Analysis of *Salmonella enterica* Enteritidis isolates from chicken and chicken meat products using PFGE, and MLST

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Abstract

Background

Salmonella is a very important food-borne pathogen causing illness in humans. The emergence of drug-resistant strains also constitutes a serious worry to global health and livestock productivity. This study investigated Salmonella isolates from poultry and poultry products using the phenotypic antimicrobial screening as well as the molecular characteristics of *Salmonella* isolates. Upon serotyping of the isolates, the antimicrobial susceptibility profiling using a panel of 9 commonly used antimicrobials was done. Subsequently, the molecular profiles of all the isolates were further determined using Pulsed Field Gel Electrophoresis (PFGE) and the Whole Genome Multi-Locus Sequence Type (wgMLST) analysis in order to obtain the sequence types.

Results

The PFGE data was input into FPQuest software, and the dendrogram generated was studied for possible genetic relatedness among the isolates. All the isolates were found to belong to the S. Enteritidis serotype with notable resistance to tetracycline, gentamycin, streptomycin, and sulfadimidine. The S. Enteritidis isolates tested predominantly subtyped into the ST11 and ST1925, which was found to be a single cell variant of ST11. The STs were found to occur in chicken meat, food, and live chicken cloacal swab, which may indicate the persistence of the bacteria in multiple foci.

Conclusion

The data demonstrate the presence of S. Enteritidis among chicken, indicating its preference and reservoir status for enteric salmonella pathogens.

Background

The continuous emergence of multidrug-resistant strains of non-typhoidal Salmonella constitutes a serious health hazard globally. In recent years, enteric Salmonella enterica associated with gastrointestinal infection in humans has been reported with increasing frequency worldwide [1]. Salmonella Enteritidis is the most common cause of food-borne infection in humans. While the majority of the infections are mild self-limiting illnesses, a small number have been reported to cause invasive infections, which is characterized by severe infections that require hospitalization [2]. The popularity of Salmonella Enteritidis is attributed to the unique ability of this serotype to contaminate chicken egg and meat without any discernible illness to the chickens [3]. Furthermore, multiple investigations have identified antimicrobial resistance phenotypes of *Salmonella Enteritidis* from among various food materials of poultry origin [4].

In Malaysia, retail chicken meat has been reported as an essential source of multiple antimicrobial-resistant Salmonella with *Salmonella enterica* serovar Enteritidis accounting for 6.7% [5]. These

multidrug-resistant (MDR) *Salmonella* are considered as serious public health problem due to tendencies for transmission of resistance to humans across the poultry production chain, thus it has become paramount to identify and characterize this important pathogen [6]. Moreover, concerns over the emergence of enteric Salmonella with increased virulence, transmissibility, and antibiotic-resistance features, has necessitated the need for highly efficient methods that can identify these variant pathogens to track their spread especially across the human, animal and environmental interface [7]. In this regard, molecular techniques including whole genome sequencing, Pulse Field Gel Electrophoresis (PFGE), and Multi-locus Sequence Typing (MLST) are among the commonly employed methods. These techniques can characterize pathogens in order to determine clonal and strain distribution across various environments and hosts.

Pulsed-field gel electrophoresis is one of the most widely used methods for the epidemiological studies of pathogenic bacterial organisms due to its high discriminatory ability [8]. With the globalization of trade, including poultry and poultry products, PFGE can be useful in understanding the diversity and evolution of infectious disease agents in order to evaluate their genetic relatedness to determine their point source during epidemiological investigations [9]. The principle of this method is based on the restriction enzyme digestion of whole DNA to produce fragment patterns that vary from strain to strain. The method relies on the distinct genomic differences between isolates that are observed as a result of the rapid accumulation of genetic variations that lead to slightly detectable differences between DNA fingerprints patterns within a clone [7].

Multi-Locus Sequence Typing (MLST) analysis, on the other hand, is best suited for longer-term as well as global epidemiologic investigations of infectious disease agents [7]. It is based on the principle of the multi-locus enzyme electrophoresis that uses a combination of discriminatory power and clonal stability, which has proven to be extremely efficient in characterizing clones within a population of bacterial organisms causing serious disease [10]. However, it uses allelic differences in the sequence of various house-keeping genes that are often exploited to differentiate strains (Wang, & Su, 2020). In this investigation, the phenotypic antimicrobial resistance profile and molecular characteristics of *Salmonella enterica* serovar Enteritidis isolated from chicken and food samples was assessed to determine the antimicrobial resistance variability and distribution.

Results

Serotyping of Salmonella and Antimicrobial Resistance

All of the 47 samples analyzed by the classical serotyping slide agglutination test comprising of food (7), chicken meat (11) and chicken cloacal swab (29), in accordance with the White–Kauffmann–Le Minor scheme and only isolates belonging to the *Salmonella enterica* Enteritidis serotype were selected for this study. Based on the phenotypic antimicrobial resistance pattern of the isolates against the nine (9) different antimicrobial drugs, 27 (57.45%) of the isolates were found to show resistance to 1 or more

antimicrobials tested (Table 1). However, out of the 20 that were susceptible to the drugs tested, 6 showed intermediate resistance to Ampicillin, Streptomycin, and Tetracycline.

Table 1
Antimicrobial resistance pattern of the 47 *Salmonella* Enteritidis isolates

New ID	Source	AMR	ST	Remark
CM&MP01	Chicken meat	-	1925	Susceptible
CM&MP02	Chicken meat	TE	1925	Tetracycline resistant
CM&MP03	Food	-	11	Susceptible
CM&MP04	Food	TE	1925	Tetracycline resistant
CM&MP05	Food	-	11	Susceptible
CM&MP06	Food	TE	1925	Tetracycline resistant
CM&MP07	Food	TE	1925	Tetracycline resistant
CM&MP08	Chicken meat	-	11	Susceptible
CM&MP09	Chicken meat	TE	1925	Tetracycline resistant
CM&MP010	Chicken meat	TE	1925	Tetracycline resistant
CM&MP011	Food	TE	1925	Tetracycline resistant
CM&MP012	Food	TE	1925	Tetracycline resistant
CM&MP013	Chicken meat	-	1925	Susceptible
CM&MP014	Chicken meat	TE	1925	Tetracycline resistant
CM&MP015	Chicken meat	-	11	Susceptible
CM&MP016	Chicken meat	-	1925	Susceptible
CM&MP017	Chicken meat	-	1925	Susceptible
CM&MP018	Chicken meat	SXT	292	Sulfamethazine/Trimeth
CCS01	Chicken swab	TE	1925	Tetracycline resist
CCS02	Chicken swab	TE	1925	Tetracycline resistant
CCS03	Chicken swab	-	1925	Susceptible
CCS04	Chicken swab	-	11	Susceptible
CCS05	Chicken swab	TE	11	Tetracycline resistant
CCS06	Chicken swab	-	11	Susceptible
CCS07	Chicken swab	AMP	11	Ampicillin resistant
CCS08	Chicken swab	AMP;CN;TE	11	**Multidrug resistant**

Key: CM&MP-Chicken Meat & Meat Products; CCS-Chicken Cloacal Swab.

New ID	Source	AMR	ST	Remark
CCS09	Chicken swab	TE	1925	Tetracycline resistant
CCS010	Chicken swab	AMP	11	Ampicillin resistant
CCS011	Chicken swab	TE	1925	Tetracycline resistant
CCS012	Chicken swab	AMP	11	Ampicillin resistant
CCS013	Chicken swab	-	1925	Susceptible
CCS014	Chicken swab	-	1925	Susceptible
CCS015	Chicken swab	-	1925	Susceptible
CCS016	Chicken swab	-	329	Susceptible
CCS017	Chicken swab	-	1925	Susceptible
CCS018	Chicken swab	TE	1925	Tetracycline resistant
CCS019	Chicken swab	AMP	11	Ampicillin resistant
CCS020	Chicken swab	AMP	11	Ampicillin resistant
CCS021	Chicken swab	-	365	Susceptible
CCS022	Chicken swab	-	1925	Susceptible
CCS023	Chicken swab	-	1925	Susceptible
CCS024	Chicken swab	-	1925	Susceptible
CCS025	Chicken swab	TE;S;AMP	2132	**Multidrug resistant**
CCS026	Chicken swab	TE	1925	Tetracycline resistant
CCS027	Chicken swab	TE	1925	Tetracycline resistant
CCS028	Chicken swab	TE	1925	Tetracycline resistant
CCS029	Chicken swab	TE	1925	Tetracycline resistant
Key: CM&MP-Chicken Meat & Meat Products; CCS-Chicken Cloacal Swab.				

When stratified by the source of samples, *S.* Enteritidis isolated from food samples exhibited the highest frequency of resistance 5/7 (71.4%), followed by cloacal swab 17/29 (58.6%) and then chicken meat with 5/11 (45.5%). However, chicken swab isolates had the overall highest percentage resistance (36.2%). Importantly, only two isolates, both from cloacal swab samples, showed multi-drug resistance (S64 = AMP; CN; TE; S81 = TE, S, AMP). Moreover, the antimicrobial agent with the most resistance across all the isolates tested was Tetracycline (46.8%); While Ampicillin had 14.8% and, Streptomycin, Sulfadimidine/trimethoprim, and gentamycin all had 2.1% respectively.

Multi-locus Sequence Analysis

For the MLST of the completely sequenced bacterial genomes, short sequence reads were first assembled to draft genomes [12]. For the whole genome MLST scheme, the MLST allele of each locus was aligned to the genome using BLAST. After that, the ST was determined by a combination of the MLST alleles after close-matching of the selected alleles. The MLST typing of all the 47 isolates was based on the comparison of internal sequences of the Salmonella seven housekeeping gene fragments (*aroC, dnaN, hemD, hisD, purE, sucA*, and *thrA*). The 47 S. Enteritidis were subtyped into six (6) different STs, with the majority assigned to ST1925 (30) followed by ST11 (12), with ST292, ST365, ST329 and ST2132 assigned to one isolate each. Worthy of note is the fact that ST1925 is a single locus variant of ST11. Additionally, while ST1925 and ST11 occurred in food materials, chicken meat, and cloacal swab, ST292 was found only in chicken while ST365, ST329 and ST2132 were all found in cloacal swab.

Pulse-field Gel Electrophoresis

The *Xba*I digestion was successfully performed on all the isolates except CCS016 and CCS025 (both from cloacal swabs), which were not type able by PFGE, hence were excluded. However, the remaining 45 selected isolated yielded 9–13 DNA bands. With a Dice Coefficient of 0.5 and a similarity index of 90%, the PFGE analysis produced 10 pulsotypes (1–10) with pulsotypes 6 and 8 being the major ones, pulsotype 1 and 5 had 3 and 2 isolates from chicken and meat isolates while 2, 3, 4, 9, and 10 appeared as singletons with 100% similarity. Moreover, the majority of the strains (17; 37.7%) belonged to pulsotype 6 and 8. Within the pulsotype 6, isolates from chicken meat, foot and cloacal swab exhibited genetic relatedness ranging from 88.9 to 100%, likewise isolates in the pulsotype 8 shared a similarity score in the region of 92–100% (Fig. 1).

Discriminatory Ability

The Simpson's index of diversity (*D*) was used to compare the bacterial typing method based on MLST and PFGE pattern of the isolates. For the 10 PFGE types, *D* was 0.96 while for the six (6) sequence types identified by MLST, *D* was 0.99. These indices imply that if two isolates are to be sampled randomly from the population, then 96% and 99% of the time they will be assigned into different types. However, it is recommended that a good index should be greater than 0.95 [13, 14].

Discussion

Salmonella is an important and one of the most frequently occurring food-borne pathogens with significant economic and public health significance globally (Abdulhaleem et al., 2019; Salihu et al., 2013; Salihu, 2015; Wattiau, & Bertrand, 2011). It is especially common in foods prepared with contaminated poultry meat or egg. Although infection with non- typhoid *Salmonella* strains is mostly associated with mild self-limiting gastroenteritis, occasionally, severe invasive infections can result [19, 20]. Moreover, the

emergence of multidrug-resistant (MDR) isolates are characterized by reduced susceptibility to the commonly used antimicrobials portend an even severe health hazard. Therefore, surveillance programs aimed at the timely detection of *Salmonella* contaminations in the entire food chain, including live animals, abattoirs, retail outlets, and food restaurants, is so much desired.

This investigation was undertaken to examine *Salmonella* isolates from food sold at restaurants, chicken meats sold at supermarkets and wet night market in Malaysia, as well as samples from live chickens from selected poultry farms located within the central region of Peninsular Malaysia in order to assess the antimicrobial susceptibility and the genetic relatedness of the *Salmonella* pathogen. In total, 47 *S*. Enteritidis were identified after culture, isolation, biochemical characterization, and serotyping was done. In order to their genetic relatedness, while genome sequencing wgMLST and PFGE were conducted.

The antimicrobial susceptibility analysis of all isolates from the food source, chicken meat, and chicken cloacal swab exhibited susceptibility and varying resistance characteristics to the antimicrobial panel tested. As mentioned above, all of the 47 isolates were confirmed to be *S*. Enteritidis serotype upon slide agglutination test. The phenotypic antimicrobial resistance result showed that the majority of the isolates were resistant (57.45%) to the antimicrobials tested. Although only two isolates had multiple resistance (resistant to 3 or more), the majority were resistant to Tetracycline. The majority of the Salmonella with a multi-drug resistant profile are resistant to Tetracycline, which has gained popularity as a clinically and agriculturally relevant antibiotic (Brunelle, & Bearson, 2013). The National Pharmaceutical Regulatory Agency (NPRA), which is the drug control authority of Malaysia under the Ministry of Health (MOH), Malaysia and the Department of Veterinary Services (DVS) under the Ministry of Agriculture have granted approvals for the use of Tetracycline in the treatment of disease, as prophylaxis and as growth promoters (Azmi et al., 2018). However, the inappropriate use of these antibiotics in the food-producing animals constitutes a serious public health hazard. More so, the emergence of multiple antibiotic resistance, as observed in 2 isolates in this study, may progressively undermine the viability of many of the routinely used antibiotics.

Sequence analysis of the isolates found that the most common sequence type (ST) observed among all the isolates were ST1925 (30) followed by ST11 (12). While novel STs reported in Malaysia for the first time (ST292, ST365, ST329, and ST2132) were also detected in one isolate each. Comparison of our result against the MLST database indicates that ST1925 is relatively common among Salmonella Enteritidis isolated from human and avian species from the United Kingdom, United States, Australia, and Malaysia (http://enterobase.warwick.ac.uk/species/senterica/search_strains?query=st_search). While ST11 had been reported in humans, poultry, food, and some wild animal species, including reptiles in many countries from Asia, Africa, South America, and European countries (http://enterobase.warwick.ac.uk/species/senterica/search_strains?query=st_search).

Importantly, ST1925 has been reported to be a single cell variant of ST11, and both sequence types are known to be geographically widespread and have previously been reported food, human and animal (Alikhan et al., 2018; Aung et al., 2019). The detection of these STs from different sources may also

indicate their ability to adapt to, and persist in, different hosts or types of samples. The detection of ST11 in food may imply possible transmission from neighboring countries like Singapore and China, where the ST type prevails [23]. In this study, the whole-genome sequencing platform was used for the MLST analysis against the traditional PCR based MLST, followed by Sanger sequencing. However, due to the availability of the new generation high-throughput sequencing, whole-genome sequence (WGS) data for typing [12]. This is because of its superior discriminatory power and efficiency in genetic detection variability between isolates, in addition to the fact that the traditional method is both costly and time-consuming [12].

ST292, ST365, and ST2132 have all been previously reported in Singapore (aquatic), India (human & environmental samples), and United States (poultry) while ST329 was reported among isolates obtained from poultry feed in Peru (Adesiji, & Karunasagar, 2014; Castellanos et al., 2018; Liu et al.,2011; Wiesner et al., 2009). In this study, ST292 was detected in chicken meat, while ST365, ST329 and ST2132 were all detected in the chicken cloacal swab. When analyzed in the MLST database, these strains were not as common as the other STs detected except for ST365, and ST2135 was primarily found among poultry in the US from Salmonella isolates belonging to the Kentucky serotype. Also, while ST365 showed no antimicrobial resistance against the drugs tested, ST292 and ST2132 showed resistance to sulfadimidine/trimethoprim as well as streptomycin and Tetracycline respectively. Therefore, having demonstrated similar antimicrobial susceptibility profiles as well as a common source, this may be suggestive of possible strain relatedness, and potential for transmission between chicken and food.

The pulsed-field gel (PFGE) analysis of the isolates revealed that most of the *S*. Enteritidis isolate examined exhibited unique genetic relatedness, albeit with some variability. Cluster analysis identified ten (10) pulsotypes with the majority belonging to pulsotype 6 and 8, which further had seventeen subtypes that shared 100% identity pattern between the poultry and food source. From the results of the PFGE analysis, it was evident that PFGE revealed more significant differentiation (10 profiles) compared to the MLST, which produced six (6) sequence types. The earlier observation supports this finding that the diversity indices with PFGE produced the highest rate of variability over MLST and the phenotypic antimicrobial susceptibility testing (Stepan et al., 2011). On the other hand, a one-to-one correlation between PFGE types and ST revealed that some isolates belonging to the same PFGE type had multiple STs and vice versa. The result of this study showed that both MLST and PFGE had a high index of discrimination (*D*) above the 0.95 recommended. The slight disparity with respect to the Simpson's index between MLST and PFGE observed in this study has previously been reported where MLST was found to exhibit higher discriminatory power with respect to the typing of ESBL *E. coli* [14]. The authors argued that such disparity may be attributed to the spectrum of changes detected by PFGE and MLST. In other words, while PFGE detect changes in nucleotide sequence associated with insertions or deletions of DNA, MLST typing detects nucleotide changes within an amplified gene fragment [14]. However, in recent years, the potentials of molecular techniques in discriminating between strains of S. enteritidis have become more pronounced. Methods with the highest discriminatory power are more specific and therefore better recommended during investigations of closely related isolates (Shaaly et al., 2005).

Furthermore, the PFGE analysis was able to delineate the genetic variability between the S. Enteritidis isolates from a different source based on the distinct DNA fingerprints generated. Except for two isolates, all other isolates were typeable and the technique reproducible, which could be very useful as an epidemiological tool for disease outbreak investigation. The present study also showed that various PFGE subtypes identified are present in both fresh chicken meat, live birds, and even cooked food ready for eating. Although the DNA profiles of most of the *Salmonella* serotype Enteritidis isolates from various sources differed, which may indicate that the isolates belong to different clones as revealed by the MLST analysis.

Conclusion

This investigation has highlighted the usefulness of molecular and phenotype analysis in understanding the genotypic and phenotypic characteristics of *S*. Enteritidis. A higher level of diversity was observed among the S. Enteritidis isolates based on the PFGE, which is an indication of the potential as the molecular choice technique for the subtyping isolates of the same serovar. The five sequence types detected in the present study with the wgMLST analysis also showed host variability by occurring in live chicken, cooked food, and fresh chicken carcass in addition to the other animal and human hosts upon comparison with the MLST database. The antimicrobial resistance pattern was equally evident in food isolates as compared to the other sources investigated in this study.

Methods

Salmonella isolates

All *Salmonella* isolates used in this study were obtained from the laboratory collection of the Food Safety Division, Ministry of Health Malaysia (7), and the Department of Veterinary Services Malaysia (40). After the differential culture, isolation and biochemical characterization, all the isolates were suspended in a Brain Heart Infusion (Oxoid) broth supplemented with 20% glycerol and then stored at -80 °C until required. All the isolates (47) comprised of 29 cloacal swab, seven food products (from chicken), and 11 fresh chicken portions of meat at retail outlets.

Serotyping And Antimicrobial Susceptibility Testing

The Salmonella serotype (S. Enteritidis) was determined using the slide agglutination assay according to the Kauffman-White scheme based on the agglutination of the bacteria with commercial Salmonella O (somatic) and H (flagellar) antisera (DIFCO, Detroit, Mich., USA) in order to identify variants of the O and H antigens. While the antimicrobial susceptibility tests were conducted using the disk diffusion method per the Clinical Laboratory Standard Institute (CLSI) protocol. The isolates were tested against ampicillin (Amp), chloramphenicol (C), gentamicin (CN), streptomycin (S), sulfamethazine/trimethoprim (SXT), Tetracycline (TE), ceftiofur (EFT), cefotaxime (Ctx), and ciprofloxacin (CIP).

Whole-genome Sequencing And MLST

The QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) was used to extract and purify the genomic DNA from all the Salmonella Enteritidis isolates. While the NGS library preparation was achieved with the aid of the Nextera XT DNA Sequencing Library Preparation Kit (FC-131-1096; Illumina, San Diego, California, USA) following the manufacturer's instructions, and the sequencing was done using the Illumina NextSeq sequencer by scanning for adapter sequences followed by removal of low-quality sequences. Finally, sound quality sequencing reads were assembled *de novo* using SPAdes software version 3.9.0 (BioEasy Sdn Bhd). The Whole Genome Sequences generated were analyzed with the aid of the software EPInod (BioEasy Sdn. Bhd. Malaysia). Multi-locus Sequence Typing was conducted within the EPInod suite by streamlining all the sequences for the isolates on the MLST program against the PubMLST database (MLST version 2.6).

PFGE analysis

PFGE analysis was performed based on the standardized protocol for the subtyping of Salmonella in the PulseNet [31]. Purified DNA was digested using *Xba*I restriction enzyme (NEB) in a final volume of 100 µl and incubated at 37 °C for 3 hrs and embedded in a 1% SeaKem Gold Agarose (Sigma Aldrich) prepared using 0.5x TBE buffer. The reaction was run for 18 hrs using the Chef Mapper XA system (Bio-Rad) in order to resolve the DNA macro-restriction fragments. *Salmonella enterica* Typhimurium was used as a control. Macro-restriction patterns were compared using the FPQuest cluster analysis based on the Dice correlation coefficient, while dendograms were constructed using the unweighted-pair group method using average linkages UPGMA.

Data analysis

Salmonella isolates were assigned sequence type (ST) according to their allelic profiles corresponding to the seven housekeeping genes, while the PFGE patterns were expressed as pulsotypes. Simpson's index of diversity (*D*) which measures the index of discrimination for the two typing methods was calculated using the formula below [13]:

$$D = 1 - \frac{1}{N(N-1)} \sum_{j=1}^{S} n_j(n_j - 1)$$

According to the formula, N is the total number of strains in the sample population, S is the total number of types described, and n_j is the number of strains belonging to the jth type.

Abbreviations

PFGE: Pulsed Field Gel Electrophoresis; wgMLST: Whole Genome Multi-Locus Sequence Type; MDR: Multidrug-resistant; MLST: Multi-locus Sequence Typing; DNA: Deoxyribonucleic acid; MOH: Ministry of Health; DVS: Department of Veterinary Services; ST: Sequence Types.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare they have no competing interests.

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Authors' contributions

ZZ, NA, NHAH and BG analyzed WGS, PFGE data and drafted the manuscript; NS, ZS, RMA, SAH SAB and LH were responsible for sampling, isolation and characterization of the various bacterial isolates; ZZ and LH conceptualized and designed the study; all authors read and approved the final manuscript.

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References

1. Ford L, Glass K, Veitch M, Wardell R, Polkinghorne B, Dobbins T, Lal A, Kirk MD. Increasing Incidence of Salmonella in Australia, 2000–2013. PLoS One. 2016;11:e0163989.
2. Brichta-Harhay DM, Arthur TM, Bosilevac JM, Kalchayanand N, Shackelford SD, Wheeler TL, Koohmaraie M. Diversity of multidrug-resistant Salmonella enterica strains associated with cattle at harvest in the United States. Appl Environ Microbiol. 2011;77:1783–96.
3. Shah DH, Elder JR, Chiok KL, Paul NC. (2017) Genetic Basis of Salmonella Enteritidis Pathogenesis in Chickens. In: Prod. Safe Eggs Microb. Ecol. Salmonella. Elsevier Inc., pp 187–208.
4. Zhao S, McDermott PF, White DG, et al. Characterization of multidrug resistant Salmonella recovered from diseased animals. Vet Microbiol. 2007;123:122–32.
5. Thung TY, Radu S, Mahyudin NA, et al. Prevalence, Virulence Genes and Antimicrobial Resistance Profiles of Salmonella Serovars from Retail Beef in Selangor, Malaysia. Front Microbiol. 2018. https://doi.org/10.3389/fmicb.2017.02697.
6. Khaltabadi RF, Shahrokhi N, Ebrahimi-Rad M, Ehsani P. Salmonella Typhimurium in Iran: Contribution of molecular and IS200 PCR methods in variants detection. PLoS One. 2019;14:e0213726.
7. Enright MC, Spratt BG. Multilocus sequence typing. Trends Microbiol. 1999;7:482–7.
8. Sharma-Kuinkel BK, Rude TH, Fowler VG. (2016) Pulse field gel electrophoresis. In: Methods Mol. Biol. Humana Press Inc., pp 117–130.
9. Blanc DS. The use of molecular typing for epidemiologica surveillance and investigation of endemic nosocomial infections. Infect Genet Evol. 2004;4:193–7.
10. Maiden MCJ. Multilocus Sequence Typing of Bacteria. Annu Rev Microbiol. 2006;60:561–88.
11. Wang J, Li J, Liu F, Cheng Y, Su J. Characterization of Salmonella enterica Isolates from Diseased Poultry in Northern China between 2014 and 2018. Pathogens. 2020;9:95.
12. Larsen MV, Cosentino S, Rasmussen S, et al. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol. 2012;50:1355–61.
13. Hunter PR, Gaston MA. Numerical index of the discriminatory ability of typing systems: An application of Simpson's index of diversity. J Clin Microbiol. 1988;26:2465–6.
14. Nemoy LL, Kotetishvili M, Tigno J, et al. Multilocus sequence typing versus pulsed-field gel electrophoresis for characterization of extended-spectrum beta-lactamase-producing Escherichia coli isolates. J Clin Microbiol. 2005;43:1776–81.
15. Wattiau P, Boland C, Bertrand S. Methodologies for Salmonella enterica subsp. Enterica Subtyping: Gold Standards and Alternatives. Appl Environ Microbiol. 2011;77:7877–85.
16. Abdulhaleem N, Garba B, Younis H, Mahmuda A, Hamat RA, Majid RBA, Lung LTT, Unyah NZ, Sattar A, Saidu B. Current trend on the economic and public health significance of salmonellosis in Iraq. Adv Anim Vet Sci. 2019;7:484–91.
17. Salihu M, Garba B, Isah Y. Sokoto Journal of Veterinary Sciences Evaluation of microbial contents of table eggs at retail outlets in Sokoto metropolis, Nigeria. Sokoto J Vet Sci J Vet Sci. 2015;13:22–8.

18. Salihu MD, Magaji AA, Garba B, Saidu B, Mamuda A, Suleiman N, Bello Shafiu Wurno. Bacteriological quality of raw meat displayed for sale at Sokoto, Sokoto state, Nigeria. Sci J Microbiol. 2013;2:134–9.
19. Stanaway JD, Parisi A, Sarkar K, et al. The global burden of non-typhoidal salmonella invasive disease: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis. 2019;19:1312–24.
20. Mohan A, Munusamy C, Tan Y-C, et al. Invasive Salmonella infections among children in Bintulu, Sarawak, Malaysian Borneo: a 6-year retrospective review. BMC Infect Dis. 2019;19:330.
21. Brunelle BW, Bearson SMD, Bearson BL. Tetracycline accelerates the temporally-regulated invasion response in specific isolates of multidrug-resistant Salmonella enterica serovar Typhimurium. BMC Microbiol. 2013;13:202.
22. Malaysia Overview
Antibiotic Use in Food Animals
Azmi M, Hassali A, Rhu H, Ashutosh Y, Verma K, Hussain R, Sivaraman S. Antibiotic Use in Food Animals: Malaysia Overview.
23. Aung C, Chau, et al. Salmonella in Retail Food and Wild Birds in Singapore—Prevalence, Antimicrobial Resistance, and Sequence Types. Int J Environ Res Public Health. 2019;16:4235.
24. Alikhan N-F, Zhou Z, Sergeant MJ, Achtman M. A genomic overview of the population structure of Salmonella. PLOS Genet. 2018;14:e1007261.
25. Liu W bing, Liu B, Zhu X, na, Yu S, jing, Shi X ming (2011) Diversity of Salmonella isolates using serotyping and multilocus sequence typing. Food Microbiol 28:1182–1189.
26. Castellanos LR, Van D Graaf-Van Bloois, Donado-Godoy L P, et al. Genomic characterization of extended-spectrum cephalosporin-resistant Salmonella enterica in the Colombian poultry chain. Front Microbiol. 2018. https://doi.org/10.3389/fmicb.2018.02431.
27. Wiesner M, Zaidi MB, Calva E, Fernández-Mora M, Calva JJ, Silva C. Association of virulence plasmid and antibiotic resistance determinants with chromosomal multilocus genotypes in Mexican salmonella enterica serovar typhimurium strains. BMC Microbiol. 2009. https://doi.org/10.1186/1471-2180-9-131.
28. Adesiji YO, Deekshit VK, Karunasagar I. Antimicrobial-resistant genes associated with Salmonella spp. isolated from human, poultry, and seafood sources. Food Sci Nutr. 2014;2:436–42.
29. Stepan RM, Sherwood JS, Petermann SR, Logue CM. Molecular and comparative analysis of Salmonella enterica Senftenberg from humans and animals using PFGE, MLST and NARMS. BMC Microbiol. 2011. https://doi.org/10.1186/1471-2180-11-153.
30. Shaaly A, Tellevik MG, Langeland N, Høiby EA, Jureen R. Comparison of serotyping, pulsed field gel electrophoresis and amplified fragment length polymorphism for typing of Streptococcus pneumoniae. J Med Microbiol. 2005;54:467–72.
31. Ribot EM, Fair MA, Gautom R, Cameron DN, Hunter SB, Swaminathan B, Barrett TJ. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of Escherichia coli O157:H7,

Salmonella, and Shigella for PulseNet. Foodborne Pathog Dis. 2006;3:59–67.

Figures

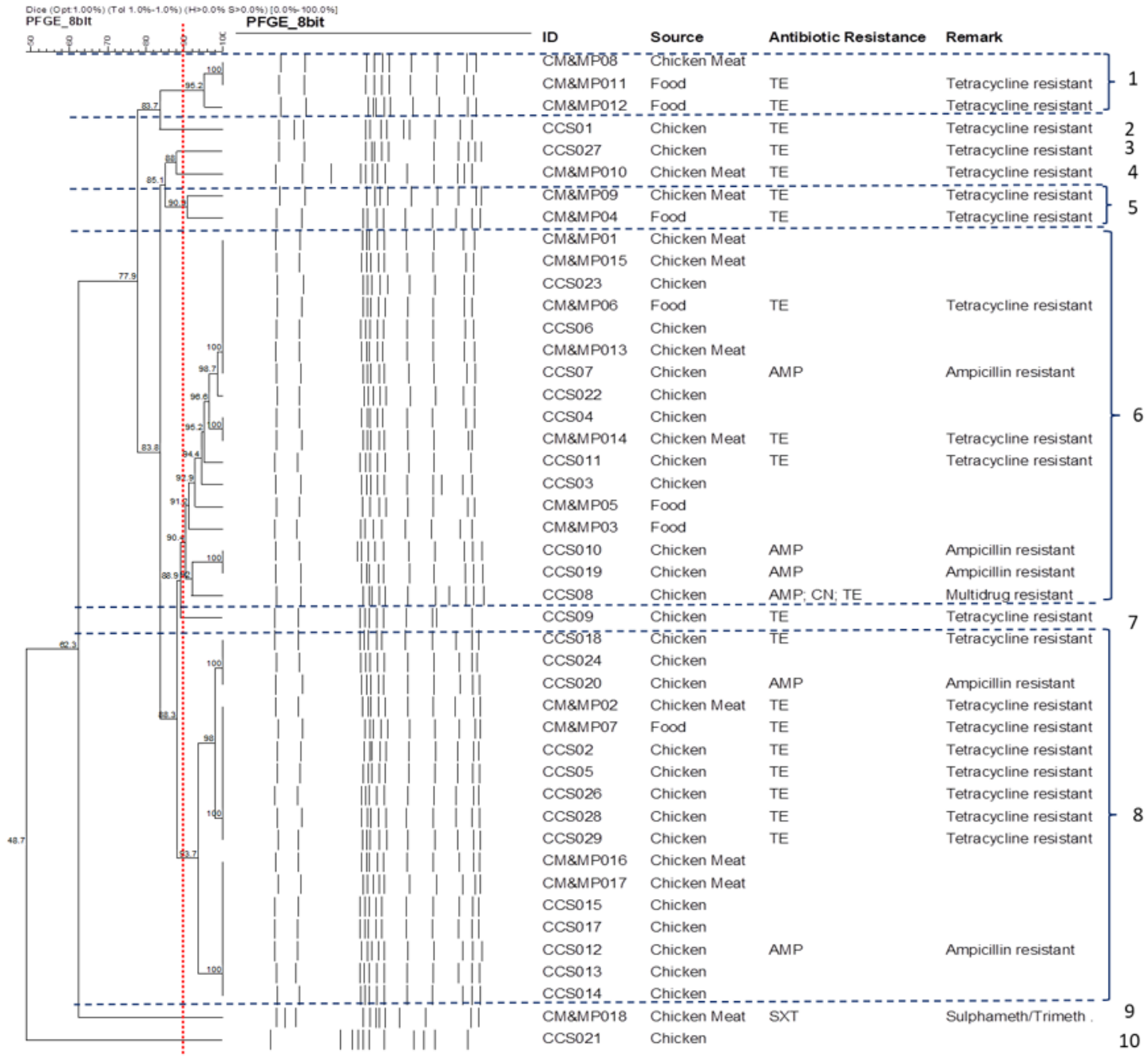

Figure 1

Pulsed-field gel electrophoresis (PFGE) patterns showing the DNA of the chicken meat, food and poultry cloacal swab isolates digested by XbaI restriction enzyme (n = 45). Nine antimicrobial susceptibility patterns and 10 PFGE pulsotypes (1-10) were identified among the 45 isolates.