Highly-prevalent multidrug-resistant Salmonella from chicken and pork meat at retail markets in Guangdong, China

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Running title: Multidrug-resistant *Salmonella* in Guangdong

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Abstract

This study aimed to investigate the prevalence, serotype distribution, and antibiotic resistance, and to characterize the extended spectrum β-lactamases (ESBLs) producing *Salmonella* isolates from chicken and pork meats from retail markets in Guangdong province, China. A total of 903 retail meat samples (475 chicken and 428 pork meats) were obtained from six cities (Guangzhou, Shenzhen, Heyuan, Shaoguan, Foshan, and Yunfu) of Guangdong province between May 2016 and April 2017. High levels of *Salmonella* contamination were detected in chicken (302/475, 63.6%) and pork (313/428, 73.1%). Thirty-eight serotypes were identified in 615 detected *Salmonella*, and the serotypes varied greatly between chicken and pork samples. Agona (55/302, 18.2%), Corvallis (45/302, 14.9%), Kentucky (38/302, 12.6%), Mbandaka (32/302, 10.6%) was the dominant serotypes in chicken samples. However, Typhimurium (78/313, 24.9%), Rissen (67/313, 24.1%), Derby (66/313, 21.1%), and London (48, 15.3%) were the most common in pork samples. High rates of antibiotic resistance were found to sulfisoxazole (468/615, 76.1%), tetracycline (463/615, 75.3%), ampicillin (295/615, 48.0%), and ofloxacin (275/615, 44.7%). Notably, antimicrobial susceptibility tests identified resistance to polymyxin B (12/615, 2.0%) and imipenem (3/615, 0.5%). Multidrug-resistance (MDR) was detected in *Salmonella* isolated from chicken (245/302, 81.1%) and pork (229/313, 73.2%). The resistance rate of different *Salmonella* serotypes varied widely. Especially, isolates such as Typhimurium, Agona, *C*orvallis and Kentucky exhibited highly resistance to antibiotics. The MDR rate of *Salmonella* isolates from chicken was significantly higher than that from pork isolates (P < 0.05). Twenty-one *Salmonella* isolates were identified as ESBLs-producing, covering six *Salmonella* serotypes and displaying different pulse field gel electrophoresis (PFGE) genotypes. *Bla*$_{OXA-1}$ was the dominant ESBLs gene (9/21, 42.9%), followed by *bla*$_{CTX-M-55}$ (5/21, 23.8%). This study indicated that *Salmonella* was widespread in chicken and pork from retail markets in Guangdong province and the isolates showed high multidrug-resistance, especially the known multidrug-resistant *Salmonella* serotypes. Therefore, it is important to focus on *Salmonella* serotypes and strengthen the long-term monitoring of MDR *Salmonella* serotypes in animal-derived foods.

Keywords: *Salmonella*, retail meat, prevalence, antibiotic resistance, ESBLs, PFGE

INTRODUCTION

Salmonella is a foodborne pathogen that causes morbidity and mortality worldwide (Scallan et al., 2011). Until now, more than 2,600 serovars have been identified among *Salmonella* worldwide and almost all *Salmonella* can cause illness in humans and animals (Guibourdenche et al., 2010). In China, about 70 to 80% of foodborne disease outbreaks are caused by *Salmonella*, and the majority of diseases are associated with the ingestion of contaminated livestock and poultry products (Wang et al., 2007). Animal-derived foods, especially chicken and pork, are considered the major reservoirs of *Salmonella* dissemination (Vo et al., 2006; Jackson et al., 2013).

At the same time, antibiotic resistance of *Salmonella* is also one of the most important public health problems worldwide. In recent years, studies have shown that because of long-term antibiotic use during animal breeding, antibiotic resistance has markedly increased. Multidrug-resistant *Salmonella* could pose a serious threat to humans through the food chain (Barza, 2002; Lai et al., 2014). In particular, extended-spectrum β-lactamases (ESBLs)-producing *Salmonella* have been frequently isolated from food animals in many countries, including China (Dahms et al., 2015; Ben Said et al., 2016; Ibrahim et al., 2016; Zhao et al., 2017).

For animal-derived foods, tracking the source of *Salmonella* infections can help to identify potential problems in food production, processing, preparation, or the sales process to prevent the introduction of other pathogens into food (Swaminathan et al., 2006; Russell et al., 2014). Guangdong is a livestock production and consumption province; furthermore, Guangdong is China's foremost livestock production area. In 2009, laboratory-based surveillance for *Salmonella* infection was established in Guangdong Province, which mainly monitors the prevalence and outbreak of *Salmonella* in various hospitals (Deng et al., 2012). However, limited information on surveillance studies of *Salmonella* in animal-derived foods from retail markets in Guangdong is available, and there is a lack of epidemiological data.

The main aim of this study was to determine the prevalence, serotype distribution, antibiotic resistance of *Salmonella*, and the phenotype and genotype of ESBLs-producing *Salmonella* isolated from chicken and pork meat from retail markets in Guangdong. The results could lay the foundation for follow-up research for public health security and food safety problems caused by *Salmonella*.

MATERIALS AND METHODS

Sample collection

A total of 903 retail meat samples (including 475 chicken and 428 pork sample) were randomly collected from retail markets in six cities (Guangzhou, Shenzhen, Heyuan, Shaoguan, Foshan, and Yunfu; chosen from three retail markets in each district) of Guangdong province between May 2016 and April 2017. Sampling methods were as follows: In Guangzhou City, different regions were sampled monthly; in other cities, samples were taken monthly from three selected retail markets. Samples were taken of different chicken types (i.e., whole chickens and chopped chickens), pork types (i.e., dressed pork) and during different seasons (i.e., spring, summer, autumn and winter). Each sample was placed in a sterile plastic sample bag and transported to the laboratory on ice. All samples were analyzed on the day of their collection.

Salmonella isolation and identification

103 *Salmonella* isolation and identification was performed according to the Standard
104 ISO-6579 (International Organization for Standardization, 2002) method, as described
105 previously (Yang et al., 2010; Ren et al., 2016) with some modifications. Briefly, 25 g
106 of samples was placed into a sterile plastic bag containing 225 mL of buffered
107 peptone water and shaken for 3 min. The suspension was then cultivated at 37 °C in a
108 Shaker at 100 rpm for 6–8 h and then 1 mL of the suspension was added to 10 mL
109 Tetrathionate Broth and Rappaport–Vassiliadis soya broth and incubated at 42 °C for
110 18-24 h, separately. After selective enrichment, the suspensions were streaked onto
111 xylose lysine tergitol 4 agar and CHROMagar™ incubated at 37 °C over night.
112 Isolates with typical *Salmonella* phenotypes were further confirmed using API 20E
113 test strips (bioMerieux, Marcy-l'Etoile, France).

115 *Salmonella* **serotyping**
116 All the *Salmonella* isolates were serotyped according to the White Kauffmann Le
117 Minor scheme or National Food Safety Standard-Food microbiological examination:
118 *Salmonella* (GB 4789.4-2016) by slide agglutination, using specific O and H antisera
119 (S&A Reagents Lab Ltd., Bangkok, Thailand).

121 **Antimicrobial susceptibility testing and screening for ESBLs strains**
122 All *Salmonella* isolates were tested for antibiotic susceptibility using the Kirby-Bauer
123 disk diffusion method, and the results were interpreted according to the standards of
124 the Clinical and Laboratory Standards Institute (CLSI, 2013).

126   Agar diffusion assays were performed on Muller-Hinton agar with disks containing
127 18 different antibiotic agents (Oxoid, Basingstoke, UK): ampicillin 10 µg (AMP),
128 amoxicillin-clavulanic acid 30 µg (AMC), cefotaxime 30 µg (CTX), ceftazidime 30
129 µg (CAZ), cefepime 5 µg (FEP), gentamicin 10 µg (GEN), streptomycin 10 µg (STR),
130 amikacin 30 µg (AMK), sulfamethoxazole-trimethoprim 23.75/1.25 µg (SXT),
131 sulfonamides 300 µg (S3), nalidixic acid 30 µg (NAL), ofloxacin 5 µg (OFX),
132 ciprofloxacin 5 µg (CIP), florfenicol 30 µg (FFC), chloramphenicol 30 µg (CHL),
133 imipenem 10 µg (IPM), polymyxin B 300 IU (PB), and tetracycline 30 µg (TET) .
134 *Escherichia coli* ATCC 25922 and ATCC 35218 were used as quality control
135 organisms. Isolates exhibiting resistance to three or more antibiotic classes were
136 defined as multidrug-resistant (MDR).

138   The *Salmonella* isolates were screened for ESBLs production using cefotaxime and
139 cefotaxime (30 µg)/clavulanic acid (10 µg) disks and ceftazidime and ceftazidime (30
140 µg)/clavulanic acid (10 µg) disks (Oxoid) according to the double-disk synergy test
141 method (Emery and Weymouth, 1997). Phenotypic presence of ESBLs of the isolates
142 was determined by detecting enhancement of the diameter of the inhibition zone of
143 the clavulanate disk and corresponding β-lactam antibiotic disk. If there was
144 difference of ≥ 5 mm between the inhibition zone of the clavulanic acid and the
145 β-lactam antibiotic disk, the isolate confirmed as having a positive ESBLs phenotype
146 (CLSI, 2013).

148 **PCR detection and DNA sequencing**
149 *Salmonella* isolates showing ESBLs phenotypes were further confirmed by PCR and
150 DNA sequencing. ESBLs encoding genes detected included bla_{TEM}, bla_{CTX-M}, bla_{CMY},
151 bla_{OXA}, bla_{PSE}, and bla_{SHV}. The primers for the ESBLs encoding gene and the reaction
152 conditions were as previously described (Li et al., 2013; Nguyen et al., 2016; Qiao et

al., 2017). The PCR products were stained with ethidium bromide, visualized under UV light after gel electrophoresis using 1% agarose, purified, and sent to Sangon Biotech Co., Ltd. (Shanghai, China) for sequencing. The ESBLs encoding gene fragments were analyzed and aligned at GenBank using the online BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/).

Pulse field gel electrophoresis (PFGE)

ESBLs-producing *Salmonella* isolates were subtyped by PFGE to determine their genetic relatedness, according to the Pulse-Net protocol recommended by the CDC (Ribot et al., 2006). PFGE was preformed after digestion of the genomic DNA with the restriction enzyme XbaI, *Salmonella enterica subsp. enterica* serovar Braenderup (CDC no. H9812) was used as standard control strain. PFGE results were analyzed using BioNumerics Software (Version 5.1; Applied-Maths, Sint-Martens-Latem, Belgium).

Statistical analysis

The isolation rates and antibiotic resistance rates among the different food types and isolates were analyzed using the Chi-squared test in SPSS Statistics 18.0 software (SPSS Inc., USA). A P-value of < 0.05 was considered statistically significant.

RESULTS

Prevalence and Serotypes of *Salmonella*

In this study, a total of 615 *Salmonella* isolates (615/903, 68.1%) were recovered from the meat samples. High levels of *Salmonella* contamination were detected: 302 (302/475, 63.6%) in chicken samples and 313 (313/428, 73.1%) in pork samples (Table 1). The isolation rate from pork was higher than that from chicken (P< 0.05).

Thirty-eight *Salmonella* serotypes were identified among the 615 *Salmonella* isolates (Table 1). More serovars were found in chicken samples (27 serovars) than in pork samples (25 serovars). The serotypes varied greatly between the chicken and pork samples. In the chicken samples, the most common serovars were Agona (55/302, 18.2%), Corvallis (45/302, 14.9%), Kentucky (38/302,12.6%), and Mbandaka (32/302, 10.6%); and in the pork samples they were Typhimurium (78/313, 24.9%), Rissen (67/313, 24.1%), Derby (66/313, 21.1%), and London (48, 15.3%). Importantly, serovar Rumford was detected in meat samples for the first time in China.

Antimicrobial susceptibility testing

The 615 isolated strains were tested for their susceptibility to 18 antibiotics (Table 2). The most resistance was to sulfisoxazole (468/615, 76.1%) and tetracycline (463/615, 75.3%), followed by ampicillin (295/615, 48.0%), ofloxacin (275/615, 44.7%), and trimethoprim/sulfamethoxazole (248/615, 40.3%). All isolates were susceptible to cefepime, ceftazidime, amikacin, and amoxicillin-clavulanic acid. Notably, the results showed very low rates of resistance to polymyxins and carbapenem antibiotics, such as polymyxin B (12/615, 2.0%) and imipenem (3/615, 0.5%).

Resistance to ampicillin, tetracycline, streptomycin, sulfisoxazole, Florfenicol, Chloramphenicol, and Ofloxacin was frequently observed in the *Salmonella* serovars isolated (Table 3): 588 (95.6%) isolates were resistant to at least one antibiotic and 474 (77.1%) were multidrug-resistant (MDR) (Table 4). Resistance to 4–9 antibiotics was detected in 331 isolates (53.8%), whereas 57 isolates (9.3%) were resistant to ≥

203 10 antibiotics. More of the serovars isolated from chicken showed MDR ≥ 3 (n=245)
204 than did the serovars isolated from pork (n=229) (P < 0.05). The MDR rates for the
205 *Salmonella* serovars are shown in Table 5. Notably, MDR was widespread serovars
206 Kentucky, Typhimurium, Derby, Agona, and Corvallis isolated from retail meat
207 samples.
208
209 **Prevalence of ESBLs-producing isolates and ESBLs genes**
210 Six serotypes were detected among 21 ESBLs-producing *Salmonella* isolates (Table
211 6). The most prevalent serotype was Indiana (7/21, 33.3%) followed by Typhimurium
212 (5/21, 23.8%), Kentucky (3/21, 14.3%), Litchfield (3/21, 14.3%), Derby (2/21, 9.5%),
213 Schwarzengrund (1/21, 4.8%). The resistance profiles are shown in Table 6. Eight
214 β-lactamases and ESBLs encoding genes, including bla_{TEM-1}, $bla_{TEM-206}$, $bla_{TEM-214}$,
215 $bla_{CTX-M-15}$, $bla_{CTX-M-55}$, $bla_{CTX-M-64}$, $bla_{CTX-M-123}$, and bla_{OXA-1} were identified in the 21
216 ESBLs-producing *Salmonella* isolates (Table 7). Among them, 42.9% (n = 9) were
217 found to harbor bla_{OXA-1}, followed by $bla_{CTX-M-55}$ (5/21, 23.8%), $bla_{TEM-206}$ (4/21,
218 19.0%), $bla_{TEM-214}$ (4/21, 19.0%), $bla_{CTX-M-123}$ (3/21, 14.3%). Moreover, 100% of
219 ESBLs-producing isolates carried at least one of the β-lactamases and/or ESBLs
220 encoding genes, including bla_{OXA-1} (n = 9), $bla_{CTX-M-55}$ (n = 5), $bla_{CTX-M-123}$ (n = 3),
221 $bla_{TEM-206}$ (n = 4), and $bla_{TEM-214}$ (n = 4). Seven isolates (33.3% were detected as
222 carrying two β-lactamases and/or ESBLs encoding genes. None of the isolates were
223 positive for bla_{SHV}, bla_{PSE}, or bla_{CMY}.
224
225 **PFGE**
226 Twenty-one PFGE patterns were identified among the 21 ESBLs-producing isolates
227 (Fig. 1). Seven ESBLs-producing Indiana isolates, five Typhimurium isolates, three
228 Kentucky isolates, three Litchfield isolates, two Derby isolates and, one
229 Schwarzengrund isolate were analyzed via PFGE with the enzyme XbaI. The PFGE
230 profile analysis demonstrated that *Salmonella* of the same serotype isolated from
231 different times and locations have markedly different genotypes. Similarly, serovars
232 isolated from the same time and place also showed diverse genotypes.
233
234 **DISCUSSION**
235 We found the high levels of *Salmonella* contamination rate in chicken (63.6%) and
236 pork (73.1%) collected from retail markets in Guangdong Province, China. These
237 prevalences were significantly higher than those reported in similar studies in other
238 districts in China, such as Hebei (Yan et al., 2010), Sichuan (Li et al., 2013), Henan
239 (Li et al., 2014), Beijing (Wang et al., 2014), Jiangsu (Bai et al., 2015), and Shandong
240 (Cui et al., 2016). Meanwhile, the *Salmonella* contamination rate was higher than that
241 of other parts of Asia, such as South Korea (Choi et al., 2015), Tokyo (Katoh et al.,
242 2015), and Northern Vietnam (Thai et al., 2012), and higher than that reported in
243 Egypt (Gharieb et al., 2015). This indicated that contamination by *Salmonella* in
244 animal-derived foods in Guangdong Province is more serious, which greatly increases
245 the risk of humans being infected. The results suggested that *Salmonella* in livestock
246 and poultry products should be a priority area for supervision in Guangdong Province.
247 It is also a reminder that animal-derived foods in the tropics should be paid more
248 attention and reflected the need for strengthening of the monitoring of *Salmonella* the
249 animal-origin food supply; for example, improving environmental sanitation in food
250 processing and health awareness among employees should be enhanced, and the
251 disinfection management of processing equipment and tableware containing food
252 should be improved.

Thirty-eight *Salmonella* serovars were found in retail meat samples, among which, serovars Typhimurium, Rissen, Derby (pork), and Agona, Corvallis, and Kentucky (chicken) were the most common serotypes, respectively. The serotypes found in the pork samples were the same as those identified in previous studies in China and abroad (Liang et al., 2015). The distribution of the serotypes in the chicken samples was very different from those in other regions. It was reported that serovars Enteritidis and Typhimurium were two predominant serotypes in chickens from other regions (Li et al., 2017). Interestingly, Agona, Corvallis, and Kentucky were reported less frequently in previous studies (Arnedo-Pena et al., 2016), whereas they were the dominant isolates in the current investigation. Additionally, serovar Agona is one of the top 10 serotypes responsible for nosocomial infections in China (Zhang et al., 2014); serovar Corvallis has been reported in Brazil, Germany, and Turkey (Kocabiyik et al., 2006; Fischer et al., 2013; Yamatogi et al., 2015); and serovar Kentucky was the most common serovar in the USA (van Duijkeren et al., 2002; Galanis et al., 2006). The results suggest that Agona, Corvallis, and Kentucky may cause public health concerns in Guangdong. Importantly, serovar Rumford was detected in animal-derived food for the first time in China. The detection of this strain not only fills the blank of the bacterial species library in China, but also provides a new basis for diagnostic tests and epidemiological investigation of this strain. Notably, Typhimurium is the most frequently isolated serovar from infants in Guangdong (Deng et al., 2012). Therefore, further study is required to determine the source of this serovar (Liang et al., 2015; O'Leary et al., 2015). Our results also showed that an association might exist between *Salmonella*-contaminated food and the sample source. Guangdong is mainly involved in yellow broiler production and its breeding, production mode, and climatic conditions are different from those in other regions, which might explain the different serotype distribution. Thus, China should strengthen yellow chicken monitoring and pay attention to changes in serotypes in different regions.

The susceptibility results showed more than 75% of the *Salmonella* isolates were resistant to tetracycline and sulfisoxazole. These high resistance rates reflect their widespread use in animal feed and are consistent with other reports (Bai et al., 2015). In addition, resistance to florfenicol and chloramphenicol in 47.0% and 48.3% of chicken isolates, in 30.7% and 31.6% of the pork isolates deserves our attention because resistance to these antibiotics may cause human resistance. In this study, all of the *Salmonella* isolates were susceptible to amoxicillin/clavulanic acid and amikacin, possibly because these antibiotics are not used for therapeutic purposes in clinical veterinary medicine; this result was consistent with previous reports (Sinwat et al., 2015). Different serotypes of *Salmonella* showed different resistance rates. In the present study, we observed a high prevalence of MDR *Salmonella* isolates among those isolated from chicken and pork. Serovars Agona, Corvallis, and Kentucky had higher resistance rates to the third-generation cephalosporins (CTX, CAZ) and fluoroquinolones than did the serovars Rissen and Derby. Serovars Agona, Corvallis, and Kentucky also exhibited higher resistance rates to ofloxacin. They also provided more information for further study related to cephalosporin or fluoroquinolones-resistant *Salmonella*. The increasing prevalence of MDR *Salmonella* involving multiple resistances to various antibiotics could cause Salmonella to evolve towards becoming a super bacteria (Campioni et al., 2014). The association between antibiotic resistance and specific serotypes should that Kentucky, Typhimurium, Corvallis, and Agona were strongly associated with MDR phenotypes. In addition,

303 resistant strains were found for first line of drug used in human medicine, such as
304 Carbapenems and Polymyxins, which showed that *Salmonella* had a tendency toward
305 drug resistance Hence, there is a need to strengthen epidemiological investigations of
306 *Salmonella* infections and further studies are required to improve our knowledge
307 concerning the development of MDR.
308
309   In the present study, the most commonly detected ESBLs encoding genes were
310 OXAs (encoding oxacillinases; 42.9%), followed by CTX-Ms (encoding
311 cefotaximases; 23.8%). This was similar to the results of previous studies in which
312 CTX-M- types were the main family of ESBLs (Nguyen et al., 2016; Qiao et al.,
313 2017). Furthermore, reports indicated that CTX-Ms have been disseminating rapidly
314 among populations of gram-negative bacteria in clinical settings in recent years
315 (Shahid et al., 2009). The 21 ESBLs-producing isolates identified in this study were
316 subjected to PFGE analysis. The resultant dendrograms of isolate patterns showed
317 clusters with a high level of diversity. This indicated that the *Salmonella* prevailing in
318 Guangdong's retail market during this period not comprised diverse serotypes, but
319 also strains of the same serotype have more complex genotypes and exhibit diverse
320 features. Thus, we should pay attention to this easily overlooked repository of
321 multidrug-resistant genes, and to the food safety issues they invoke in terms of
322 consumers' health. Moreover, more research into the characteristics of the
323 dissemination of *Salmonella* and antibiotic resistance genes is warranted.
324
325 **CONCLUSION**
326 In conclusion, our results indicated the diversity of *Salmonella* serotypes and the high
327 prevalence and antibiotic resistance that existed among *Salmonella* recovered from
328 chicken and pork meat at retail markets. In particular, multidrug-resistant *Salmonella*
329 serotypes *Agona*, *Corvallis*, and *Kentucky* may indicate the potential risk of resistant
330 *Salmonella* foodborne infections in Guangdong, China. Thus, this study suggests that
331 epidemic trends information about the *Salmonella* serovars in Guangdong province
332 should be further monitored and studied.
333

334 **AUTHOR CONTRIBUTIONS**
335 ML, LZ, contributed to the conception of the study; JZ, LZ, contributed significantly
336 to analysis and manuscript preparation; LZ, performed the data analyses and wrote the
337 manuscript; YF, ZX, YM: helped perform the analysis with constructive discussions;
338 YW, XQ and HZ collected samples and conducted the experiments.
339

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527

Table 1. Prevalence and serotypes of *Salmonella* isolated from chicken and pork meat at retail markets in Guangzhou, China

Source of samples	No. of positive samples /No. of total sample(%)	Serovar of isolates (number of samples)	Number of Serovars
Chicken	302/475 (63.6)	Agona (55), Corvallis (45), Kentucky (38), Mbandaka (32), Braenderup (21), Enteritidis (21), Rissen (14), Indiana (12), Derby (9), London (9), Meleagridis (7), Typhimurium (6), Stanley (5), Cerro (5), Albany (4), Hadar (4), Senftenberg (3), Litchfield (3), Weltevreden (1), Anatum (1), Muenster (1), Infantis (1), Haifa (1), Rumford (1), Saintpaul (1), Thompson (1), Schwarzengrund (1).	27
Pork	313/428 (73.1)	Typhimurium (78), Rissen (67), Derby (66), London (48), Meleagridis (13), Stanley (9), Give (4), Weltevreden (3), Krefeld (3), Newport (3), Mbandaka (2), Senftenberg (2), Anatum (2), Goldcoast (2), Agona (1), Corvallis (1), Muenster (1), Infantis (1), Alachua (1), Bovismorbificans (1), Brandenburg (1), Chailey (1), Kedouguo (1), Kottbus (1), Reading (1).	25
Total	615/903 (68.1)		38

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Table 2. Antibiotic resistance of *Salmonella* isolates from retail meats in Guangdong, China.

Antibiotic	Number of resistant isolates (%) from:		
	Chicken (n = 302)	Pork (n = 313)	Total (n = 615)
β-lactams			
Ampicillin	96 (31.8)	199 (63.6)	295 (48.0)
Amoxicillin/clavulanic acid	4 (1.3)	3 (1.0)	7 (1.1)
Cefotaxime	30 (9.9)	10 (3.2)	40 (6.5)
Ceftazidime	10 (3.3)	4 (1.3)	14 (2.3)
Cefepime	8 (2.6)	9 (2.9)	17 (2.8)
Aminoglycosides			
Gentamicin	34 (11.3)	66 (21.1)	100 (16.3)
Streptomycin	84 (27.8)	127 (40.6)	211 (34.3)
Aamikacin	6 (2.0)	2 (0.6)	8 (1.3)
Sulfonamides			
Trimethoprim/sulfamethoxazole	103 (34.1)	145 (46.3)	248 (40.3)
Sulfisoxazole	212 (70.2)	256 (81.8)	468 (76.1)
Quinolones			
Nalidixic acid	112 (37.1)	65 (20.8)	177 (28.8)
Ofloxacin	192 (63.6)	83 (26.5)	275 (44.7)
Ciprofloxacin	37 (12.3)	37 (11.8)	74 (12.0)
Phenicols			
Florfenicol	142 (47.0)	96 (30.7)	238 (38.7)
Chloramphenicol	146 (48.3)	99 (31.6)	245 (39.8)
Carbapenems			
Iimipenem	0	3 (1.0)	3 (0.5)
Polymyxins			
Polymyxin B	7 (2.3)	5 (1.6)	12 (2.0)
Tetracyclines			
Tetracycline	216 (71.5)	247 (78.9)	463 (75.3)

Table 3. Antibiotic resistance for the most common *Salmonella* serotypes isolated from pork and chicken meats in Guangdong

Antibiotic	Number (%)								
	Typhimurium (n = 84)	Rissen (n = 81)	Derby (n = 75)	London (n = 57)	Agona (n = 56)	Corvallis (n = 46)	Kentucky (n = 38)	Mbandaka (n = 34)	Others (n = 144)
AMP	70 (83.3)	63 (77.8)	42 (56.0)	35 (61.4)	9 (16.1)	8 (17.4)	8 (22.2)	0 (0)	60 (41.7)
AMC	2 (2.4)	0 (0)	2 (2.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	3 (2.1)
CTX	8 (9.5)	1 (1.2)	4 (5.3)	0 (0)	3 (5.4)	4 (8.7)	4 (11.1)	1 (2.9)	15 (10.4)
CAZ	2 (2.4)	1 (1.2)	0 (0)	0 (0)	1 (1.8)	2 (4.3)	0 (0)	1 (2.9)	7 (4.9)
FEP	5 (6.0)	0 (0)	3 (4.0)	0 (0)	0 (0)	1 (2.2)	0 (0)	0 (0)	8 (5.6)
GEN	13 (15.5)	2 (2.5)	27 (36.0)	33 (57.9)	0 (0)	0 (0)	3 (8.3)	1 (2.9)	21 (14.6)
STR	50 (59.5)	16 (19.8)	27 (36.0)	39 (68.4)	9 (16.1)	8 (17.4)	5 (13.9)	25 (73.5)	32 (22.2)
AMK	1 (1.2)	0 (0)	1 (1.3)	0 (0)	1 (1.8)	0 (0)	1 (2.8)	1 (2.9)	3 (2.1)
SXT	23 (27.4)	63 (77.8)	32 (42.7)	40 (70.2)	10 (17.9)	8 (17.4)	26 (72.2)	2 (5.9)	44 (30.6)
S3	75 (89.3)	63 (77.8)	69 (92.0)	44 (77.2)	23 (41.1)	34 (73.9)	35 (97.2)	34 (100.0)	91 (63.2)
NAL	25 (29.8)	6 (7.4)	30 (40.0)	1 (1.8)	11 (19.6)	20 (43.5)	11 (30.6)	1 (2.9)	72 (50.0)

OFX	25 (29.8)	7 (8.6)	32 (42.7)	9 (15.8)	51 (91.1)	40 (87.0)	25 (69.4)	21 (61.8)	65 (45.1)
CIP	11 (13.1)	1 (1.2)	25 (33.3)	0 (0)	1 (1.8)	8 (17.4)	11 (30.6)	1 (2.9)	16 (11.1)
FFC	30 (35.7)	5 (6.2)	34 (45.3)	23 (40.4)	49 (87.5)	25 (54.3)	30 (83.3)	0 (0)	42 (29.2)
CHL	34 (40.5)	7 (8.6)	33 (44.0)	24 (42.1)	50 (89.3)	24 (52.2)	30 (83.3)	0 (0)	43 (29.9)
IPM	1 (1.2)	0 (0)	0 (0)	2 (3.5)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
PB	2 (2.4)	1 (1.2)	0 (0)	1 (1.8)	1 (1.8)	1 (2.2)	1 (2.8)	1 (2.9)	4 (2.8)
TET	69 (82.1)	77 (95.1)	61 (81.3)	49 (86.0)	27 (48.2)	37 (80.4)	33 (91.7)	33 (97.1)	77 (53.5)

* AMP: ampicillin, AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, FEP: cefepime, GEN: gentamicin, STR: streptomycin, AMK: amikacin, SXT: trimethoprim/sulfamethoxazole, S3: sulfisoxazole, NAL: nalidixic acid, OFX: ofloxacin, CIP: ciprofloxacin, FFC: florfenicol, CHL: chloramphenicol, IPM: imipenem, PB: polymyxin B, TET: tetracycline

Table 4. Multidrug-resistant (MDR) *Salmonella* isolates from retail meats in Guangdong, China.

No. of antibiotics	Number (%) of resistant isolates:		
	Chicken (n = 302)	Pork (n = 313)	Total (n = 615)
0	7 (2.3)	20 (6.4)	27 (4.4)
1–3	102 (33.8)	98 (31.3)	200 (32.5)
4–6	130 (43.0)	113 (36.1)	243 (39.5)
7–9	38 (12.6)	50 (16.0)	88 (14.3)
≥ 10	25 (8.3)	32 (10.2)	57 (9.3)
Resistance ≥ 1	295 (97.7)	293 (93.6)	588 (95.6)
MDR ≥ 3	245 (81.1)	229 (73.2)	474 (77.1)

Table 5. Multidrug resistance (MDR) of the most prevalent *Salmonella* serovars isolates from retail meats.

Serovars	Number (%) of antibiotics resistance				
	1–3	4–6	7–9	≥10	Total (resistance ≥ 3)
Typhimurium (84)	20 (23.8)	32 (38.1)	16 (19.0)	12 (14.3)	74 (88.1)
Rissen (81)	26 (32.1)	51 (63.0)	3 (3.7)	1 (1.2)	66 (81.5)
Derby (75)	31 (41.3)	12 (16.0)	11 (14.7)	20 (26.7)	54 (72.0)
London (57)	11 (19.3)	22 (38.6)	19 (33.3)	1 (1.8)	42 (73.7)
Agona (56)	19 (34.0)	31 (55.4)	5 (9.0)	1 (1.8)	53 (94.6)
Corvallis (46)	16 (34.8)	18 (39.1)	9 (19.6)	2 (4.3)	38 (82.6)
Kentucky (38)	5 (13.2)	22 (57.9)	8 (21.1)	3 (7.9)	35 (92.1)
Mbandaka (34)	17 (50.0)	17 (50.0)	0 (0)	0 (0)	32 (94.1)
Braenderup (21)	17 (81.0)	1 (4.8)	1 (4.8)	2 (9.5)	7 (33.3)
Enteritidis (21)	7 (33.3)	13 (61.9)	1 (4.8)	0 (0)	17 (81.0)
Meleagridis (20)	6 (30.0)	6 (30.0)	3 (15.0)	0 (0)	9 (45.0)
Stanley (14)	6 (42.9)	0 (0)	0 (0)	0 (0)	0 (0)
Indiana (12)	0 (0)	0 (0)	0 (0)	12 (100)	12 (100)

18

Table 6. *Salmonella* carrying ESBLs encoding genes and corresponding antibiotic resistance profile.

Number of isolates	Sample types	Serotypes	Resistance phenotype	ESBLs genes
7	Pork	Derby	AMP+CTX+FEP+NAL+TET	$bla_{CTX-M-55}$
	Pork	Derby	AMP+CTX+FEP+NAL+TET	$bla_{CTX-M-55}$
	Pork	Typhimurium	AMP+CTX+CAZ+FEP+STR+S3+OFX+TET	$bla_{TEM-206}$
	Pork	Typhimurium	AMP+CTX+GEN+STR+SXT+S3+FFC+CHL+TET	$bla_{TEM-214}$
	Pork	Typhimurium	AMP+CTX+FEP+STR+SXT+S3+OFX+FFC+CHL+TET	$bla_{TEM-206}$, $bla_{CTX-M-55}$
	Pork	Typhimurium	AMP+CTX+STR+S3+TET	$bla_{TEM-214}$, $bla_{CTX-M-55}$
	Pork	Typhimurium	AMP+CTX+FEP+STR+S3+NAL+OFX+CHL+TET	$bla_{CTX-M-55}$
14	Chicken	Indiana	AMP+CTX+GEN+STR+SXT+S3+NAL+OFX+CIP+FFC+CHL	$bla_{TEM-214}$, bla_{OXA-1}
	Chicken	Indiana	AMP+CTX+FEP+GEN+STR+AMK+SXT+S3+NAL+OFX+CIP+FFC+CHL+TET	$bla_{CTX-M-123}$
	Chicken	Indiana	AMP+CTX+CAZ+FEP+GEN+AMK+SXT+S3+NAL+OFX+CIP+FFC+CHL+TET	bla_{OXA-1}
	Chicken	Indiana	AMP+CTX+FEP+GEN+STR+AMK+SXT+S3+NAL+OFX+CIP+FFC+CHL+TET	bla_{TEM-1}, bla_{OXA-1}
	Chicken	Kentucky	AMP+CTX+GEN+SXT+S3+NAL+OFX+CIP+FFC+CHL+TET	$bla_{TEM-214}$
	Chicken	Indiana	AMP+CTX+GEN+STR+SXT+S3+NAL+OFX+CIP+FFC+CHL+TET	bla_{OXA-1}

Chicken	Litchfield	AMP+CTX+CAZ+FEP+GEN+STR+SXT+S3	$bla_{CTX-M-123}$
Chicken	Schwarzengrun	AMP+CTX+GEN+S3+NAL+OFX+CIP+FFC+CHL+TET	bla_{OXA-1}
Chicken	Indiana	AMP+CTX+CAZ+FEP+GEN+STR+SXT+S3+NAL+OFX+CIP+FFC+CHL+TET	$bla_{CTX-M-64}$, bla_{OXA-1}
Chicken	Indiana	AMP+CTX+CAZ+FEP+GEN+SXT+S3+NAL+OFX+CIP+FFC+CHL+TET	$bla_{CTX-M-15}$, bla_{OXA-1}
Chicken	Litchfield	AMP+CTX+CAZ+FEP+GEN+STR+SXT+OFX+FFC+CHL+TET	$bla_{CTX-M-123}$, $bla_{TEM-206}$
Chicken	Litchfield	AMP+CTX+GEN+STR+SXT+S3+OFX+FFC+CHL+TET	$bla_{TEM-206}$
Chicken	Kentucky	AMP+CTX+S3+OFX+FFC+CHL	bla_{OXA-1}
Chicken	Kentucky	AMP+CTX+GEN+S3+NAL+OFX+CIP+FFC+CHL	bla_{OXA-1}

* AMP: ampicillin, AMC: amoxicillin-clavulanic acid, CTX: cefotaxime, CAZ: ceftazidime, FEP: cefepime, GEN: gentamicin, STR: streptomycin, AMK: amikacin, SXT: trimethoprim/sulfamethoxazole, S3: sulfisoxazole, NAL: nalidixic acid, OFX: ofloxacin, CIP: ciprofloxacin, FFC: florfenicol, CHL: chloramphenicol, IPM: imipenem, PB: polymyxin B, TET: tetracycline

Table 7. Prevalence of *Salmonella* carrying β-lactamase and ESBLs encoding genes (n= 21).

Genes		Number of isolates	Percentage of isolates
bla$_{TEM}$	*bla*$_{TEM-1}$	1	4.8
	bla$_{TEM-206}$	4	19.0
	bla$_{TEM-214}$	4	19.0
bla$_{CTX}$	*bla*$_{CTX-M-15}$	1	4.8
	bla$_{CTX-M-55}$	5	23.8
	bla$_{CTX-M-64}$	1	4.8
	bla$_{CTX-M-123}$	3	14.3
bla$_{OXA}$	*bla*$_{OXA-1}$	9	42.9

1   **Fig 1.** Dendrogram of pulse field gel electrophoresis profiles of 21 extended spectrum
2   β-lactamases (ESBLs)-producing *Salmonella* isolates with the respective ESBL genes
3   from chicken and pork meat.

Figure 01.TIF

Percentage similarity	PFGE profiles	Isolates	Serotype	Time	Sample type	Sample source	ESBLs genes
		668	Litchfield	2016.11	Chicken	Nanshan District, Shenzhen	CTX-M-123, TEM-206
		669	Litchfield	2016.11	Chicken	Nanshan District, Shenzhen	TEM-206
		610	Indiana	2016.10	Chicken	Haizhu District, Guangzhou	CTX-M-64, OXA-1
		629	Indiana	2016.10	Chicken	Baiyun District, Guangzhou	CTX-M-15, OXA-1
		1	Derby	2016.3	Pork	Tianhe District, Guangzhou	CTX-M-55
		3	Derby	2016.3	Pork	Tianhe District, Guangzhou	CTX-M-55
		586	Indiana	2016.10	Chicken	Liwan District, Guangzhou	OXA-1
		590	Litchfield	2016.10	Chicken	Liwan District, Guangzhou	CTX-M-123
		473	Indiana	2016.9	Chicken	Heyuan	TEM-1, OXA-1
		595	Schwarzengrund	2016.10	Chicken	Yuexiu District, Guangzhou	OXA-1
		270	Typhimurium	2016.7	Pork	Yuexiu District, Guangzhou	TEM-206
		467	Typhimurium	2016.9	Pork	Foshan	TEM-206, CTX-M-55
		564	Typhimurium	2016.10	Pork	Liwan District, Guangzhou	TEM-214, CTX-M-55
		449	Typhimurium	2016.9	Pork	Baiyun District, Guangzhou	TEM-214
		566	Typhimurium	2016.10	Pork	Liwan District, Guangzhou	CTX-M-55
		348	Indiana	2016.8	Chicken	Liwan District, Guangzhou	OXA-1
		55	Indiana	2016.5	Chicken	Liwan District, Guangzhou	CTX-M-123
		36	Indiana	2016.5	Chicken	Haizhu District, Guangzhou	TEM-214, OXA-1
		671	Kentucky	2016.11	Chicken	Nanshan District, Shenzhen	OXA-1
		672	Kentucky	2016.11	Chicken	Nanshan District, Shenzhen	OXA-1
		485	Kentucky	2016.9	Chicken	Yunfu	TEM-214