Antibiogram Profiles and Risk Factors for Multidrug Resistance of *Salmonella enterica* Recovered from Village Chickens (*Gallus gallus domesticus* Linnaeus) and Other Environmental Sources in the Central and Southern Peninsular Malaysia

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Received: 26 June 2020; Accepted: 8 October 2020; Published: 15 October 2020

**Abstract:** The emergence of multidrug resistance (MDR), including colistin resistance, among Enterobacteriaceae recovered from food animals poses a serious public health threat because of the potential transmission of these resistant variants to humans along the food chain. Village chickens or Ayam Kampung are free-range birds and are preferred by a growing number of consumers who consider these chickens to be organic and more wholesome. The current study investigates the antibiogram profiles of *Salmonella* isolates recovered from village chicken flocks in South-central Peninsular Malaysia. A total of 34 isolates belonging to eight serotypes isolated from village chickens were screened for resistance towards antimicrobials including colistin according to the WHO and OIE recommendations of critical antibiotics. *S. Weltevreden* accounted for 20.6% of total isolates, followed by serovars Typhimurium and Agona (17.6%). The majority of isolates (73.5%) demonstrated resistance to one or more antimicrobials. Eight isolates (23.5%) were resistant to ≥3 antimicrobial classes. Colistin resistance (minimum inhibitory concentrations: 4–16 mg/L) was detected among five isolates (14.7%), including *S. Weltevreden*, *S. Albany*, *S. Typhimurium*, and *Salmonella* spp. Univariable analysis of risk factors likely to influence the occurrence of MDR *Salmonella* revealed that the flock size, poultry production system, and use of antibiotics in the farm were not significantly (*p > 0.05*) associated with MDR *Salmonella*. The current study highlights that MDR *Salmonella* occur at a lower level in village chickens compared to that found in live commercial chickens. However, MDR remains a problem even among free-range chickens with minimal exposure to antibiotics.

**Keywords:** chicken; antibiotic; resistance; *Salmonella*; colistin; Malaysia

1. Introduction

Increasing antibiotic resistance among foodborne pathogens is an emerging problem of global health importance [1] and the overuse of antibiotics in food animal production has been reported as one of its major drivers [2]. Colistin is a critically important antimicrobial in veterinary medicine [2], and is considered the drug of last resort against the emergent multidrug resistant (MDR) Gram-negative bacterial infections in humans, especially the carbapenem-resistant Enterobacteriaceae [3]. However, the overuse of colistin in the animal industry is reported to play an important role in the global emergence of colistin resistance [2]; as such, its use in livestock is being reconsidered in order to preserve drug efficacy [4,5]. Consequently an increasing number of consumers are choosing to consume...
meat from organically-grown sources or sourced from environments where there is less selective pressure that promotes the development of antibiotic resistance, thus reducing the transmission of resistant agents through food consumption [6,7]. Such environments typically include free-range production arrangements for food animals.

*Salmonella* is one of the most common foodborne bacteria worldwide [8]. *Salmonella* is a Gram-negative, rod-shaped bacilli and facultative anaerobe of the family Enterobacteriaceae, which can be broadly classified into two species based on their 16S rRNA sequence analysis: *Salmonella enterica* and *Salmonella bongori* [9]. There are more than 2500 serovars belonging to *S. enterica*, the majority of which are pathogenic and cause diseases in both animals and humans [10,11]. The global burden of non-typhoidal *Salmonella* (NTS) is increasing, with one study reporting approximately 94 million cases of NTS gastroenteritis, which is responsible for 155,000 deaths globally each year [8]. According to the study, the majority of the NTS burden is found in the Southeast Asian and Western Pacific region [8,12]. Of the 94 million cases reported, an estimated 80.3 million are thought to be of foodborne origin [13]. Poultry and poultry products (e.g., eggs and food products containing eggs) are commonly linked to NTS and have been demonstrated to serve as primary vehicles for human salmonellosis [8,14].

In Malaysia, several deaths and illnesses in recent years have been linked to foodborne NTS involving contaminated chicken and related products [15]. *Salmonella* contamination of various food products—sourced from either wet, retail markets or processing plants—such as chicken carcasses, chicken portions, various chicken organs (e.g., liver and gizzards), ready-to-eat foods, fruits, and vegetables, and other environmental sources, have been widely reported [16–18]. Only one of these studies was conducted on live birds in commercial poultry farms [19]. Nevertheless, no study to date has focused on village chickens in Malaysia.

Malaysian consumers increasingly prefer safer, wholesome, organic foods [20,21]. Moreover, in light of growing concerns over the transmission of antibiotic resistance via the food chain, the demand for these organic food products will likely increase over time. Therefore, village chicken production is an emerging niche market catering for this preference [22], with village chickens raised in a more “organic” free-range environment as compared to commercial broiler chickens. In this study, we described patterns of antibiotic resistance, including colistin resistance, and associated risk factors for the development of MDR in *Salmonella* isolates isolated from live local village chickens in South-central Peninsular Malaysia.

2. Results

2.1. Antimicrobial Resistance and Antibiogram Profiles of the Salmonella Isolates

Tables 1 and 2 illustrate the isolate antibiotic resistance profile, multiple antibiotic resistance (MAR) index, and resistance phenotypes. Among the isolates, 26.5% (*n* = 9) were susceptible to all antibiotics, while 73.5% (*n* = 25) were resistant to at least one tested antibiotic. Multidrug resistance was displayed by eight isolates (23.5%). Ciprofloxacin (100%), gentamicin (97.1%), norfloxacin (97.1%), cefotaxime (97.1%), and ceftriaxone (97.1%) were effective against most isolates. The highest level of resistance was observed for tetracycline (35.3%) and streptomycin (35.3%; Table 1).
Table 1. Antibiogram of *Salmonella* isolates recovered from village chickens of the central and Southern Peninsular Malaysia.

| Antimicrobial Agents                  | No. Tested | Resistant (%) | Intermediate (%) | Sensitive (%) |
|--------------------------------------|------------|---------------|------------------|--------------|
| Gentamicin                           | 34         | 00            | 1 (2.9)          | 33 (97.1)    |
| Amoxicillin-Clavulanate              | 34         | 2 (5.9)       | 2 (5.9)          | 30 (88.2)    |
| Nitrofurantoin                       | 34         | 4 (11.8)      | 0                | 30 (83.8)    |
| Ciprofloxacin                        | 34         | 00            | 0                | 34 (100)     |
| Kanamycin                            | 34         | 1 (2.9)       | 2 (5.9)          | 31 (91.2)    |
| Trimethoprim                         | 34         | 7 (20.6)      | 0                | 27 (79.4)    |
| Norfloxacin                          | 34         | 00            | 1 (2.9)          | 33 (97.1)    |
| Tetracycline                         | 34         | 12 (35.3)     | 1 (2.9)          | 21 (61.8)    |
| Nalidixic acid                       | 34         | 5 (14.7)      | 0                | 29 (85.3)    |
| Chloramphenicol                      | 34         | 4 (11.8)      | 0                | 30 (88.2)    |
| Ampicillin                           | 34         | 6 (17.6)      | 0                | 28 (82.4)    |
| Cefotaxime                           | 34         | 1 (2.9)       | 0                | 33 (97.1)    |
| Streptomycin                         | 34         | 12 (35.3)     | 11 (32.4)        | 11 (32.4)    |
| Sulfonamides                         | 34         | 10 (29.4)     | 0                | 24 (70.6)    |
| Ceftiofur                            | 34         | 0             | 1 (2.9)          | 33 (97.1)    |
| Colistin 1                           | 34         | 5 (14.7)      | 0                | 29 (85.3)    |

1 Minimum inhibitory concentrations (MICs) was determined by the microbroth dilution method using the MIC-Strip kit (MERLIN Diagnostika GmbH, Bornheim, Germany).

Table 2. Antibiotic resistance patterns of *Salmonella* recovered from village chickens in the South-central Peninsular Malaysia.

| Sources          | Resistance Profiles | *Salmonella* Serovars | Colistin MIC 1 | MAR Index 2 |
|------------------|---------------------|------------------------|----------------|-------------|
| Cloacal swab     | AmpTeWS2SFNaCt      | *Salmonella* spp.      | 4 R            | ≥0.2        |
| Flies            | TeWS2SFCtxAmc       | S. Molade              | 2 S            | ≥0.2        |
| Cloacal swab     | AmpS2WNaCn          | S. Weltevreden         | 8 R            | ≥0.2        |
| Cloacal swab     | AmpS2WNaCn          | S. Weltevreden         | 8 R            | ≥0.2        |
| Drinking water   | AmpTeSSCn           | S. Albany              | 16 R           | ≥0.2        |
| Cloacal swab     | TeWS2              | S. Corvallis           | 0.25 S         | ≥0.2        |
| Cloacal swab     | TeS2S              | *Salmonella* spp.      | 2 S            | ≥0.2        |
| Drinking water   | TeNa                | S. Albany              | 2 S            | ≥0.2        |
| Drinking water   | TeNa                | *Salmonella* spp.      | 2 S            | <0.2        |
| Drinking water   | AmpTe               | S. Weltevreden         | 2 S            | <0.2        |
| Drinking water   | AmpS2               | S. Weltevreden         | 0.5 S          | <0.2        |
| Cloacal swab     | TeS                | S. Molade              | 0.5 S          | <0.2        |
| Cloacal swab     | TeW                | S. Typhimurium         | 4 R            | <0.2        |
| Cloacal swab     | TeW                | S. Agona               | 2 S            | <0.2        |
| Cloacal swab     | Ct                  | S. Enteritidis         | 2 S            | <0.2        |
| Cloacal swab     | Te                  | *Salmonella* spp.      | 0.25 S         | <0.2        |
| Cloacal swab     | Te                  | S. Typhimurium         | 0.25 S         | <0.2        |
| Cloacal swab     | Te                  | S. Typhimurium         | 0.25 S         | <0.2        |
| Feed             | Amp                  | S. Typhimurium         | 0.5 S          | <0.2        |
| Feed             | Amp                  | S. Albany              | 0.25 S         | <0.2        |
| Cloacal swab     | S                   | S. Agona               | 0.25 S         | <0.2        |
| Cloacal swab     | S                   | S. Agona               | 0.25 S         | <0.2        |
| Cloacal swab     | S                   | S. Agona               | 0.25 S         | <0.2        |
| Cloacal swab     | S                   | S. Agona               | 0.25 S         | <0.2        |

1 MIC, minimum inhibitory concentration (mg/L) according to the MIC-strip microbroth dilution method, 2 MAR, multiple antibiotic resistance index, MAR index = No. of resistance antibiotic types/total number of antibiotic types tested, 3 R/I/S, Resistant, Intermediate or Susceptible according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (http://www.eucast.org/clinical-breakpoints/) (Te, Tetracyclines; Amp, Ampicillin; S, Streptomycin; W, Trimethoprim; S, Sulfonamides; Amc, Amoxicillin-clavulanate; Na, Nalidixic acid; Ct, Chloramphenicol; Ctx, Cefotaxime; Cn, Gentamicin; F, Nitrofurantoin; K, Kanamycin).
Figures 1–3 show the frequency distribution of isolates resistant to commonly used antimicrobials according to the various sample sources, farms utilizing antimicrobials for treatment, and farm production systems. Generally, higher percentages of resistance were observed among isolates from farms that use antibiotics as compared to those farms that did not, and from the free-range system compared to the other systems. Most isolates found to manifest antimicrobial resistance were recovered from cloacal samples (Table 2). Table 3 presents the distribution and percentages of MDR *Salmonella* recovered from cloacal swabs, drinking water, and flies caught at the farm.

![Graph showing frequency of resistance of *Salmonella* isolates resistant to various antimicrobials](image1)

**Figure 1.** Frequency of *Salmonella* isolates recovered from various sources resistant to commonly used antibiotics from the South-central Peninsular Malaysia. (Te, Tetracycline; S, Streptomycin; W, Trimethoprim; S3, Sulfonamides; Amp, Ampicillin; Na, Nalidixic acid; Cn, Gentamicin; Ct, Chloramphenicol; F, Nitrofurantoin; Ctx, Cefotaxime; Amc, Amoxicillin-clavulanate).

![Graph showing frequency of resistance of *Salmonella* isolates from farms with and without antibiotic use](image2)

**Figure 2.** Frequency of resistance of *Salmonella* isolates recovered from village chickens of the South-central Peninsular Malaysia against antimicrobials according to the flocks with or without antibiotic use (Te, Tetracycline; S, Streptomycin; W, Trimethoprim; S3, Sulfonamides; Amp, Ampicillin; Na, Nalidixic acid; Cn, Gentamicin; Ct, Chloramphenicol; F, Nitrofurantoin; Ctx, Cefotaxime; Amc, Amoxicillin-clavulanate).
Figure 2. Frequency of resistance of *Salmonella* isolates recovered from village chickens of the South-central Peninsular Malaysia according to the flocks with or without antibiotic use (Te, Tetracycline; S, Streptomycin; W, Trimethoprim; S3, Sulfonamides; Amp, Ampicillin; Na, Nalidixic acid; Cn, Gentamicin; Ct, Chloramphenicol; F, Nitrofurantoin; Ctx, Cefotaxime; Amc, Amoxicillin-clavulanate).

Figure 3. Frequency of resistance of *Salmonella* isolates from village chickens of the South-central Peninsular Malaysia according to the chicken production systems (Te, Tetracycline; S, Streptomycin; W, Trimethoprim; S3, Sulfonamides; Amp, Ampicillin; Na, Nalidixic acid; Cn, Gentamicin; Ct, Chloramphenicol; F, Nitrofurantoin; Ctx, Cefotaxime; Amc, Amoxicillin-clavulanate).

Table 3. Distribution of multidrug resistant (MDR) *Salmonella* isolates recovered from native chickens of the South-central Peninsular Malaysia.

| Sources          | No. of MDR *Salmonella* | % Positive ¹ |
|------------------|-------------------------|--------------|
| Cloacal swabs    | 5                       | 29.4%        |
| Drinking water   | 2                       | 22.2%        |
| Flies            | 1                       | 33.3%        |

¹ Fisher’s exact test, *p* > 0.05 (not significant).

For colistin, the minimum inhibitory concentrations (MICs) of the *Salmonella* isolates ranged from 0.25 to 16 mg/L (Table 2). Five (14.7%) of the *Salmonella* isolates had MICs of 4–16 mg/L. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) MIC breakpoints for colistin, MIC 4 mg/L is considered resistant to colistin (http://www.eucast.org/clinical-breakpoints/). These isolates comprise of *S.* Weltevreden, *S.* Typhimurium, *S.* Albany, and *Salmonella* spp. (Table 2). The two former serovars originated from free range (*n* = 3), while the latter two were from semi-intensive (*n* = 2) production systems. All but *S.* Typhimurium were MDR (Table 2).

2.2. Analysis of Risk Factors for MDR Salmonella

Simple logistic regression analysis of the risks factors associated with the development of MDR *Salmonella* revealed that none of the factors investigated (including flock size, poultry production system, and use of antibiotics) were not significantly associated (*p* > 0.05) with MDR *Salmonella* (Table 4).
Table 4. Risk factors associated with the occurrence of MDR Salmonella amongst village chickens from the South-central Peninsular Malaysia.

| Variables                               | Frequency | MDR (%) | OR (95% CI) | p-Value |
|-----------------------------------------|-----------|---------|-------------|---------|
| Flock size (number of birds)            |           |         |             |         |
| <500                                    | 22        | 6 (27.3)| 1.84 (0.32–15.52) | 0.533 |
| ≥500                                    | 12        | 2 (16.7)| Reference   | -       |
| Poultry production system               |           |         |             |         |
| Free-range                              | 22        | 5 (22.7)| 0.78 (0.14, 4.90) | 0.779 |
| Semi-intensive/Intensive                | 12        | 3 (25.0)| Reference   | -       |
| Use of antibiotics in the farm          |           |         |             |         |
| Yes                                     | 20        | 6 (30.0)| 1.96 (0.34–16.20) | 0.478 |
| No                                      | 14        | 2 (14.3)| Reference   |         |

1 OR, Odds ratio, CI, Confidence interval.

3. Discussion

Food safety issues, such as the presence of residual chemicals and antibiotic resistance, have resulted in an increased willingness among consumers to pay more for organically raised foods. Many Malaysians consume raw village chicken eggs because they believe it is medicinal, nutritious, safe, and antibiotic-free [6]. Salmonella isolates found in this study were susceptible to most of the antibiotics tested. High sensitivity levels were demonstrated against gentamicin, ciprofloxacin, cefotaxime, norfloxacin, and ceftiofur. This finding is consistent with similar studies that report a minimum level of resistance against the aforementioned antibiotics from local commercial chickens and chicken carcasses [23–26]. Complete susceptibility against cefotaxime, ciprofloxacin, and gentamicin was also demonstrated by Salmonella isolates recovered from Spanish broiler flocks [27]. In the present study, we found the highest levels of resistance were against tetracyclines (35.3%), streptomycin (35.3%), sulfonamides (29.4%), and trimethoprim (20.6%). In contrast, these levels were much lower than those reported against the same antibiotics (34–100%) for Salmonella isolates originating from commercial chickens in Malaysia [24,26]. The highest levels of resistance manifested by the isolates from the aforementioned studies were against tetracycline (100%), ampicillin (100%), clindamycin (100%), and ciprofloxacin (83%). Moreover our Salmonella isolates were susceptible to ciprofloxacin in contrast to that reported elsewhere as being between 30.8% and 96% [28–31].

Other studies around the world have shown the presence of antimicrobial resistant Salmonella recovered from backyard or free-range chickens. In most of these studies, resistance was demonstrated against ampicillins and tetracyclines [32–36]. Eight (23.5%) of the Salmonella isolates in this study were resistant to three or more antimicrobial agents (MDR; Table 3). This is comparatively lower than those reported in previous studies in commercial chickens in Malaysia, where a 100% and 75% of Salmonella recovered from cloacal swabs and chicken carcasses/products [26] respectively exhibited MDR [37]. In China, a similar level of MDR (26.3%) of Salmonella was also reported among free-range chickens [35].

Elsewhere, in commercial poultry as well as in retail chicken meats, or other related products, reports of the frequency of MDR Salmonella vary widely. For example, MDR Salmonella has been found in 80% of chicken carcasses and other related products in Egypt [28], with a similar level found in retail chicken meats in China [29]. Conversely, Spanish broiler flocks [27], as well as poultry houses [38] and broiler chicken farms [39] in Brazil reported MDR Salmonella as lower than 20%. MDR has been well documented among epidemiologically important serovars, such as S. Typhimurium, which can exhibit resistance to up to 13 antibiotics [25], and S. Enteritidis, which can demonstrate resistance to up to six antibiotics [24]. In our study, isolates of the aforementioned serotypes were not MDR (Table 3).
In Iran, a similar study found that *S. Typhimurium* isolates recovered from backyard chickens exhibited resistance against tetracycline, sulfamethoxazole, and trimethoprim [36].

The level of MDR found in this study, although meaningfully lower than those reported in commercial chickens locally and elsewhere, is worrying given that antibiotics are not routinely used in the production of village chickens. However, recent reports of the discovery of antibiotic resistance among animals in geographically remote locations where commercial antibiotics have not been applied may help to explain our findings [40]. The practice of free-range farming, allowing birds to roam freely, may result in their exposure to natural environmental hazards, such as untreated water and soil, which have been well documented to harbor drug-resistant foodborne pathogens [41–44]. Recent reports suggest that wild birds and gulls, creatures not exposed to the selective pressure of antibiotic use, have also been found to harbor MDR organisms in light of their continuous exposure to the natural environment [45–48]. It is also possible that feeding village chicken table scraps, which may contain resistant bacteria or materials, has additionally contributed to our observation of the phenomena. We found isolated MDR *Salmonella* from the cloacal swabs, drinking water and flies (Table 3). These findings further support the role of the environment in the dissemination and recirculation of MDR *Salmonella* [49,50]. Shang et al. (2018) found a significantly higher isolation rate of MDR *Salmonella* from litter samples compared to other samples in broiler farms, indicating the significance of poultry litters in the in-door environmental transmission of MDR *Salmonella* [31].

Five (14.7%) of the *Salmonella* isolates were resistant to colistin, with MICs in the range of 4–16 mg/L (Table 2). All of these isolates originated from free-range and semi-intensive production systems where chickens spend much of their time free grazing. Since the first report of the plasmid-mediated colistin resistance gene (*mcr-1*) from China in 2015, reports on resistance have emerged from more than 30 countries across several continents. These resistant isolates were recovered from several sources, such as environmental samples, food-producing animals, ready-to-eat foods, fruits, and vegetables, and humans [51–60]. Colistin resistance has been reported worldwide [58]; nevertheless, the burden of colistin resistance is highest in Asian countries. For example, several reports have documented the identification of MCR-1-producing *E. coli* isolates from samples of chickens and chicken meat, pigs and piglets, cattle, calves, turkeys, and humans in Cambodia [61,62], South Korea and China [53,63,64], Japan [65,66], Laos [67], Nepal [68], Pakistan [69,70], India [71], Thailand [67,72], and Vietnam [73,74]. In 2015, a study in Laos reported a possible clonal transmission of colistin-resistant *E. coli* between a domesticated pig and a human [67]. Although the direction of the transmission cannot be ascertained, the findings indicate that the fluidity of resistant agent transmission between species is of animal and public health significance.

4. Materials and Methods

4.1. Source of the Isolates

The design of this study, sampling, and data collection, and the process of isolation and identification have been described in our previous publication [75]. Briefly, isolates and data were obtained from a cross sectional study involving 35 village chicken farms across 4 states in central and Southern Peninsular Malaysia. Table 5 shows the distribution of *Salmonella* serotypes analyzed in this study. All work was carried out at the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia.
Table 5. Distribution of Salmonella serovars isolated across different samples from village chicken flocks in the South-central Peninsular Malaysia.

| Serovars Isolated       | Sources                          | Number (%) |
|-------------------------|----------------------------------|------------|
| Salmonella Weltevreden   | Cloacal swabs, feed, water       | 7 (20.6)   |
| Salmonella Typhimurium   | Cloacal swabs, feeds             | 6 (17.6)   |
| Salmonella Agona         | Cloacal swabs                    | 6 (17.6)   |
| Salmonella Enteritidis   | Cloacal swabs, water, feeds      | 3 (8.8)    |
| Salmonella Albany        | Water, feeds                     | 3 (8.8)    |
| Salmonella Molade        | Cloacal swabs, flies             | 2 (5.9)    |
| Salmonella Corvallis     | Cloacal swabs                    | 2 (5.9)    |
| Salmonella Schleissheim  | Flies                            | 1 (2.9)    |
| Salmonella spp.          | Cloacal swabs, water             | 4 (11.8)   |

4.2. Antimicrobial Susceptibility Testing

Antibiotic sensitivity testing of the isolates against 15 antibiotics was performed using the agar disc diffusion method [76] using antibiotic discs; ampicillin (10 µg), nalidixic acid (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), nitrofurantoin (300 µg), trimethoprim (5 µg), tetracycline (30 µg), kanamycin (30 µg), amoxicillin–clavulanate (20/10 µg), cefotaxime (30 µg), norfloxacine (10 µg), sulfonamides (300 µg), streptomycin (10 µg), and ceftiofur (30 µg). For colistin, the minimum inhibition concentration were determined by broth microdilution, using the MIC-Strip Colistin (MERLIN Diagnostika GmbH, Bornheim, Germany), in accordance with the international standard reference method (ISO 20776-1), and as recommended by the EUCAST subcommittee [77]. Test procedures were performed according to the manufacturer’s instructions, and interpretative MIC breakpoints were based on the EUCAST criteria (http://www.eucast.org/clinical-breakpoints/).

Antibiotics were selected based upon the recommendations of the World Health Organization (WHO) and World Organization for Animal Health for the use of antimicrobials in both human and food-producing animals. Colistin was recently added to the list of critically important antibiotics used in food-producing animals [2,78].

The diameter of the zone of inhibition (mm) were interpreted according to the criteria of the The Clinical and Laboratory Standards Institute (CLSI) [76]. Strains were subsequently evaluated according to the CLSI breakpoints as susceptible, intermediate, or resistant. Digital Vernier calipers were used to measure the diameter of the zones of inhibition. S. Typhimurium (ATCC 29213) and S. Enteritidis (ATCC 25922) were used as reference strains for antibiotic disc control. A resistant isolate was defined as an isolate resistant to one or more of the agents tested, whereas isolates resistant to three or more classes of antimicrobials were classified as multidrug resistant (MDR) [79]. The multiple antibiotic resistance (MAR) index was defined as the proportion formed by the number of antibiotic types to which a particular isolate displayed resistance, to the total number of antibiotics to which the isolate had been evaluated for susceptibility [80,81]. MAR is a good tool for assessing health risk, and is used to determine whether an isolate originates from a region of high or low antibiotic usage [81]. An MAR index of >0.2 reflects a bacteria from a high risk source of contamination where several antibiotics or growth promoters are used, whereas <0.2 represent those from a low risk source or source with less antibiotic use [80,81].

MAR index = a/b, where “a” is the number of antibiotics to which the isolates were resistant, and “b” is the total number of antibiotics to which the isolate was exposed [80,81].

4.3. Data Analysis

Data generated from the study was subjected to descriptive analysis using MS Excel (version 2011) to obtain percentages and proportions. SPSS (version 22.0, IBM, Armonk, NY: IBM Corp.) was used for all analyses. Chi-square, Fisher’s exact test, or simple logistic regression was used for the univariable exploratory analysis to identify risk factors associated with the outcome variable. The outcome variable
was the presence or absence of multidrug resistant isolates (Salmonella isolates manifesting resistance to ≥3 classes of antimicrobial agents). The statistical significance level was set at $\alpha = 0.05$.

5. Conclusions

The present study found that the level of resistance to antibiotics among isolates recovered from local village chickens was much lower than those found in commercial chickens and their products. Therefore, village chickens could be an alternative to those consumers seeking to reduce exposure to antibiotic resistant pathogens via the food chain. The findings also highlight the presence of MDR isolates, including those resistant to colistin, despite minimal antibiotic usage in free-range chicken production systems. We conclude that prolonged exposure to the natural environment plays a critical role in the transmission of resistance. Nevertheless, the role of the environment in the perpetuation and transmission of agents of resistance requires further study in light of emerging global trends in free-range food animal production.

Author Contributions: S.M.J. executed the field and laboratory work, analyzed the data and wrote the manuscript. L.H. devised the project design and main conceptual ideas, facilitated data analyses and interpretation of data and co-wrote the manuscript. S.A.A. and Z.Z. facilitated the laboratory technical work and analyses. J.A. supervised technical fieldwork and communication with farmers. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by UNIVERSITI PUTRA MALAYSIA (UPM), grant number GP IPS/2017/9522900 and grant number GP-IPB/2019/9676500.

Acknowledgments: The authors thank Madam Fauziah Nordin for her laboratory technical assistance.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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