Maludam	Dry	Dry	Rural	5	High
Miri	Dry	Wet	Urban	8	High
Kuching	Wet	Dry	Urban	7	High
Semarang	Dry	Dry	Rural	7	Low
Sepinang	Dry	Wet	Rural	4.5	Low
Sarikei	Dry	Wet	Urban	5	High
Sibu	Dry	Wet	Urban	3	High

Legend: S1: First sampling; S2: Second sampling; Bird nests production rate: High (more than 2 kg per harvest); Low (less than 2 kg per harvest)

Table 3 shows that most of the faecal bacteria were resistant to penicillin G and ampicillin antibiotics. This shows that most faecal bacteria produced β-lactamase enzyme, which may deactivate the antibiotics' attacking mechanism (Sibhghatulla *et al.*., 2015). The result is in agreement with Sanlibaba *et al.* (2018), who stated that *Enterococcus* sp was highly resistant to rifampicin and vancomycin mainly due to acquired resistance through mutation of genes. Streptomycin has been widely used as a first-line antimicrobial treatment. Thus, the extensive usage of streptomycin against *Enterobacter* sp may cause the development of resistance.

Table 3: The percentage of resistance to 23 types of antibiotics among faecal bacteria isolated from Kota Samarahan, Saratok, Betong, Maludam, Miri, Kuching, Semarang, Sepinang, Sarikei and Sibu in Borneo

Antibiotic	Resistance rate (%)										Mean
	Kota Samarahan (n=100)	Saratok (n=100)	Betong (n=100)	Maludam (n=100)	Miri (n=100)	Kuching (n=100)	Semarang (n=100)	Sepinang (n=100)	Sarikei (n=100)	Sibu (n=100)	
Penicillin											
Ampicillin	71	29	33	25	22	63	5	31	41	45	36.50±19.49^{ab}
Penicillin G	71	54	50	27	31	64	10	31	40	44	42.20±18.35^{b}
Cephalosporin											
Ceftriaxone	61	4	6	9	0	1	1	6	20	7	11.50±18.31^{c}
Ceftazidime	67	9	12	14	0	1	22	15	27	14	18.10±19.06^{ac}
Cephalothin	63	10	8	7	10	3	8	11	30	8	15.80±18.08^{c}
Phenicol tetracycline											
Chloramphenicol	0	8	0	3	10	0	1	0	0	0	2.20±3.74^{c}
Quinolones											
Norfloxacin	2	8	0	0	0	0	0	1	0	1	1.20±2.49^{c}
Fluroquinolone											
Ciprofloxacin	2	8	2	0	2	1	0	0	0	0	1.50±2.46^{c}
Macrolide											
Erythromycin	0	17	1	9	2	10	1	31	0	0	7.10±10.18^{c}
Aminoglycoside											
Gentamycin	27	11	0	0	0	0	1	16	26	10	9.10±10.83^{c}
Kanamycin	2	5	2	0	0	12	0	0	0	0	2.10±3.84^{c}
Amikacin	0	5	0	0	0	0	4	0	0	2	1.10±1.91^{c}
Tobramycin	0	5	0	0	0	11	0	0	0	2	1.80±3.61^{c}
Carbapenem											
Imipenem	0	4	0	0	0	0	0	0	0	0	0.40±1.26^{c}
Nitrofuran											
Nitrofurantoin	0	13	0	0	10	15	2	0	1	0	4.10±6.06^{c}
Ansamycin											
Rifampicin	25	16	8	12	12	21	11	14	30	8	15.70±7.38^{c}
Tetracycline											
Tetracycline	4	17	0	1	3	12	4	0	14	19	7.40±7.34^{c}
Doxycycline	2	14	0	1	3	12	1	0	29	21	8.30±10.26^{c}

Sulfonamide											
Sulphamethoxazole/ Trimethoprim	62	9	8	10	0	0	3	17	20	7	13.60±18.22[c]
Only applicable to *Enterobacter* bacteria											
Penicillin											
Piperacillin	100	0	0	50	0	0	40	0	100	0	29.00±41.75[A]
Quinolones											
Nalidixic acid	100	0	0	0	0	0	0	0	0	100	20.00±42.16[A]
Ansamycin											
Streptomycin	100	0	0	100	0	0	100	0	100	0	40.00±51.64[A]
Only applicable to *Enterococcus* bacteria											
Glycopeptide											
Vancomycin	0	75	0	100	100	100	100	100	100	100	77.50±41.58

abc Means with different superscript letters in a column are significantly different at 5% level.

Analysis Among the Factors and Mean Bacterial Count in the Faeces

The multiple regression analysis of the mean FB count with the intrinsic and extrinsic factors, shows that faecal sample acidity (pH), sampling season, mean daily temperature, site location and faecal moisture content were the best predictors of this model. The R-squares show that 40.4% of the variance was accounted for by "faecal pH" alone, 46.4% of the variance was accounted for by "faecal pH" and "sampling season", 59% of the variance was accounted for by "faecal pH", "sampling season" and "mean daily temperature", 64.6% of the variance was accounted for by "faecal pH", "sampling season", "mean daily temperature" and "site location" and lastly 66.9% of the variance was accounted for by "faecal pH", "sampling season", "mean daily temperature", "site location" and "faecal moisture content". The results of this study show that there was a significant regression ($P<0.0005$, $N=100$). Therefore, the relationship between the mean bacterial colony count ($\log_{10}$ cfu/g) and the significant predictors can be expressed as Equation [2]:

Predicted mean bacterial colony count

= -9.935 + 1.26 (pH) - 2.784 (Season)
+ 0.413 (mean daily temperature) + 0.569
(Site location) - 0.029 (Moisture content) [2]

Analysis of the Fctors and Swiftlet Faecal Bacteria Antibiotic Resistance

A logistic regression analysis was conducted. A test of the full model against a constant model was not statistically significant, indicating that the predictors were not reliably distinguished between the low and high incidence of antibiotic resistance (chi square = 10.843, $P>0.05$ with $df = 7$). Nagelkerke's R2 of 0.137 indicated a moderately strong relationship between the prediction and grouping. Prediction success overall was 59% (66.7% for low incidence and 51.9% for high incidence. The Wald criterion demonstrated that only faecal acidity (pH) made a significant contribution to the prediction ($P = 0.012$). All the other predictors were not significant. EXP (B) value indicates that when the faecal acidity (pH) was raised by one unit the odd ratio is 0.50 times as large. Therefore, antibiotic resistance is 0.50 more times likely to happen. The logistic regression equation of the probability of occurrence of antibiotic resistance in swiftlet faecal bacteria (P) can be expressed as Equation [3]:

$$P = \frac{e^{\,(-0.761\,(\text{faecal pH}) + 0.22\,(\text{faecal moisture content}) + 0.072\,(\text{mean daily temperature}) + 1.022\,(\text{season}) - 0.526\,(\text{site location}) - 0.0366\,(\text{age of the house}) - 0.293\,(\text{bird nest production rate}) + 2.969)}}{1 - e^{\,((-0.761\,(\text{faecal pH}) + 0.22\,(\text{faecal moisture content}) + 0.072\,(\text{mean daily temperature}) + 1.022\,(\text{season}) - 0.526\,(\text{site location}) - 0.366\,(\text{age of the house}) - 0.293\,(\text{bird nest production rate}) + 2.969))}} \quad [3]$$

A logistic regression analysis predicted that bacteria could grow well under conditions of higher faecal acidity (pH), dry season, higher mean daily temperature and faecal moisture content of the swiftlet houses built in urban areas. This may indicate that bacteria would multiply faster in the urban areas than rural areas. In urban areas, higher population density and more human activities may cause higher contamination of microorganisms growing in the swiftlet houses, thus, resulting in an increase in the bacterial number and species isolates. According to British standards (2014) microbial growth was greatly influenced by the temperature, pH, dissolved gases, osmotic pressure and water availability. It shows that the increase in the bacterial colony count in the faecal samples with increasing faecal acidity (pH) in the urban area and the increase in the mean daily temperature (°C) during the dry season would also increase bacterial growth. Probability of occurrence of antibiotic resistance was positively affected only by faecal acidity. The results of this study are in agreement with Isnawati and Trimulyono (2018), who stated that most bacteria actually grow optimally in pH ranging from 6 to 8 which are slightly acidic to slightly alkaline. All living organisms, including bacteria, need a physiological pH inside their cells in order to survive. Previously (Ratzke & Gore, 2018) also mentioned that most pathogens are more likely to adapt to slightly alkaline pH in different microbial systems. Therefore, more pathogenic bacteria are more likely to be isolated from the slightly alkaline pH condition of the faecal and airborne samples of the swiftlet houses in the present study.

Conclusion

The effects of environmental factors on FB growth were experimentally investigated. Thus, FB can survive, multiply and proliferate under the mean pH range of pH 6.69 to 8.27, mean water content ranging from 18.10% to 41.24% and total mean daily temperature range of 27.17°C to 33.83°C in urban areas. The probability of the development of antibiotic resistance (%) increased 0.50 times if the faecal acidity (pH) increased by one unit. Logistic regression is a good model for predicting the relationship between antibiotic resistance in FB and environmental parameters. Understanding how these FB grow and react to environmental parameters following this model allowed us to estimate their interaction outcomes, especially in urban cities. We may be able to control FB pollution by minimizing FB growth which may pose a health hazard to people.

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