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Research

Salmonella Isolated From Raw Chicken Meats at Selected Slaughterhouses in Peninsular Malaysia; Their Antibiotic Resistance Profiles and Biofilm Formation on Nutrient-Limited Media

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ABSTRACT

Salmonella is one of the pathogens responsible for foodborne diseases. Antibiotic resistance of *Salmonella*, particularly multidrug-resistant (MDR) strains have emerged and are becoming more prevalent, which is a very serious issue worldwide. This study sought to determine the antibiotic resistance profiles of *Salmonella* isolated from raw chicken meats, which were collected at selected slaughterhouses in Peninsular Malaysia and evaluating its biofilm-forming capability on surfaces. Antibiotic resistance of 135 *Salmonella* isolates against 12 antibiotics were investigated via disk diffusion method. The biofilm-forming ability of the isolates was evaluated by crystal violet staining using two media; a tryptic soy broth (TSB) and a 1/20 TSB with incubation periods of 24 and 48 h at 37 °C. A total of 118 strains of *Salmonella* showed higher resistance to erythromycin (87.41%), followed by tetracycline (85.19%;); 93 of the isolates (68.88%) were multi-drug resistant. A greater quantity of *Salmonella* was able to produce biofilm when grown in 1/20-TSB (90.37%) compared to the growth in TSB (88.15%), respectively. The findings in this study showed high prevalence, antibiotic resistance, and the biofilm forming ability of *Salmonella* strains isolated from raw chicken meats, suggesting that effective measures are required to ensure food safety in the poultry industry.

Key words: Antibiotic resistance, chicken meat, *Salmonella*, biofilm formation, slaughterhouse

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INTRODUCTION

Salmonella is one of the prevalent bacteria that has been linked to foodborne illness across the world (Cui *et al*., 2016; Grant *et al*., 2016;). The disease caused by *Salmonella* bacteria has been regarded as a concerning problem in a variety of human infections and may contribute to a significant cause of morbidity, mortality, and economic loss (Sallam *et al*., 2014). Non-typhoidal *Salmonella* is responsible for about 93.8 million illnesses; of which an estimated 80.3 million are foodborne and bring about 155,000 deaths every year, which is a global burden concern to public health (Majowicz *et al*., 2010). In 2017, it was estimated that 535 000 (95% uncertainty) cases of non-typhoidal salmonella invasive disease occurred, 77 500 (46400–123 000) deaths and remaining 59100 (33 300–98100) deaths not attributable to HIV accounted for 4.26 million (2·38–7·38) disability-adjusted life-years (DALYs) (Stanaway *et al*., 2019). The Malaysian Ministry of Health (MOH) has published a report

indicating that the national incidence rate of food poisoning due to *Salmonella* infections was 45.7 per 100,000 population in 2018 (MOH, 2019).

Poultry has been considered as one of the major reservoirs of *Salmonella* dissemination (Vo *et al*., 2006; Jackson *et al*., 2013). As an affordable source of protein, Malaysians consume a lot of chicken and have become self-sufficient in meeting the demands of consumers (Ariffin *et al*., 2014). The incidence of *Salmonella* contamination in chicken farms has been previously reported (Thung *et al*., 2016; Nidaullah *et al*., 2017; Shafini *et al*., 2017). Thus, prevention and control measures are needed to reduce the dissemination of this pathogenic bacteria.

Antibiotic resistance of *Salmonella*, particularly multi-drug-resistant (MDR) strains is becoming more prevalent and is a global public health concern. Antibiotic resistance in *Salmonella*, particularly multi-drug-resistant (MDR) strains, is becoming increasingly common across the world. In the poultry sector, antibiotics are used for the promotion of growth, therapeutics (treating clinically sick animals), and prophylaxis (preventing or reducing the incidence of infectious disease) purposes (Thai *et al*., 2012). The use of antibiotics leads to genomic selective pressure by killing susceptible bacteria, which causes antibiotic-resistant bacteria to survive and multiply (Kemal *et al*., 2016). The occurrence of multi-drug resistant *Salmonella* isolated from raw chicken meat was previously reported (Thai *et al*. 2012; Ren *et al*. 2016)(Thai *et al*. 2012; Akbar & Anal 2013; Mir, Kashyap & Maherchandani 2015; Alcaine *et al*. 2016; Ren *et al*. 2016). Ta *et al*., (2014) reported that these bacteria most frequently develop resistance to tetracycline, ampicillin, chloramphenicol, streptomycin, nalidixic acid, trimethoprim, and sulphonamides. Therefore, it is important to monitor resistance among bacteria found in both animals and food products.

Salmonella has the propensity to develop biofilm on food contact surfaces. The clusters of bacterial cells adhered firmly together, and to surfaces embedded in a matrix of extracellular polymeric substances (Zhou *et al*., 2013), and became hard to eliminate once biofilm is formed on surfaces. In the food processing environment, biofilm formation by *Salmonella* on surfaces can occur through contaminated foods or food handlers and may become a source of contamination for the food. This could lead to spoilage of food products, lowered shelf life, and transmission of disease, affecting food safety and contributing to economic losses (Manijeh *et al*., 2008). In addition, the formation of biofilm could result in bio-fouling in the pipelines, rusting, and impedance of the heat transfer process or mechanical blockage. Among the common sites for the presence of *Salmonella* in food manufacturing areas are floors, drains, pipelines, walls, conveyors, and racks. Furthermore, bacteria can attach to surfaces such as plastic, glass, stainless steel, or rubber (Sinde & Carballo, 2000; Agarwal *et al*., 2011; Nillian *et al*., 2016).

To manage the risk of *Salmonella* infection to human health, an investigation on the level of cross-contamination of the pathogenic bacteria in slaughterhouses is needed. To date, the study of the occurrence of multi-drug resistant *Salmonella* isolated from raw chicken meat from slaughterhouses in Malaysia has concentrated more on certain states only (Thung *et al*., 2016; Nidaullah *et al*., 2017; Shafini *et al*., 2017; Sukri *et al*. 2021) while studies focused on every state in Malaysia is lacking. This research extends the body of work in this area by including samples from all states in Peninsular Malaysia.

This study aimed to determine the antibiotic resistance profile of *Salmonella* isolated from raw chicken meats and the pattern of multi-resistant isolates. Additionally, the biofilm-forming ability of *Salmonella* in different growth media and incubation periods was also investigated. The information on the trends of contamination of Salmonella in raw chicken meat could help in establishing prevailing serotypes in this bacterial community. The antibiotic resistance profiles of *Salmonella* isolates and the ability of *Salmonella* to form biofilm could promote awareness of controlling *Salmonella* at all production stages of raw chicken meats.

MATERIALS AND METHODS

Preparation of *Salmonella* isolates

Preliminary testing has detected a total of 135 Salmonella isolates out of 790 samples (17.09%) from raw chicken meats samples, which were collected from selected slaughterhouses in Peninsular Malaysia. The sampling areas of the slaughterhouses were divided into four different zones: the Northern Zone (States of Perlis, Kedah, Penang & Perak); the Central Zone (States of Selangor, Negeri Sembilan & Melaka); the Southern Zone (State of Johor); and the Eastern Zone (States of Terengganu, Kelantan & Pahang). Two pieces of raw chicken carcasses were collected from each slaughterhouse and inspected by a Veterinary Inspector (Department of Veterinary Services, Malaysia). The collection of five samples per slaughterhouse in the sampling plan was based on the General Guidelines on Sampling (CAC, 2004). Stocks were stored in Tryptic soy broth (TSB; Merck, Darmstadt, Germany)

containing 20% glycerol stock at -20 °C. Working cultures were grown on Tryptic Soy Agar (TSA; Merck, Darmstadt, Germany) and incubated at 37 °C overnight.

Antibiotic susceptibility testing

The antibiotic susceptibility testing was conducted using a standard disk diffusion method following a procedure by the Clinical and Laboratory Standard Institute (CLSI, 2015). The *Salmonella* isolates obtained from raw chicken meat were tested against 12 antibiotics. The antibiotics tested are shown in Table 1. All of the antibiotic discs were purchased from Oxoid (England). *Salmonella* was streaked on a tryptic soy agar (TSA) (Merck, Darmstadt, Germany) and grown for 24 hr at 37 °C. Five single colonies were picked and suspended in 10 mL of normal saline (Merck, Darmstadt, Germany). The normal saline with *Salmonella* colonies was swirled and the concentration was adjusted until 0.5 McFarland turbidity

Then, the culture was transferred onto a Mueller Hinton (MH) (Merck, Darmstadt, Germany) agar using a sterile cotton swab. The antibiotic disks were placed evenly using an antibiotic disc dispenser onto the agar plate surface. The space between the disks should not be narrower than 24 mm and the distance to the edge should be no less than 1 cm to prevent overlapping of inhibition zones. The plates were then incubated at 37 °C for 24 hr.

The results were interpreted as sensitive, intermediate, or resistant according to the CLSI (2015). *Escherichia coli* ATCC®35218™ was used as the negative control strain in the antibiotic susceptibility test. Isolates exhibiting resistance to at least three classes of antibiotics were classified as multi-drug resistant (MDR) (Tadesse *et al*., 2016). The diameters of inhibition zones, including the diameter of the disc were measured in millimeters (mm). Each isolate was interpreted as susceptible (S), intermediate (I), or resistant (R) according to the CLSI guidelines (CLSI, 2015). MAR indexing was analyzed for each isolate. MAR indexing is defined as a/b where 'a' indicates the number of resistant antibiotics while 'b' indicates the total number of tested antibiotics (Krumperman, 1983).

Serotyping

All *Salmonella* isolates were serotyped at the Veterinary Research Institute, Ipoh, Perak, Malaysia according to the Kauffman-White Classification Scheme. *Salmonella* serotyping was done using slide-agglutination and undertaken to identify surface antigens (lipopolysaccharides, O-antigens) and flagella (H-antigens) (Bio-Rad, Hercules, CA).

Quantification of biofilm

Preparation of bacteria culture

Salmonella strains (*n*=135) isolated from raw chicken meats were stored at -20 °C in tryptic soy broth (TSB; Merck, Darmstadt, Germany) with 20% glycerol. Isolates of *Salmonella* were transferred from stock cultures and grown on TSA (Merck, Darmstadt, Germany) incubated overnight at 37°C. Single mauve of colony was inoculated into TSB (Merck, Darmstadt, Germany) and then incubated at 37°C for 18 hr to 24 hr. Grown cultures were inoculated into TSB or diluted tryptic soy broth (1/20 TSB) in the 96-well microtiter plate.

Crystal violet assay

Tryptic soy broth (TSB) (Merck, Darmstadt, Germany) and 1/20 of diluted TSB (1/20 TSB) were used in this study. TSB is a laboratory medium, which is optimal for *Salmonella* growth, while 1/20-TSB is diluted TSB, which is prepared to mimic food industry conditions (Stepanović *et al*., 2004). The 1:20 dilution of TSB was prepared in the amount of 20 mL. Then, 1 mL of stock solution of TSB was measured and transferred to 19 mL of distilled water. All media were autoclaved at 121 °C for 15 min.

Quantification of biofilm production was performed using microtiter plate procedures with some modifications (Stepanović *et al*., 2004). The modifications were on the amounts of the media used in the microtitre plate, the phosphate buffer saline, amounts of methanol, and the measurement of optical density. First, sterile flat-bottomed 96-well polystyrene microtiter plates (Merck, Darmstadt, Germany) were filled with 180 µL of media (TSB or 1/20 TSB). Then, 20 µL of the grown culture of *Salmonella* isolates (preparation of bacterial culture) was added into each well. Next, 135 *Salmonella* isolates were tested in triplicate.

The negative-control wells contained only 200 µL of media (TSB or 1/20 TSB) per well. The microtiter plates were sealed and incubated at 37 °C for two different incubation times (24 hr & 48 hr). The content of the wells was poured out and the wells were washed three times with 250 µL phosphate buffer saline

(Vivantis Technologies, Selangor, Malaysia). The remaining attached bacteria were fixed using 200 µL methanol (Merck, Darmstadt, Germany) for 15 min and air-dried at room temperature.

Subsequently, each well was stained with 250 µL of 1% crystal violet for 10 min. The excess stain was rinsed off by filling the well with sterile distilled water. This water was then discarded by inverting the plates a total of three times. The microtiter plates were then tapped vigorously and air-dried. The crystal violet (Sigma-Aldrich, Seelze, Germany) bound to the formed biofilm mass was solubilized in 250 µL of decoloring solution (33% glacial acetic acid) (Sigma-Aldrich, Seelze, Germany) for 15 min and then added into each well. The optical density of the wells was measured at 590 nm (OD590 nm) using an automated microtiter reader (Bio-Rad Laboratory, Hercules, CA).

The *Salmonella* isolates were classified into the following categories; non-biofilm producer, or weak, moderate, or strong biofilm producer based on the average value of the optical density (OD) produced by the bacterial films. The cut-off OD (ODc) was defined as three standard deviations above the mean OD of the negative control. The isolates were classified as follows: OD ≤ ODc = non-biofilm producer; ODc < OD ≤ (2 × ODc) = weak biofilm producer; (2 × ODc) < OD ≤ (4 × ODc) = moderate biofilm producer; and (4 × ODc) < OD = strong biofilm producer. All tests were carried out in triplicate and the results averaged out.

Statistical analysis

Statistical analyses were carried out using SPSS (Statistical Program for Social Sciences, Chicago, IL) Software version 16.0. One-way ANOVA (Analysis of Variance) was used to analyze the average values and the significant differences between means of optical density of *Salmonella* isolates in both nutrient media (TSB and 1/20TSB) and incubation periods (24 hr & 48 hr). The relationship between the antibiotic-resistant phenotype and the biofilm formation in TSB and 1/20-TSB was analyzed via the Chi-square test. For all analyses, a *P*-value < 0.05 was considered significant.

RESULTS

Determination of antibiotics resistance profile among *Salmonella* isolates

The antibiotic resistance of 135 *Salmonella* strains isolated from raw chicken meat samples was classified into resistant (R), intermediate (I), or susceptible (S) (Table 1). Among 12 antibiotics tested, the highest percentage of resistance was found to be against erythromycin (87.41%), tetracycline (85.19%), and sulphamethoxazole/trimethoprim (55.55%), followed by streptomycin (29.63%) and ampicillin (26.63%). The isolates showed the most resistance to both antibiotics, with the resistance rate having increased compared to 1997, as reported by NARMS (National Antimicrobial Resistance Monitoring System) (Centre of Disease Control and Prevention (CDC), 2020).

The isolates demonstrated lower resistance against gentamicin (7.41%), cephalothin (5.96%), ceftriaxone (3.70%), and amoxicillin-clavulanic acid (2.22%). This research found that all the *Salmonella* isolates were susceptible to ciprofloxacin. However, lower resistance to enrofloxacin (22.96%) and nalidixic acid (17.04%) was observed although both are from the same class of antibiotics. Antibiotics from the fluoroquinolone class are the drugs of choice for treating severe *Salmonella* infections caused by multi-drug resistant isolates in adults (Parry & Threlfall 2008).

Of the 135 *Salmonella* isolates, 132 (97.78%) were found resistant to one or more of the antibiotics tested. Specifically, 8 isolates and 31 isolates were resistant to at least one and two antibiotic categories of the five antibiotic categories tested, respectively. In this research, 93 (68.88%) isolates of *Salmonella* were resistant to three or more antibiotic agents (MDR). The antibiotic resistance profiles of the S*almonella* isolates from various States around Peninsular Malaysia are shown in Table 2.

All *Salmonella* isolates (100%) from Melaka, N. Sembilan, and Perak showed resilience against erythromycin. Meanwhile, *Salmonella* isolates from Selangor, Kelantan, Penang, and Johor had between 80% and 98% resistance to erythromycin. *Salmonella* also showed a high percentage of resistance to tetracycline. In the results of the study, all *Salmonella* isolates from Penang, Perlis, and Melaka were 100% resistant to this antibiotic. *Salmonella* isolates from other States in Peninsular Malaysia showed 75% to 93% resistance towards tetracycline, quite a significant percentage compared to other antibiotics, although this antibiotic is one of the common therapeutic agents used in animal husbandry.

In addition, Pahang, Melaka, Perak, and Perlis showed resistance towards sulphamethoxazole/trimethoprim while the isolates in Perlis and Melaka were resistant against ampicillin. Most of the isolates were highly resistant to erythromycin from the macrolide group, tetracycline and sulphamethoxazole/trimethoprim from the sulphonamide group, and ampicillin from the β-lactam class. All isolates from Perlis and Melaka showed resistance towards three or more classes of antibiotics and were therefore

classified as isolates with multi-drug resistance. In contrast, less than 50% of the *Salmonella* isolates were resistant to the rest of the antibiotics. The results were tested for significance using the Chi-square test, which showed a significant variation between antibiotic resistance profiles for different States in Peninsular Malaysia (P<0.05).

Table 1. Antibiotic resistance profile of 135 *Salmonella* strains isolated from raw chicken meat samples

Antibiotic agents	Disk content (µg)	Number of isolates that were: (%)		
		Resistant (%)	Intermediate (%)	Susceptible (%)
Macrolides				
Erythromycin (E)	15	118 (87.41)	16 (11.85)	1 (0.74)
Tetracyclines				
Tetracycline (TE)	30	115 (85.19)	5 (3.70)	15 (11.11)
Sulfonamides				
Sulphamethoxazole/ Trimethoprim (SXT)	25	75 (55.55)	17 (12.60)	43 (31.85)
B-lactams				
Ampicillin (AMP)	10	40 (26.63)	1 (0.74)	94 (69.63)
Amoxycillin/ Clavulanic acid (AMC30)	30	3 (2.22)	3 (2.22)	129 (95.56)
Ceftriaxone (CRO)	30	5 (3.70)	1 (0.74)	129 (95.56)
Cephalothin (KF)	30	8 (5.96)	3 (2.22)	124 (91.85)
Aminoglycosides				
Streptomycin (S)	25	40 (29.63)	19 (14.07)	76 (56.30)
Gentamicin (CN)	10	10 (7.41)	1 (0.74)	124 (91.85)
Quinolones and fluoroquonolone				
Enrofloxacin (ENR)	5	31 (22.96)	48 (35.56)	56 (41.48)
Nalidixic acid (NA)	30	23 (17.04)	57 (42.22)	55 (40.74)
Ciprofloxacin (CIP)	5	0	0	135 (100)

The results of antibiotic resistance of 12 *Salmonella* serovars against 12 antibiotics (Table 3) show that *Salmonella* Corvallis was highly resistant against tetracycline (84.78%) and erythromycin (82.61%). This serovar was identified in a higher number of isolates compared to other serovars (46 isolates). Two isolates from *Salmonella* Corvallis were susceptible to all antibiotics. In Malaysia, *Salmonella* Corvallis is a non-typhoidal serovar commonly detected in food animals (Thong *et al*., 2015). The other most highly detected serovar was *Salmonella* Brancaster. This isolate was found resistant to tetracycline (100%) and erythromycin (94.44%) and had the same resistance to sulphamethoxazole/trimethoprim and ampicillin (88.89%). All of the isolates from *Salmonella* Indiana *and Salmonella* Cyprus were highly resistant to erythromycin (100%) and tetracycline (100%).

One isolate from *Salmonella* Hiddudify and *Salmonella* Hindmarsh was found resistant to erythromycin and sulphamethoxazole/trimethoprim. *Salmonella* Albany, on the other hand, was highly resistant to tetracycline. In addition, this research also identified one isolate from *Salmonella* Duesseldorf that was resistant to 8 antibiotics and discovered that *Salmonella* Bellevue was resistant to 2 antibiotics. The Chi-square test was used to analyze the results of the antibiotic resistance profiles of 7 major *Salmonella* serovars (82 *Salmonella* isolates) and a significant variation between both variables was detected (P<0.05) (Appendix 1).

A total of 32 resistance patterns were observed among the *Salmonella* isolates (Table 4), with the predominant resistance pattern being erythromycin + tetracycline (n=19). The most frequent pattern of multi-resistance was erythromycin + sulphamethoxazole/trimethoprim + tetracycline (n=15). The MAR index ranged from 0.08 to 0.75 for all *Salmonella* isolates. A MAR index >0.2 denotes a high-risk contaminated source (Kruperman, 1983).

Table 2. Antibiotic resistance profile of *Salmonella* strains recovered from various States in Peninsular Malaysia (*n*=135)

A.	State										
	Prls[b] (%)	Pen (%)	Kdh (%)	Prk (%)	Slgr (%)	N.S (%)	Mlka (%)	Jhr (%)	Phg (%)	Trg (%)	Kltn (%)
E[a]	1/2 (50)	5/6 (83.33)	7/9 (77.78)	9/9 (100)	32/33 (97.97)	18/18 (100)	1/1 (100)	24/27 (88.89)	1/4 (25)	7/12 (58.33)	13/14 (92.86)
TE	2/2 (100)	6/6 (100)	8/9 (88.89)	7/9 (77.78)	28/33 (84.85)	15/18 (83.33)	1/1 (100)	22/27 (81.48)	3/4 (75)	10/12 (83.33)	13/14 (92.86)
SXT	2/2 (100)	6/6 (100)	8/9 (88.89)	9/9 (100)	6/33 (18.18)	11/18 (61.11)	1/1 (100)	14/27 (51.85)	4/4 (100)	10/12 (83.33)	4/14 (28.57)
AMP	2/2 (100)	2/6 (33.33)	2/9 (22.22)	5/9 (55.56)	5/33 (15.15)	8/18 (44.45)	1/1 (100)	6/27 (22.22)	1/4 (25)	4/12 (33.33)	4/14 (28.57)
AMC	0 (0)	0 (0)	0 (0)	0 (0)	1/33 (3.03)	1/18 (5.56)	0 (0)	1/27 (3.7)	0 (0)	0 (0)	0 (0)
CRO	0 (0)	0 (0)	0 (0)	0 (0)	2/33 (6.06)	2/18 (11.11)	0 (0)	1/27 (3.7)	0 (0)	0 (0)	0 (0)
KF	0 (0)	0 (0)	0 (0)	0 (0)	5/33 (15.15)	2/18 (11.11)	0 (0)	1/27 (3.7)	0 (0)	0 (0)	0 (0)
S	0 (0)	4/6 (66.66)	5/9 (55.59)	2/9 (22.22)	10/23 (44.48)	6/18 (33.33)	1/1 (100)	3/27 (11.11)	1/4 (25)	1/12 (8.33)	7/14 (50)
CN	0 (0)	1/6 (16.67)	0 (0)	0 (0)	2/33 (6.06)	5/18 (27.78)	0 (0)	1/27 (3.7)	0 (0)	0 (0)	1/14 (7.14)
ENR	0 (0)	0 (0)	3/9 (33.33)	0 (0)	11/33 (33.33)	5/18 (27.78)	0 (0)	2/27 (7.41)	0 (0)	1/12 (8.33)	9/14 (64.29)
NA	0 (0)	2/6 (33.33)	0 (0)	0 (0)	7/33 (21.21)	7/18 (35)	0 (0)	1/27 (3.7)	0 (0)	3/12 (25)	3/14 (21.43)
CIP	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

A=Antibiotics.

[a]E=Erythromycin, TE=Tetracyline, SXT=Sulphamethoxazole/Trimethoprim, AMP=Ampicillin, AMC=Amoxycillin, CRO=Ceftriaxone, KF=Cephalothin, S=Streptomycin, CN=Gentamicin, ENR=Enrofloxacin, NA=Nalidixic Acid, CIP=Ciprofloxacin.

[b]Prls=Perlis, Pen=Penang, Kdh=Kedah, Prk=Perak, Slgr=Selangor, N.S=Negeri Sembilan, Mlka=Melaka, Jhr=Johor, Phg=Pahang, Trg=Terengganu, Kltn=Kelantan

Table 3. Number of Salmonella serovars (%) resistant to 12 different antibiotics (*n*=87)

A.	Serovar											
	S. C[b]	*S.* B	*S.* A	*S.* I	*S.* Cy	*S.* Br	*S.* T	*S.* E	*S.* Bell	*S.* D	*S.* H	*S.* Hind
E[a]	38[a]/46 (82.61)	17/18 (94.44)	5/7 (71.43)	4/4 (100)	3/3 (100)	1/2 (50.0)	2/2 (100)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)	1/1 (100)
TE	39/46 (84.78)	18/18 (100)	7/7 (100)	4/4 (100)	3/3 (100)	1/2 (50.0)	2/2 (100)	1/1 (100)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)
SXT	15/46 (32.61)	16/18 (88.89)	4/7 (57.14)	1/4 (25.00)	2/3 (66.67)	1/2 (50.0)	2/2 (100)	1/1 (100)	0/1 (0)	1/1 (100)	1/1 (100)	1/1 (100)
AMP	8/46 (17.39)	16/18 (88.89)	1/7 (14.23)	0/4 (0)	0/3 (0)	0/2 (0)	1/2 (50)	0/1 (0)	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)
AMC	3/46 (6.52)	0/18 (0)	0/7 (0)	0/4 (0)	0/3 (0)	0/2 (0)	0/2 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
CRO	5/46 (10.87)	0/18 (0)	0/7 (0)	0/4 (0)	0/3 (0)	0/2 (0)	0/2 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
KF	5/46 (10.87)	0/18 (0)	0/7 (0)	0/4 (0)	0/3 (0)	0/2 (0)	0/2 (0)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)
S	12/46 (26.07)	3/18 (16.67)	3/7 (42.86)	0/4 (0)	0/3 (0)	1/2 (50)	2/2 (100)	0/1 (0)	1/1 (100)	1/1 (100)	0/1 (0)	0/1 (0)
CN	4/46 (8.70)	4/18 (22.22)	1/7 (14.23)	0/4 (0)	0/3 (0)	0/2 (0)	0/2 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
ENR	14/46 (30.43)	2/18 (11.11)	1/7 (14.23)	0/4 (0)	0/3 (0)	0/2 (0)	0/2 (0)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)
NA	8/46 (17.39)	4/18 (22.22)	2/7 (28.57)	0/4 (0)	0/3 (0)	0/2 (0)	0/2 (0)	0/1 (0)	0/1 (0)	1/1 (100)	0/1 (0)	0/1 (0)
CIP	0/46 (0)	0/18 (0)	0/7 (0)	0/4 (0)	0/3 (0)	0/2 (0)	0/2 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)
*x/y (%)	151/552 (27.36)	80/216 (37.04)	24/84 (28.57)	9/48 (18.75)	8/36 (22.22)	4/24 (16.67)	9/24 (37.5)	3/12 (25.0)	2/12 (16.67)	8/12 (66.67)	2/12 (16.67)	2/12 (16.67)

A. =Antibiotics.

[a]E=Erythromycin, TE=Tetracyline, SXT=Sulphamethoxazole/Trimethoprim, AMP=Ampicillin, AMC= Amoxycillin, CRO=Ceftriaxone, KF=Cephalothin, S=Streptomycin, CN=Gentamicin, ENR=Enrofloxacin, NA=Nalidixic Acid, CIP=Ciprofloxacin.

[b]S.C = S. Corvallis, S. B = S. Brancester, S. A = S. Albany, S. I = S. Indiana, S. Cy = S. Cyprus, S. Br =S. Braenderup, S. T =S. Typhimurium, S. E = S. Enteritidis, S. Bell = S. Bellevue, S. D = S. Duesseldorf, S. H = S. Hiddudify, S. Hind = S. Hindmarsh.

Table 4. Resistance patterns of *Salmonella* and the respective MAR index (*n*=135)

Resistance pattern	No. of isolates	MAR Index
AMP[a]-E-SXT-TE-AMC-CN-KF-CRO-NA	2	0.75
AMP-E-SXT-TE-CN-S-KF-CRO-NA	1	0.75
E-ENR-SXT-TE-AMC-S-KF-CRO-NA	1	0.75
AMP-E-ENR-SXT-TE-S-KF-NA	1	0.67
AMP-E-SXT-TE-S-KF-CRO-NA	1	0.67
AMP-E-ENR-SXT-TE-S-NA	1	0.58
AMP-E-ENR-SXT-TE-KF-NA	1	0.58
AMP-E-SXT-TE-CN-S-NA	2	0.58
AMP-E-SXT-TE-S-NA	1	0.42
AMP-E-SXT-TE-CN-NA	1	0.42
AMP-E-SXT-AMC-KF-CRO	1	0.42
AMP-E-ENR-SXT-TE	2	0.42
AMP-E-SXT-TE-S	5	0.42
AMP-E-SXT-TE-NA	3	0.42
AMP-E-SXT-TE-CN	3	0.42
E-ENR-SXT-TE-S	2	0.42
E-ENR-TE-S-NA	2	0.42
AMP-E-SXT-TE	10	0.33
AMP-E-SXT-NA	3	0.33
E-SXT-TE-S	5	0.33
E-ENR-TE-S	11	0.33
E-ENR-SXT-TE	1	0.33
E-TE-S-NA	1	0.33
AMP-SXT-TE	2	0.25
E-ENR-SXT	1	0.25
E-SXT-TE	15	0.25
E-TE-KF	1	0.25
E-TE-S	3	0.25
E-TE-CN	1	0.25
E-TE-NA	1	0.25
E-ENR-TE	8	0.25
SXT-TE-S	2	0.25
AMP-S	1	0.17
E-TE	19	0.17
E-SXT	2	0.17
SXT-TE	6	0.17
SXT-NA	1	0.17
E	5	0.08
SXT	1	0.08
NA	1	0.08
TE	1	0.08

[a]E=Erythromycin, TE=Tetracyline, SXT=Sulphamethoxazole/Trimethoprim, AMP=Ampicillin, AMC=Amoxycillin, CRO=Ceftriaxone, KF=Cephalothin, S=Streptomycin, CN=Gentamicin, ENR=Enrofloxacin, NA=Nalidixic Aci

Quantification of biofilm formation of *Salmonella* isolates

The *Salmonella* isolates were cultivated in two growth media (TSB and 1/20-TSB) with ΔOD_{590} under two different incubation periods (24 hr & 48 hr) at 37 °C. The results of the *Salmonella* isolates after growth in TSB and 1/20TSB are summarised in Table 5 (TSB) and Table 6 (1/20-TSB) for the incubation period of 24 hr and 48 hr, respectively.

More than 85% of the Salmonella isolates formed biofilm within ΔOD_{590} values ranging from 0.093 ± 0.250 to 0.627 ± 0.172 in TSB and 1/20-TSB growth media, respectively. For TSB, a cut-off value of 0.156 at OD590 nm was used to categorize the isolates as non-biofilm producers, or weak, moderate, or strong biofilm producers. The ΔOD_{590} values for the *Salmonella* isolates growth in TSB media was 0.305 ± 0.115 after 24 hr of incubation, subsequently decreasing to 0.269 ± 0.161 after 48 hr incubation. Most of the *Salmonella* isolates identified were classified as weak biofilm producers in TSB at 45.19% and 40.0%, followed by moderate biofilm producers at 25.19% and 30.37%, and only a small number produced strong biofilm at 18.52% and 17.04%, respectively in 24 hr and 48 hr (Table 5).

The results of biofilm formation of *Salmonella* isolates grown in 1/20-TSB are presented in Table 6. For 1/20-TSB, a cut-off value of 0.175 at OD590nm was used to categorize the isolates as non-biofilm

producers or weak, moderate, or strong biofilm producers. The OD_{590} values found in 1/20-TSB were higher compared to the TSB OD_{590} values.

Table 5. Biofilm formation of *Salmonella* in TSB medium at 24 hr and 48 hr incubation periods

Incubation period	24 hr		48 hr	
Biofilm formation	No. of isolates (%)	Av. ± STD[a] 590 nm	No. of isolates (%)	Av. ± STD 590 nm
Strong biofilm producer	25 (18.52)	0.585 ± 0.227[b]	23 (17.04)	0.484 ± 0.352
Moderate biofilm producer	34 (25.19)	0.223 ± 0.088	41 (30.37)	0.215 ± 0.097
Weak biofilm producer	61 (45.19)	0.107 ± 0.031	54 (40.00)	0.109 ± 0.034
Total biofilm produced	120/135 (88.89)	0.305 ± 0.115	118/135 (87.41)	0.269 ± 0.161

[a]Average ± standard deviation.

[b]Values are expressed as the average ± standard deviation of the three replicate

Table 6. Biofilm formation of *Salmonella* in 1/20-TSB at 24 hr and 48 hr incubation periods

Incubation period	24 hr		48 hr	
Biofilm formation	No. of isolates (%)	Av. ± STD[a] 590 nm	No. of isolates (%)	Av. ± STD 590 nm
Strong biofilm producer	34 (25.19)	0.379 ± 0.110[b]	37 (27.41)	0.627 ± 0.172
Moderate biofilm producer	59 (43.70)	0.206 ± 0.064	27 (20.00)	0.193 ± 0.094
Weak biofilm producer	35 (25.93)	0.093 ± 0.025	52 (38.52)	0.099 ± 0.031
Total biofilm produced	128/135 (94.81)	0.226 ± 0.066	116/135 (85.93)	0.306 ± 0.099

[a]Average ± standard deviation.

[b]Values are expressed as the average ± standard deviation of the three replicates

In 1/20-TSB, the average OD_{590} values for *Salmonella* isolates tested were 0.226 ± 0.066 and 0.306 ± 0.161 for 24 hr and 48 hr of incubation, respectively. Most *Salmonella* isolates were classified as moderate biofilm producers (43.7%) at 24 hr followed by 25.93% as weak biofilm producers and 25.19% as strong biofilm producers. After 48 hr incubation, most of the *Salmonella* isolates formed weak biofilm in 1/20-TSB (38.52%). 27.41% were strong biofilm producers followed by 20.0% as moderate biofilm producers. The number of *Salmonella* isolates forming biofilm decreased from 94.81% to 85.93% after 48 hr. There was a significant difference ($P<0.05$) between the OD_{590} values of the TSB and 1/20-TSB growth media, however, the difference was not significant ($P>0.05$) between the OD_{590} values for the two incubation periods (24 hr & 48 hr) at a 95% confidence interval.

Among the 25 strong biofilm producers in the TSB medium after 24 hr of incubation, 76% were MDR isolates and 24% were non-MDR. The 61 weak biofilm producers consisted of 81.97% MDR isolates while 18.03% were non-MDR isolates. The 15 isolates that were non-biofilm producers consisted of 73.3% MDR and 26.67% non-MDR isolates. After 48 hr of incubation, 23 strong biofilm producers were found consisting of 73.91% MDR isolates and 26.09% non-MDR.

Among the 54 weak biofilm producers, 66.67% were MDR isolates and 33.33% were non-MDR. The 17 isolates that had negative biofilm formation consisted of 70.59% MDR and 29.41% non-MDR (Table 7). These results reveal that most of the biofilm formed in the TSB medium contained a larger proportion of MDR isolates. The statistical analysis showed no significant difference ($P>0.05$) between the resistance phenotype and type of biofilm producers of *Salmonella* in the TSB (Appendix 2).

Table 7. Distribution of resistance phenotype for different biofilm producers among *Salmonella* isolates grown in TSB

	TSB			
Incubation period	24 hr		48 hr	
Resistance phenotype	MDR[a] (%)	Non-MDR (%)	MDR (%)	Non-MDR (%)
Strong biofilm producer	19/25 (76%)	6/25 (24%)	17/23 (73.91)	6/23 (26.09%)
Moderate biofilm producer	21/34 (61.76%)	13/34 (38.24)	28/41 (68.29%)	13/41 (31.71%)
Weak biofilm producer	50/61 (81.97%)	11/61 (18.03%)	36/54 (66.66%)	18/54 (33.33%)
None biofilm producer	11/15 (73.33%)	4/15 (26.67)	12/17 (70.59%)	5/17 (29.41)

[a]multidrug-resistant

Salmonella isolates grown in 1/20-TSB showed that 34 isolates were strong biofilm formers after 24 hr of incubation, among which 82.35% were MDR isolates and 17.65% were non-MDR. The 35 weak biofilm producers consisted of 60.00% MDR isolates and 40.00% non-MDR isolates. The 7 isolates with no biofilm formation consisted of 71.43% MDR and 14.29% non-MDR isolates. Thirty-seven strong biofilm producers after 48 hr of incubation consisted of 73.91% MDR isolates and 35.16% non-MDR. Among the 52 weak biofilm-formers, 65.38% were MDR isolates and 34.62% were non-MDR. None biofilm producers consisted of 19 isolates comprising 68.42% MDR and 31.58% non-MDR (Table 8).

Table 8. Distribution of resistance phenotype for different biofilm producers of *Salmonella* isolates grown in 1/20-TSB

1/20-TSB				
Incubation period	24 hr		48 hr	
Resistance phenotype	MDR[a] (%)	Non-MDR (%)	MDR (%)	Non-MDR (%)
Strong biofilm producer	28/34 (82.35)	6/34 (17.65)	24/37 (64.86)	13/37 (35.16)
Moderate biofilm producer	38/59 (64.41)	21/59 (35.59)	23/27 (85.19)	4/27 (14.81)
Weak biofilm producer	21/35 (60.00)	14/35 (40.00)	34/52 (65.38)	18/52 (34.62)
None biofilm producer	5/7 (71.43)	2/7 (14.29)	13/19 (68.42)	6/19 (31.58)

[a]multidrug-resistant

These results show that MDR *Salmonella* isolates growth in 1/20-TSB tend to be classified as strong and moderate biofilm producers. The 1/20-TSB medium is, therefore, an effective medium for promoting biofilm formation among *Salmonella* isolates although it is a nutrient-limited medium. The findings also show that the biofilm quantities of *Salmonella* increased when 1/20-TSB was used as a growth medium. 1/20-TSB does not provide many nutrients compared to rich laboratory media; however, the chosen 1/20-TSB is considered to mimic conditions in the food industry (Stepanović *et al*., 2004). No significant difference ($P>0.05$) between the resistance phenotype and type of biofilm producers of *Salmonella* in 1/20-TSB was detected based on the Chi-square test (Appendix 3).

A correlation test was carried out using Goodman and Kruskal's Gamma correlation coefficient to determine the relationship between resistance phenotype (MDR and non-MDR *Salmonella* isolates) and the ability of the *Salmonella* isolates to form biofilms. The finding showed a positive correlation between biofilm-forming ability and the number of antibiotic-resistant isolates; however, this correlation was not significant ($r_s=0.077$, $P>0.05$; Table 9).

Table 9. Biofilm forming ability of *Salmonella* isolates with different antibiotic resistance phenotype

Resistance Phenotype	OD_{590} [a]	r_s	P-value
Non-MDR	0.2186 ± 0.077	0.05	>0.05
MDR[b]	0.2190 ± 0.080		

[a]OD_{590}, optical density at 590nm; data shown in average ± standard deviation

[b]MDR, multi-drug resistant

DISCUSSION

Salmonella Corvallis was identified in a higher number of isolates compared to other serovars (46 isolates). Thong and Modarressi (2011) also found that *Salmonella* Corvallis in raw meat was the most resistant to antibiotics. In Malaysia, *Salmonella* Corvallis is a non-typhoidal serovar commonly detected in food animals (Thong *et al*., 2015). Notably, this research discovered *Salmonella* Typhimurium and *Salmonella* Enteritis as multi-resistant isolates. Both serovars are commonly encountered in food animals, primarily in poultry, and are common causes of salmonellosis (Modarressi & Thong 2010). Thus, raw chicken meat is a primary source of non-typhoidal *Salmonella* that can spread to humans.

According to Krumperman (1983), the MAR index >0.2 denotes a high-risk contaminated source. Overall, 70.37% of *Salmonella* isolated from raw chicken meat exhibited more than 0.2 MAR index. The broader MAR index for *Salmonella* isolates indicates these isolates often used antibiotics in animal feeds as growth promoters (Krumperman, 1983; Adzitey *et al*., 2012). The MAR index reported by Wang *et al*. (2013) was 0.09 to 0.91, which is higher than the results of this research.

The finding in this research is slightly similar to that of Yoke-Kqueen *et al*. (2008), which reported the resistance of *Salmonella* against erythromycin (100%) and tetracycline (85%). This *Salmonella* was isolated from poultry. A study by Gharieb *et al*. (2015), discovered *Salmonella* isolates that were resistant to erythromycin (100%), as well as tetracycline (100%) followed by sulphamethoxazole (83.3%). Yildirilm *et al*. (2011) also found that *Salmonella* could grow in the presence of erythromycin (89.7%) but had lower rates of growth in tetracycline (67.6%). Erythromycin has a large molecular size

that can pass through the outer membrane of the bacterial cell (Najwa *et al*., 2015); therefore, it is unable to affect *Salmonella.* Besides that, the increase in *Salmonella* resistance towards tetracycline has been frequently observed in line with the frequent use of this antibiotic as an antibiotic agent in animal production (Ta *et al*., 2014).

Carramiñana *et al*. (2004) and Lampang *et al*. (2013) reported the susceptibility of *Salmonell*a isolates to ciprofloxacin. According to Lampang *et al*. (2013), ceftriaxone and ciprofloxacin are classified as critically important human medicines based on the World Health Organisation (WHO). Quinolones have been the choice of antibiotics for treating infections with MDR (multi-drug resistance) *Salmonella* (Goncuoglu *et al*., 2016). Although *Salmonella* is still susceptible to ciprofloxacin, CDC (2013) reported an outbreak of *Salmonella* resistant to quinolone in the United States. On the other hand, Chia *et al*. (2009) found a low incidence of *Salmonella* isolates that were resistant to ciprofloxacin (3%).

Mariappan *et al*. (2021), reported the Malaysian government's goal to strengthen antibiotic surveillance and monitoring, engage in more research, and provide education and awareness to farmers, the general public, and users of antimicrobials in the animal health industry. The contribution to resistant bacteria could be caused by different sample sizes, the nature of the drug, characteristics of the bacteria, development of resistant genes, a lack of prevention and control, as well as the low number of research on chicken, with a focus only on bacteria resistance (Beyene *et al*. 2016). The extensive use of antibiotics in animal husbandry has also promoted the appearance of antibiotic-resistant bacteria (Van *et al*. 2007).

There is a remarkable variation in the resistance of *Salmonella* to a wide range of antibiotic agents as per research conducted all over the world. In Spain, *Salmonella* isolates with resistance as high as 100% (Carramiñana *et al*. 2004) were observed. A report by Chotinun *et al*. (2015) showed that *Salmonella* was 68.4% resistant to at least one antibiotic, which is lower than the results obtained in this research. According to Rincon-Gamboa *et al*. (2021) exemplified that the predominant antimicrobial-resistant isolates of Non-Typhoidal Salmonella (NTS) isolated from beef, pork, chicken meat, and other meat products came from poultry. Enteritidis and Typhimurium were the most reported serovars by minimum inhibitory concentration (MIC).

In this research, 68.88% of the *Salmonella* was resistant to three or more antibiotic agents (MDR). This result shows *Salmonella* with a higher percentage resistance compared to the results of Bacci *et al*. (2012) and Chotinun *et al*. (2015), which were 40.0% and 50.6%, respectively. According to Capita *et al*. (2007), the high level of resistant isolates in many publications could be due to the overuse of antibiotics in different fields worldwide, leading to enormous pressure in selecting antibiotics that bacterial pathogens are not resistant to. The appearance of multi-resistant *Salmonella* isolates is a serious problem affecting public health. Besides, certain bacteria have inherent characteristics that resist antibiotics. Continuous surveillance and more prudent use of antibiotics added into animal feed are among the wise suggestions to diminish multi-resistant bacteria (Carramiñana *et al*., 2004).

As mentioned, the presence of antibiotic-resistant *Salmonella* isolates may cause serious infections in humans and livestock. Effective strategies and new legislation must, therefore, be established to ensure a reduction of bacteria resistant to antibiotics. Studies on antibiotic-resistant *Salmonella* conducted in many countries provide an understanding of the potential for *Salmonella* bacteria to disseminate around the world. Poor sanitation, inadequate health care systems, and abuse of antibiotics are the factors that contribute to the multi-drug resistance of *Salmonella* (Franco *et al*., 2009).

Salmonella can adapt and respond to a varied range of adverse environmental conditions. These bacteria are also notable for attaching to either abiotic or biotic surfaces to create biofilm (Giaouris & Nesse, 2015). According to Chmielewski and Frank (2003), the adhesive properties of fimbriae on *Salmonella* at food contact surfaces possibly contribute to serious potential risks in food safety cases because of possible cross-contamination. Biofilm-forming capacity, which is one of the properties of *Salmonella*, has been extensively studied and investigated in correlation with several surfaces commonly encountered in food facilities (Nguyen *et al*., 2014). In this research, most of the *Salmonella* isolates had the ability to form a biofilm on plastic surfaces. Djordjevic *et al*. (2002) also noted the ability of *Salmonella* isolates to form biofilms on plastic surfaces. According to Sinde and Carballo (2000), plastic is a hydrophobic material, so it is easy for bacteria to attach to it. This is because plastic has a hydrophobic nonpolar nature with little or no surface charge, which makes it easy for bacteria to attach to its surface. Adhesion is the initial step in the biofilm formation process after which bacteria will produce a biofilm in high numbers, especially on plastic surfaces (Donlan 2002).

The majority of *Salmonella* isolates obtained in this research were weak to moderate biofilm producers. This result is consistent with the findings of Ghasemmahdi *et al*. (2015), which reported that the majority of *Salmonella* isolates (60.52%) were incapable of biofilm formation. Specifically, 26.31%,

7.89%, and 5.26% of the isolates were weak, strong, and moderate biofilm producers, respectively. According to Nair *et al*. (2015), weak biofilm producers especially isolates from poultry, might be able to produce new genetic traits, plasmids, or other external selective pressures, which may expand their pathogenic potential over some time. Agarwal *et al*. (2011) reported that the majority of *Salmonella* serotype strains (57.61%) were found to be moderate biofilm producers, while 22.52% and 19.21% strains were weak and strong biofilm producers on plastic surfaces, respectively. Other previous studies showed that *Salmonella* that was able to form a biofilm on plastic surfaces were normally classified as strong biofilm produces (Stepanović *et al*., 2003; Solomon *et al*., 2005). The strong and moderate biofilm-producing isolates were more resistant to various antibiotics and colonises the environment compared to the weak or non-biofilm producers (Singh *et al*., 2017).

The OD values generated from an ELISA reader showed results between 24 hr and 48 hr of incubation periods. As shown in Table 5 and Table 6, at 24 hr, the ΔOD values detected for TSB and 1/20-TSB were similar, but the quantity of *Salmonella* isolates identified were greater in 1/20-TSB than TSB (94.81% & 88.89%, respectively). This result is in agreement with the findings of Stepanović *et al*. (2004), which reported that although the diluted TSB (1/20-TSB) selected as the medium for biofilm formation was low in nutrients, the bacteria were still able to adapt and survive the stressful conditions of that particular environment. TSB is a laboratory medium, which is optimal for *Salmonella* growth, while 1/20-TSB is diluted TSB, which is a different approach for quantifying the biofilm formation of *Salmonella*. Nevertheless, the 1/20-TSB medium is often used to mimic food industry conditions (Stepanović *et al*., 2004). The attachment of *Salmonella* may result in a serious problem for the food industry. The nutrient content or composition of media influenced the ability of bacteria to form biofilms (Hood & Zottola, 1997). Besides, bacteria may be exposed to different levels of nutrients depending on the location of processing. Using a diluted growth medium might enhance the expression of the promoter, agfD, which is involved in the *Salmonella* spp. biofilm formation (Keelara *et al*., 2016).

The quantity of *Salmonella* isolates producing biofilm decreased after 48 hr incubation at 37 °C for both the TSB and 1/20-TSB growth media. The bacteria were unable to survive after 48 hr although biofilm had already been formed. This might be due to the depletion of nutrients in the growth media. However, other research reported that 48 hr of incubation was optimal for *Salmonella* spp. to form a biofilm (Agarwal *et al*., 2011). Incubation time is, therefore, one of the factors that influence the biofilm formation of bacteria. Meanwhile, according to Čabarkapa *et al*. (2015), biofilm in its initial phase was formed after 24 hr accompanied by slight cell aggregation while more intensive cell aggregation followed by the formation of a microcolony occurred after 48 hr.

When there is a lower level of nutrients or when the bacterial cells are in the stationary phase, the maximum expression of aggregative fimbriae will take place, causing biofilm to form (Gerstel & Römling 2001). Another Gram-negative organism, *E. coli*, developed biofilm faster when the organism was grown in low-nutrient media, resulting in the recovery of a higher number of adherent cells (Stepanović *et al*. 2004). In other words, nutrient limitation can lead to increased biofilm formation as the bacteria can adapt to stressful conditions.

Stepanović *et al*. (2004) reported growth media as statistically significant for the formation of biofilm, similar to this current research. However, 1/20-TSB proved the most effective medium for biofilm production based on the tested *Salmonella* isolates (average O.D. was 0.51 ± 0.177), followed by TSB (0.286 ± 0.065) (Stepanović *et al*. 2004). Wang *et al*. (2013) also reported both the types of growth media and incubation time were significant factors in influencing the formation of biofilm. There was a positive correlation between the formation of biofilm and antibiotic resistance phenotype, which contradicts the previous study (Wang *et al*., 2013). Therefore, findings are sometimes inconsistent and correlations are species-independent.

This research showed that the identified *Salmonella* isolates could form a biofilm on plastic surfaces. The amount of biofilm formation of the *Salmonella* isolates was significantly influenced by the growth media used in this study. Therefore, *Salmonella* can form biofilm even if only a single species is involved. Hence, using mixed microbial populations to study the formation of a biofilm could produce novel findings on the growth and interaction of these bacteria. Therefore, the selection of the material type for surfaces of production lines such as cutting boards or tables is of paramount importance in slaughterhouses to assure the safety and quality of the processed chicken meat.

Salmonella was isolated from raw chicken meat, which is a potential reservoir of AMR *Salmonella* dissemination. The surveillance of resistant *Salmonella* in the food chain and the resistance gene would be needed for further study. Therefore, further studies on the molecular characterization of the isolates and resistance genes should be pursued to determine the mechanism of AMR development. A polystyrene plate could be an ideal surface for the attachment of *Salmonella* and could, therefore,

promote the growth of biofilm. The result from this study demonstrated that although *Salmonella* could adhere to polystyrene surfaces, its ability to form a biofilm was categorized as weak to moderate in the TSB and 1/20- TSB media. However, the finding also indicates the capacity of MDR *Salmonella* isolates as a possible major contributor to biofilm formation which could potentially cause a devastating outbreak if control practices that could mitigate the occurrence of salmonellosis were not established in the chicken slaughterhouses.

CONCLUSION

The present study investigated the occurrence, antibiotic resistance profile, and biofilm-forming ability of *Salmonella* isolated from raw chicken meats from slaughterhouses in Peninsular Malaysia. The result demonstrated that most of the isolates were resistant to one or more of the antibiotics tested, with a considerably high number of *Salmonella* isolates being resistant to three or more antibiotic agents (MDR). This suggests that better antibiotic stewardship is needed to reduce the multidrug-resistant isolates of *Salmonella*. This study also detected *Salmonella* Corvallis as the predominant serovar. The information on the trends of contamination of *Salmonella* in raw chicken meat could help in establishing prevailing serotypes in this bacterial community. The study on the biofilm-forming ability of *Salmonella* on polystyrene plate surfaces revealed that the type of growth medium used had significantly affected the amount of biofilm formed but incubation time had no significant effect. Although *Salmonella* could adhere to plastic surfaces, its ability to form a biofilm was categorized as weak to moderate in the tested growth media. Further studies investigating the relationship between the multi-drug-resistant isolates and the biofilm formation of *Salmonella* can be further explored, as there is still a need to understand the mechanismthat the resistant isolates use in biofilm formation.

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ETHICAL STATEMENT

The authors declare no conflict of interest.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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