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Running title: Antimicrobial resistance of *Salmonella* in 2020 in China

Antimicrobial Resistance in Non-typhoidal *Salmonella* from Retail Foods Collected in 2020 in China

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ABSTRACT

Objective:

Non-typhoidal *Salmonella* (NTS) is a major cause of human salmonellosis globally and food animals are major reservoirs. The rising trend of antimicrobial resistance (AMR) of foodborne NTS led to clinical treatment failure. We tested the antimicrobial susceptibility of 1,256 NTS cultured from retail foods in 2020 in China to better understand the prevalence and characterization of AMR foodborne NTS of China.

Method:

The antimicrobial susceptibility of 26 antimicrobial agents representing 12 classes was evaluated by the broth-microdilution; the presence of ten *mcr* genes was screened by multi-PCR. The complete closed genomes of *mcr*-gene-carrying isolates were obtained by whole genome sequencing using both PacBio and Illumina platforms. Genomic features and genetic environments of *mcr-1* gene were analysed.

Results:

The whole drug resistance rate was 92.28% and the multiple drug resistance (MDR) rate was 76.53%. Resistance was the highest to nalidixic acid (63.38%); 341 AMR profiles were recorded. Among 887 MDR NTS, 232 showed co-resistance to cefotaxime and ciprofloxacin, and 25 were resistant to ten classes of antimicrobials. The resistance of NTS isolated from different

regions varied. Isolates of raw chicken source showed most frequent resistance. Four NTS carried *mcr-1* gene and they represented four different serotypes. Four *mcr-1* gene-harboring plasmids from the four *Salmonella* isolates were denoted as two replicon types (IncI2 and IncHI2A). Two *mcr-1* genes on IncI2 type plasmids were found to be located between a PAP2 family protein-encoding gene and a relaxase-encoding gene, while the other two *mcr-1* gene structures located at IncHI2A type plasmids varied with different presence of insertion sequences.

Conclusion:

Our data demonstrated a severe AMR for foodborne NTS isolated from food in China, and highlighted the importance of antimicrobial susceptibility surveillance to reduce resistance dissemination, especially to the concerned critically important drugs for human medicine.

Keywords：Non-typhoidal *Salmonella* (NTS), antimicrobial resistance(AMR), multi-drug resistance (MDR), *mcr-1*

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INTRODUCTION

Foodborne diseases remain a global public health challenge with a significant burden. Unsafe food causes an annual 600 million cases of foodborne illnesses and 420,000 deaths by 31 hazards in 2010 worldwide, and 40% of these deaths occur among children younger than 5 years of age **[1]**. The most frequent causes of these foodborne illnesses were diarrhoeal agents that caused 230,000 deaths, particularly non-typhoidal *Salmonella* (NTS). NTS is a major cause of foodborne infections that give rise to over 93 million cases of gastroenteritis annually and 155,000 deaths globally, resulting in about 4 million disability-adjusted life years **[2]**. In the United States, it is estimated that 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths are attributed to NTS, leading to more than $400 million of medical costs each year **[3]**. In 2019, 27 European Union member states reported 5,175 foodborne outbreaks, NTS was the most commonly identified agent that accounts for 17.9% of the total outbreaks **[4]**. From 2002 to 2017, China reported 2,815 foodborne disease outbreaks related to meat and meat products, resulting in 52,122 illnesses, 25,361 hospitalizations and 96 deaths. Among which, NTS was the most common cause of outbreaks (420/2815, 14.92%) and hospitalizations (7641/25,361, 30.13%). Hence, NTS ranks as the most reported bacterial species causing human gastrointestinal infections globally. Food animals mainly poultry is a major reservoir of NTS and contaminated animal-based products are frequently associated with

human salmonellosis.

The emergence and spread of antimicrobial resistant (AMR) NTS has become great public health concerns over the past two decades. Of particular concern is the occurrence of extended spectrum beta-lactamase genes in plasmids of NTS and reports of carbapenemase-containing NTS isolates **[5,6]**, both of which confer resistance to highly important antimicrobials. Acquisition of AMR genes to both antimicrobials by foodborne NTS along the food chain has been increasing. Treatment options of salmonellosis in animals and humans have become more difficult due to the antimicrobial resistant NTS. Data from China showed that the prevalence of multidrug-resistant (MDR) NTS increased from 20-30% in the 1990s to 70% in the early 2000s, and the overall incidence of foodborne antimicrobial resistant NTS was higher than 70% between 2015 and 2016, particularly, strains carrying *mcr-1* gene on plasmid mediated resistance to colistin **[7]**. Food workers that are infected with antimicrobial resistant NTS after consuming or handling contaminated food may serve as reservoirs and pose a high risk for further food contamination. To reduce the prevalence of NTS in foods and consequently the burden of human salmonellosis, China implemented a foodborne pathogen monitoring and control program across the country. In this study, the antimicrobial susceptibility of 1256 NTS isolates cultured from retail foods in 2020 in China was tested. All isolates were subsequently screened for the presence of *mcr* genes by polymerase

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chain reaction (PCR) followed by whole genome sequencing of the *mcr* gene-positive strains for further confirmation with the aim of insight into the genome of antimicrobial mechanisms.

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MATERIALS AND METHODS

Bacterial strains. A total of 1256 foodborne NTS isolates were cultured from various retail foods, mainly meat and meat-based products collected from 30 provinces (municipalities or autonomous regions) in China in 2020. The presumptive colonies were confirmed as *Salmonella* by both morphology and amplification of the *invA* gene by PCR as described previously, the ones with negative amplification would be further confirmed with GN card and Vitek2 compact (BioMérieux, France) **[8]**. All isolates confirmed to be *Salmonella* were preserved in brain heart infusion (BHI) broth with 40% (v/v) glycerol (HopeBio, Qingdao, China) at -80°C before analysis. *Escherichia coli* ATCC®25922 was used as the control for the antimicrobial susceptibility testing.

Antimicrobial susceptibility testing (AST). All *Salmonella* isolates were performed on AST using the Biofosun® Gram-negative panels (Fosun Diagnostics, Shanghai, China) by the broth microdilution method. The panel of 26 antimicrobial compounds including ampicillin (AMP), ampicillin/sulbactam (SAM), cefepime (FEP), ceftazidime (CAZ), ceftriaxone (CRO), cefoxitin (FOX), cefotaxime (CTX), aztreonam (ATM), ertapenem (ETP), imipenem (IMP), meropenem (MEM), colistin (CT), polymyxin B (PB), gentamicin (GEN), amikacin (AK), tetracycline (TET), doxycycline (DC), tigecycine (TGC), ciprofloxacin (CIP), nalidixic acid (NAL), sulfamethoxazole-trimethoprim(SXT), sulfonamides (SMX), trimethoprim (TMP), chloramphenicol

(CHL), florfenicol (FFC), nitrofurantoin (NIT) that represent 12 classes were selected. Data obtained were interpreted following recommendations described by Clinical and Laboratory Standards Institute guidelines (CLSI, M100-S32, version 2022) **[9]**. Additionally, CLSI (M31-A3, version) and European Committee on Antimicrobial Susceptibility Testing documents were consulted for FFC and TGC, respectively **[10,11]**.

***mcr* genes screening.** All 1256 foodborne NTS isolates were screened for the presence of *mcr* genes (from *mcr-1* to *mcr-10*) by multi-target PCR methods as previously reported **[12]**. Isolates that carry any *mcr* genes were selected for further whole genome sequencing.

Whole genome sequencing. DNA extraction and whole genome sequencing were conducted for *mcr*-gene-carrying isolates to obtain complete genomes. Briefly, a single colony for NTS isolate was cultured in BHI broth and incubated at 37°C overnight. A TIANamp bacterial DNA extraction kit (DP302, TIANGEN BIOTECH, Beijing, China) was used to extract the bacterial genomic DNA according to the manufacturer's instructions, followed by a library preparation with NEBNext® Ultra DNA Library Prep Kit for Illumina (NEB#E7370) and sonication fragmentation (350bp insert). Sequencing was performed commercially using Illumina HiSeq platform with PE 150 sequencing strategy (Novogene, Beijing, China) using a HiSeq X Ten Reagent Kit v2.5 (Illumina, San Diego, CA). The *mcr* gene carrying isolates were also sequenced with the SMRT® Pacific Biosciences

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(PacBio) Sequel platform (Tianjin Biochip Corporation, Tianjin, China) with a 10-kbp template library preparation step with PacBio® Template Prep Kit. SMRT Analysis v2.3.0 was used for de novo assembly according to RS Hierarchical Genome Assembly Process (HGAP) workflow v3.0. Subsequently, Consed version 28.0 was used to manually inspect and trim duplicate ends to generate single, complete and closed sequences for each chromosome and plasmid. For data error correction, Pilon v1.23 was used with Illumina MiSeq sequencing read data. The closed genomes were then annotated using prokka (version 1.14.6).

Bioinformatic Analysis. The predicted serotype and multi-locus sequence typing (MLST) types were identified using the *Salmonella* In Silico Typing Resource (SISTR). Plasmid replicon types (Incompatibility groups or Inc groups) were identified through the Center for Genomic Epidemiology (CGE) website with PlasmidFinder (v2.0). All genes, plasmids and chromosome sequences used in this study were managed, aligned and analyzed by Geneious prime (v2023.1.2) software. Genetic environments of *mcr-1* gene were analysed and displayed using Easyfig (v2.2.2).

RESULTS

Antimicrobial resistance of 1256 NTS isolates. The key antimicrobial resistance trends in 1256 NTS isolates recovered from various foods is shown in **Table 1**. Totally, 1159 (1159/1256, 92.28%) isolates exhibited resistance to at least one antimicrobial compound, while 97 (97/1256, 7.72%) isolates had no resistance to any of the antimicrobial compounds tested. Resistance in strains studied was most frequent to nalidixic acid (796/1256, 63.38%), sulfonamides (782/1256, 62.26%), tetracycline (714/1256, 56.85%), doxycycline (710/1256, 56.53%), ampicillin (705/1256, 56.13%), ampicillin/sulbactam (530/1256, 42.20%), florfenicol (463/1256, 36.86%), chloramphenicol (455/1256, 36.23%), trimethoprim (427/1256, 34.00%), followed by resistance to some drugs with the prevalence lower than 30% (**Table 1**). Lower resistance to amikacin (93/1256, 7.40%) and cefoxitin (33/1256, 2.63%) were observed. It is necessary to point out that high prevalence of resistance to drugs categorized as critically important antimicrobials for human medicine by World Health Organization **[13]** including cephalosporin, quinolones, aminoglycosides, lipopeptides, monobactams, penicillins were found in these foodborne NTS (**Table 1**). In particular, higher resistance to the first line antimicrobial compounds for salmonellosis treatment, cephalosporin including the 3rd generations cefotaxime (322/1256, 25.64%), ceftriaxone (316/1256, 25.16%), ceftazidime (223/1256, 17.75%) and the 4th generation cefepime (282/1256, 22.45%), as

well as quinolones like ciprofloxacin (333/1256, 26.51%), respectively were observed. These findings indicated that the resistance of NTS to both cephalosporin and quinolones were largely increased in comparison with the results published previously **[7]**. In addition, high percentage of intermediate resistance to polymyxin B (944/1256, 75.16%), colistin (933/1256, 74.28%), ciprofloxacin (655/1256, 52.15%) and slightly low incidence of intermediate resistance to nitrofurantoin (353/1256, 28.11%), chloramphenicol (240/1256, 19.11%), ampicillin/sulbactam (150/1256, 11.94%) and cefoxitin (117/1256, 9.32%) were found, illustrating that the resistance to the above mentioned antimicrobial agents will much increased in the near future. No isolate was resistant to tigecycine and any of carbapenem compounds tested (ertapenem, imipenem and meropenem).

Co-resistance and antimicrobial resistance profiles. Of 1159 resistant NTS isolates, MDR (resistant to $\geqslant 3$ antimicrobial classes) was present in around 76.53% (887/1159) of isolates on average. Among which, 146 (146/887, 16.46%), 138 (138/887, 15.56%), 140 (140/887, 15.78%), 127 (127/887, 14.32%), 88 (88/887, 9.92%), 95 (95/887, 10.71%), 128 (128/887, 14.43%) and 25 (25/887, 2.82%) isolates were resistant to 3, 4, 5, 6, 7, 8, 9 and 10 classes of antimicrobials tested, respectively. Especially, 248 isolates (27.96%, 248/887) were resistant to as many as eight or more classes of antimicrobials. It is worthy of noting that 232 (26.16%, 232/887) MDR isolates were co-resistant to cefotaxime and ciprofloxacin, which are the first-line

antimicrobial agents to treat human salmonellosis clinically. In total, 341 antimicrobial resistant profiles were recorded. The top five antimicrobial resistant profiles were AMP-SAM-FEP-CAZ-CRO-CTX-ATM-GEN-AK-TET-DC-CIP-NAL-SXT-SMX-TMP-CHL-FFC (4.23%, 49/1159), TET-DC (3.71%, 43/1159), AMP-SAM-CT-PB-NAL-SMX-NIT (3.62%, 42/1159), CT-PB-NAL (3.62%, 42/1159) and SMX (3.62%, 42/1159) (**Table S1**).

Geographical distribution of antimicrobial resistant NTS isolates. In total, 30 provinces were selected as the sampling sites. By sampling locations, the frequency of antimicrobial resistance ranged from 78.57% to 100%, with an average of 92.28%. The geographical distribution of AMR frequency is given in **Figure 1**. As shown in **Figure 1**, more than half NTS isolates was MDR with a range between 56.45% to 100% (average76.53%, 887/1159). In particular, the frequency of MDR NTS in 11 provinces including Hebei, Anhui, Ningxia, Yunnan, Liaoning, Henan, Jiangsu, Inner Mongolia, Shanxi, Jiangxi and Chongqing were all higher than 80% (range: 80.95% - 92.42%); whereas, between 70.37% and 78.48% were found in 11 provinces including Shandong, Gansu, Beijing, Heilongjiang, Guizhou, Zhejiang, Sichuan, Shaanxi, Guangxi, Fujian and Tianjin, and between 56.45% and 69.01% for Hunan, Guangdong, Shanghai and Hubei, respectively. Although isolates obtained in Qinghai, Hainan and Xinjiang were all MDR (and 75.00% for Jilin), variations may be due to a few numbers of isolates cultured in these regions. Regarding the 18 provinces with the isolate

number higher than 35, the highest total resistant frequency of 100.00% (38/38) together with MDR frequency of 76.32% (29/38) were found in Gansu, followed by Hebei (98.51, 66/67) for the total and 92.42, 61/66 for that of MDR) and Jiangsu (96.77%,60/62 for the total and 83.33%, 50/60 for that of MDR).

Antimicrobial resistance of NTS from different food samples. NTS were recovered from six categories of foods in this study. By food categories, resistance to any of the 26 tested compounds was most frequent in raw chicken sources (approximately 93.85% resistant to ≥1 class of agent, 565/602), followed by other raw poultry sources (about 92.04%, 104/113), and raw duck (88.19%, 254/288). Whereas, NTS cultured from prepared meat exhibited the same trend of resistance to one class of drug (93.55%, 58/62) for red meat source and 93.85%, 122/130 for poultry meat source) in comparison with those from raw poultry meat. Proportionately, MDR was most frequent in isolates from prepared poultry meat (76.15%, 99/130) and raw chicken (74.42%, 448/602), followed by prepared red meat (72.58%, 45/62), other raw poultry meat (66.37%, 75/113) and raw duck (61.46%, 177/288). Although high prevalence of MDR (78.38%, 29/37) in isolates from other foods (including sushi, cake and bread, milk, beverage and processed algae) was found, a small number of NTS isolates may contribute to this big variation. Among 25 isolates resistant to ten classes of antimicrobials, 24 of them were recovered from raw poultry samples and only one from prepared

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beef (**Table 2**). These findings showed that NTS from poultry sources tend to be more resistant than those from other sources.

***mcr* genes screening.** PCR results together with whole-genome sequencing data notified that 4 out of 1256 (0.32%) NTS isolates carried the *mcr-1* gene. No other *mcr* genes (from *mcr-2* to *mcr-10*) were detected. All the four *mcr-1* positive NTS were recovered from prepared meat samples. Samples information and resistance phenotypes against a panel of 26 antimicrobial compounds were shown in **Table 3**. It is worthy of pointing out that three strains coded 2020s302, 2020s327 and 2020s329 cultured from two prepared chicken samples and one prepared beef sample, respectively, were from the same region (Quzhou, Zhejiang province), while the rest strain coded 2020s542 was isolated from prepared chicken meat in Suizhou, Hubei province. All these four *mcr-1* positive isolates showed resistance to ampicillin, ceftriaxone, cefotaxime and colistin but susceptible to cefoxitin, ertapenem, imipenem, meropenem, amikacin, tigecycine and nitrofurantoin. This demonstrated an MDR phenotype of the four *mcr-1* positive strains against at least three classes of antimicrobial compounds. Strains of 2020s302, 2020s327, 2020s329 and 2020s542 were resistant to 3, 9, 10 and 8 classes of antimicrobials with the AMR profiles of AMP-FEP-CRO-CTX-CT-PB, AMP-SAM-FEP-CRO-CTX-CT-PB-GEN-TET-DC-CIP-NAL-SXT-SMX-CHL-FFC, AMP-SAM-FEP-CAZ-CRO-CTX-ATM-CT-GEN-TET-DC-CIP-NAL-SMX-CHL-FFC and AMP-SAM-CRO-CTX-ATM-CT-PB-TET-DC-SXT-SMX-

TMP-CHL-FFC, respectively.

Genome features of *mcr-1*-harboring *Salmonella* isolates

Whole genome sequence blending Illumina data and PacBio data showed that the serovar (MLST type) for these four *mcr-1* positive isolates were *S.* Bredeney (ST241) for 2020s302, *S.* Schwarzengrund (ST 241) for 2020s327, *S.* Kentucky (ST198) for 2020s329 and *S*. Newport (ST45) for 2020s542, based on the prediction by the SISTR platform. Each isolate consisted of one single circular chromosome (4.59~4.82M) and at least one plasmid (30~263k), in particular, *S*. Newport 2020s542 contained four plasmids (one big plasmid of 258k and three small plasmids of 30~85k). Four *mcr-1* gene-harboring plasmids were denoted as two replicon types (IncI2 and IncHI2A). The genome information including serotype, MLST type, genome size, GC content and incompatibility (Inc) group of each sequences of these four *Salmonella* isolates were listed in **Table 4**.

To better understand the genetic environment of the *mcr-1* loci of the plasmids harboring *mcr-1* gene, sequences extracted from different plasmids including plasmids from this study and previous studies, belonging to two replicon types, were compared and analysed (Figure 2). This analysis revealed that the *mcr-1* genes in five IncI2 type plasmids (pCFSA244-2, pCFSA664-3, pHNSHP45, p2020s542-3 and p2020s302-1) were found to be located between a PAP2 family protein-encoding gene (arrowed in yellow) and a relaxase-encoding gene (arrowed in dark green). In the case of plasmid

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pHNSHP45 (KP347127), an IS*30* family element IS*Apl1* was followed by its relaxase-encoding gene downstream. In terms of plasmid p2020s542-3 and p2020s302-1 in this study and together with pCFSA244-2, pCFSA664-3, the *mcr-1* genes were identified locating with a PAP2 family protein-encoding gene distal to this site, but without any ISs.

In comparison with these IncI2 type plasmids, seven IncHI2A plasmids (two plasmids in this study and five other plasmids from previous studies) did not have a relaxase-encoding gene upstream of the *mcr-1* gene but processed some hypothetical proteins and some ORFs. Besides the ORF differences, the main difference between gene structures near *mcr-1* of these plasmids is that they varied with different presence of insertion sequences. pCFSA1096 had no IS; pCFSA122-1, pCFSA629, pHNSHP45-2 (KU341381) and p2020S329-2 obtained only one IS*Apl1;* a tellurium resistance gene cluster was located downstream PAP2 coding gene of pCFSA629, pHNSHP45-2 and p2020S329-2. pWW012 (CP022169), the *mcr-1*-carrying plasmid from our previous study, together with the p2020s329-2 from this study, consisted of an IS-*mcr-1*-PAP2-IS module which is an IS*Apl1*-flanked composite transposon (Tn6330). And it should be noted that the PAP2 coding gene of p2020s329-2 were exactly the same sequence as the same gene of pWW012 but in opposite direction.

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Discussion

Antimicrobial resistance poses an important, complex, and priority global public health challenge. China has one of the largest food animal production economies in the world. For the sake of lowering the potential consequences of foodborne AMR risk to humans, animal and plant health, China works to implement a national antimicrobial resistance monitoring system and the status of resistance in *Salmonella* can be assessed from a large number of samples annually, most of which target retail meat products. In this study, we characterized NTS isolates cultured in 2020 that were tested for the resistance to a panel of 26 antimicrobial agents. The results showed that the drug resistance rate of food-borne NTS in 2020 was 92.28% and the multiple drug resistance rate was 76.53%, and these were consistent with those reported in 2015 **[7]**. Meanwhile, a higher resistant frequency was found in some antimicrobial agents like cephems, quinolones and fluoroquinolones, lipopeptides, penicillins, aminoglycosides that have a long history of usage in food production chains in China. Quinolone are the preferred front-line drugs for clinical treatment or prevention/prophylaxis of *Salmonella* disease. The frequency of drug resistance to nalididic acid and ciprofloxacin was 63.38% and 26.51%, respectively, slightly increased in comparison with the results obtained in 2016 (52.5% and 21.3%) **[14]**. Therefore, much more attention should be paid to that continuous increase of quinolone resistance will lead to the risk of clinical treatment failure.

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Antimicrobial resistance varies among the regions and food categories. Foodborne NTS showed regional differences in drug resistance with the range between 100% and 78.57% in this study. Totally, 341 antimicrobial resistance profiles were found in NTS tested, showing a high polymorphism. Additionally, data showed that more than 90% of the *Salmonella* isolates were found to be resistant to at least one antimicrobial agent, and resistance to the traditional compounds including ampicillin, ampicillin-sulbactam, nalidixic acid, and tetracycline was recorded frequently among substantial numbers of the study isolates. Particularly, frequency of resistance to ciprofloxacin (26.51%) and extended-spectrum cephalosporins including ceftazidime (17.75%) and cefotaxime (25.64%) were largely increased in comparison with the results of NTS (16.47% for ciprofloxacin, 4.71% for ceftazidime, 11.18% for cefotaxime, respectively) cultured from raw chicken carcasses conducted between 2011 and 2012[8]. Since the food category had a big influence on antimicrobial resistance of *Salmonella*, isolates from raw chicken samples collected after 2020 might have higher drug resistance. Hence, it is necessary to strengthen the supervision and management of the use of antimicrobial agents in the food production chain. In addition, carbapenems are not used in Chinese agriculture, nor are they approved for food-producing animals in any country. No carbapenemases-producing *Salmonella* has been found in the present study, suggesting that carbapenems were still be effective when tested in vitro but we have to keep

our eyes on the resistance to carbapenem compounds since it might not be effective as the ultimate drugs for the clinical treatment of *Salmonella* infection in vivo.

Polymyxins are important lipopeptide antibiotics that serve as the last-line defense against multidrug-resistant Gram-negative bacterial infections. Worryingly, the clinical utility of polymyxins is currently facing a serious threat with the global dissemination of MCR, mobile colistin resistance and the relevant gene *mcr*. The *mcr* genes are the main polymyxin resistance determinant in *Escherichia coli* and high prevalence remains in agriculture globally, especially in China, due to high polymyxin usage. The transferability of *mcr* is of considerable concern due to the potential of MDR Gram-negative bacteria to acquire *mcr*-harboring plasmids, negating antimicrobial therapy with the important last-line polymyxins. A gene named *mcr-1* was first reported in November 2015 in China **[15]**. Although the use of colistin as a feed additive for animals has been banned for agriculture purposes in China from April 30th 2017 **[16]**, NTS carrying the *mcr-1* gene were still isolated from lettuce, beef and pork products various foods with a frequency of 1.07% (3/280) in 2017, and from goose egg and field snails with a frequency of 0.69% (4/579) in 2018, respectively (data not published). No *mcr-1* positive foodborne NTS was detected in 2019. In the present study, *mcr-1* in foodborne NTS collected in 2020 was still detected at a low level of 0.32% (4/1256), similar to that of 0.23% (6/2555) in isolates reported by Hu et al **[17]**.

Meanwhile, compared with our previous data on food source of *mcr-1*-harbouring *Salmonella* isolates (pork, chicken, egg, and dumpling) **[17]**, the four strains of *Salmonella* carrying *mcr-1* gene in this study were cultured from either poultry or beef, indicating that *Salmonella*, as a reservoir of *mcr*-1 gene, may have complex diversity in the source of food and the *mcr-1* positive clone may be most limited to meat. Additionally, the widespread of *mcr-1* positive *Salmonella* among chicken, beef, pork, egg, vegetables also suggested the potential transmission via the food chain, particularly by chicken.

The most common *mcr-1* gene locus structure has been known to be an 2609bp DNA sequence consisting of an *mcr-1* gene and a putative PAP2 super family protein gene, along with two copies of IS*Apl1* which is a member of the IS*30* family, and this structure has made up a kind of composite transposon Tn6330 which is IS*Apl1*-flanked and which is also thought to mediate the initial *mcr-1* gene mobilization event **[18, 19]**. Though this transposon Tn6330 was so common and was found to be existing among a lot of *mcr-1*-harboring isolates, the *mcr-1* gene could also be disseminated with just a single end of IS*Apl1* or by other means devoid of this IS element, and such circumstance could occur on different plasmid replicon types, including IncI2-, P-, X4- and HI-type plasmids, and this also contributes to four general *mcr-1* structures identified to date **[18, 20]**. In this study, the *mcr-1* region located on p2020s327-1 possessed a very similar

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single-ended Tn6330 variant structure and also tellurium resistance coding gene region to other two *mcr-1* locus structure on pCFSA629 and pHNSHP45-2, which relate to a *S*. Typhimurium and an *E.coli* isolate, respectively. At the same time, both the *mcr-1* regions belonging to p2020s329-2 and pWW012 (*S*. Typhimurium) obtained more than one single IS, however, the one from pWW012 was the standard Tn6330 structure but there existed one difference in terms of p2020s329-2 reported in this study, that is PAP2 coding gene of opposite direction, which might be attributable to the gene rearrangement caused by the loss and gain of IS*Apl1* from the transposon occurring during multiplication. It was also reported that plasmid-to-chromosomal transfer of *mcr-1* could have occurred recently and that Tn6330 on the chromosome would provide a more stable *mcr-1* state with the loss of IS*Apl1* from the transposon occurring during the mobilization event and this would present an additional challenge in the fight to combat the dissemination of colistin resistant bacteria **[21]**.

This report showed that all 4 *mcr-1* positive *Salmonella* isolates were of multidrug resistance phenotypes, and 3 of them even resistant to more than 7 (with the highest of 10) out of 12 antibacterial classes. As polymyxins are the last therapeutic option for life-threatening infections caused by Gram-negative ‘superbugs’, every effort must be made to minimize the emergence of resistance, in particular due to *mcr*. Hence, an integrated monitoring and surveillance of foodborne antimicrobial usage as well as antimicrobial

resistance in humans, animals and plants/crops, availability of food consumption and agriculture production, based on a “One Health” approach which is a strong multi-sectoral collaborative and institutional system **[22]**, should be taken into account in China. Furthermore, more studies should be focused on resistance and transmission mechanism of food-borne *Salmonella* to important antimicrobial drugs in order to provide theoretical basis for rational use of antimicrobial drugs and government supervision in order to ensure food safety.

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CONFLICTS OF INTEREST

The authors declare that this research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCE

1. World Health Organization. WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. World Health Organization. Available from: [https://apps.who.int/iris/handle/10665/199350] [Accessed on 2023-01-02]
2. Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'Brien SJ, et al. The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin Infect Dis 50:882–889.
3. Centers for Disease Control and Prevention (ECDC). Antimicrobial resistance threats in the United States. Available from: [https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf][Accessed on 2023-01-02].
4. European Food Safety Authority and European Centre for Disease Prevention and Control. 2021. The European Union One Health 2019 Zoonoses Report. EFSA J. 2021;19(2):e06406.
5. Denagamage TN, Wallner-Pendleton E, Jayarao BM, et al. Detection of CTX-M-1 extended-spectrum beta-lactamase among ceftiofur-resistant *Salmonella* enterica clinical isolates of poultry. J Vet Diagn Invest. 2019;31(5):681-687.
6. Nordmann P, Poirel L, Mak JK, White PA, McIver CJ, Taylor P. Multidrug-resistant *Salmonella* strains expressing emerging antibiotic resistance

determinants. Clin Infect Dis. 2008;46(2):324-325.

7. Hu Y, Wang W, Yan S, Gan X, Lyu H, Liu C, Yang D, Li FQ and Xu J. Resistance analysis of 1070 *Salmonella* strains isolated from food sample in mainland China, 2015. Chinese Journal of Food Hygiene. 2017;29(6): 647-652.
8. Hu Y, He Y, Wang Y, Fanning S, Cui S, Chen Q, Liu G, Chen Q, Zhou G, Yang B, Huang J, Li F. Serovar diversity and antimicrobial resistance of non-typhoidal *Salmonella enterica* recovered from retail chicken carcasses for sale in different regions of China. Food Control, 2017:81:46-54.
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI supplement M100-S32. Wayne: CLSI, 2022.
10. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 8.0, 2018. Available from: [https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_8.0_Breakpoint_Tables.pdf] [Accessed on 2023-01-02].
11. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. M31-A3[M]. Wayne: CLSI, 2008.
12. Hu Y, He Y, Nguyen SV, Liu C, Liu C, Gan X, Wang W, Dong Y, Xu J, Li F,

Fanning S. Antimicrobial resistance of *Salmonella* Indiana from retail chickens in China and emergence of an *mcr-1*-harboring isolate with concurrent resistance to ciprofloxacin, cefotaxime, and colistin. Front Microbiol. 2022;13:955827.

13. World Health Organization. (2019) . WHO list of critically important antimicrobials for human medicine (WHO CIA list) . World Health Organization. Available from: [https://apps.who.int/iris/handle/10665/325036] [Accessed on 2023-01-02]
14. Hu Y, Liu C, Wang M, Gan X, Xu J, Li F, Yang D, Pei X. Resistance characteristic analysis for foodborne *Salmonella* isolates from China, 2016. Chinese Journal of Food Hygiene. 2018;30(5): 456-461.
15. Liu Y, Wang Y, Walsh TR, Yi L, Zhang R, Spencer J, et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis. 2016; 16: 161–168.
16. Ministry of Agriculture and Rural Affairs of the People's Republic of China. Cessation of colistin as a growth promoter (feed additive) in animals. Ministry of Agriculture announcement (number 2428). Available from: [http://www.moa.gov.cn/govpublic/SYJ/201608/t20160801_5224428.htm] [Accessed on 2023-01-02].
17. Yujie Hu, Se´amus Fanning, Xin Gan,Chang Liu, Scott Nguyen, Meimei Wang, Wei Wang, Tao Jiang, Jin Xu and Fengqin Li. *Salmonella*

harbouring the *mcr-1* gene isolated from food in China between 2012 and 2016. The Journal of antimicrobial chemotherapy, 2019, 74(03) : 826-828.

18. Snesrud E, McGann P, Chandler M. The birth and demise of the ISApl1-*mcr-1*-IS*Apl1* composite transposon: the vehicle for transferable colistin resistance. mBio, 2018; 9: e02381-17.
19. Hu Y, Fanning S, Nguyen SV, Wang W, Liu C, Cui X, Dong Y, Gan X, Xu J, Li F. Emergence of a *Salmonella* enterica serovar Typhimurium ST34 isolate, CFSA629, carrying a novel *mcr-1.19* variant cultured from egg in China. J Antimicrob Chemother. 2021, 76(7):1776-1785.
20. Li R, Xie M, Zhang J, Yang Z, Liu L, Liu X, Zheng Z, Chan EW, Chen S. Genetic characterization of *mcr-1*-bearing plasmids to depict molecular mechanisms underlying dissemination of the colistin resistance determinant. J Antimicrob Chemother. 2017, 72: 393–401.
21. Yamaguchi T, Kawahara R, Hamamoto K, Hirai I, Khong DT, Nguyen TN, Tran HT, Motooka D, Nakamura S, Yamamoto Y. High prevalence of colistin-resistant Escherichia coliwith chromosomally carried *mcr-1* in healthy residents in Vietnam. mSphere. 2020, 5: e00117-20.
22. Erkyihun GA, Alemayehu MB. One Health approach for the control of zoonotic diseases. Zoonoses, 2022, 2(1): 37.

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Table 1. Antimicrobial susceptibility of 1256 *Salmonella* isolates against to 26 antimicrobial agents representing 12 classes

Antimicrobial class	Antimicrobial agent	AST results, number of strains (%)			WHO Category*
		Resistant	Intermediate	Susceptible	
Penicillins	AMP	705 (56.13)	1 (0.08)	550 (43.79)	CIA
β-Lactam combination agents	SAM	530 (42.20)	150 (11.94)	576 (45.86)	HIA
	FEP	282 (22.45)	28 (2.23)	946 (75.32)	CIA
Cephems	CAZ	223 (17.75)	14 (1.11)	1019 (81.13)	CIA
	CRO	316 (25.16)	1 (0.08)	939 (74.76)	CIA
	FOX	33 (2.63)	117 (9.32)	1106 (88.06)	HIA
	CTX	322 (25.64)	2 (0.16)	932 (74.20)	CIA
Monobactams	ATM	302 (24.04)	19 (1.51)	935 (74.44)	CIA
Carbapenems	ETP	0 (0.00)	0 (0.00)	1256 (100.00)	CIA
	IMP	0 (0.00)	1 (0.08)	1255 (99.92)	CIA
	MEM	0 (0.00)	0 (0.00)	1256 (100.00)	CIA
Lipopeptides	CT	323 (25.72)	933 (74.28)	-	CIA
	PB	312 (24.84)	944 (75.16)	-	CIA
Aminoglycosides	GEN	309 (24.60)	7 (0.56)	940 (74.84)	CIA
	AK	93 (7.40)	4 (0.32)	1159 (92.28)	CIA
Tetracyclines	TET	714 (56.85)	7 (0.56)	535 (42.60)	HIA
	DC	710 (56.53)	22 (1.75)	524 (41.72)	HIA
	TGC	0 (0.00)	1 (0.08)	1255 (99.92)	HIA
Quinolones and fluoroquinolones	CIP	333 (26.51)	655 (52.15)	268 (21.34)	CIA
	NAL	796 (63.38)	-	460 (36.62)	CIA
Folate pathway antagonists	SXT	351 (27.95)	-	905 (72.05)	HIA
	SMX	782 (62.26)	-	474 (37.74)	HIA
	TMP	427 (34.00)	-	829 (66.00)	HIA
Phenicols	CHL	455 (36.23)	240 (19.11)	561 (44.67)	HIA
	FFC	463 (36.86)	106 (8.44)	687 (54.70)	HIA
Nitrofurans	NIT	229(18.23)	353(28.11)	674(53.66)	IA

* CIA: Critically important antimicrobials; HIA: Highly important antimicrobials; IA: Important antimicrobials

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Table 2. Distribution in antimicrobial resistance of 1256 NTS isolates recovered from different sample sources

No. of antimicrobial class resistant	Raw poultry meat(n=890)			Prepared meat(n=192)		Others * (n=37)	Information unavailable (n=24)	Total(n=1256)
	Raw chicken (n=602)	Raw duck (n=288)	Others (n=113)	red meat (n=62)	poultry meat (n=130)			
0	37(6.15)	34(11.81)	9(7.96)	4(6.45)	8(6.15)	2(5.41)	3(12.5)	97(7.72)
1	56(9.30)	45(15.63)	16(14.16)	6(9.68)	7(5.38)	5(13.51)	2(8.33)	137(10.91)
2	61(10.13)	32(11.11)	13(11.50)	7(11.29)	16(12.31)	1(2.70)	5(20.83)	135(10.75)
3	72(11.96)	41(14.24)	13(11.50)	1(1.61)	13(10.00)	3(8.11)	3(12.5)	146(11.62)
4	64(10.63)	29(10.07)	11(9.73)	13(20.97)	17(13.08)	2(5.41)	2(8.33)	138(10.99)
5	72(11.96)	19(6.60)	10(8.85)	11(17.74)	19(14.62)	7(18.92)	2(8.33)	140(11.15)
6	58(9.63)	25(8.68)	11(9.73)	3(4.84)	22(16.92)	5(13.51)	3(12.5)	127(10.11)
7	38(6.31)	16(5.56)	9(7.96)	8(12.90)	12(9.23)	5(13.51)	0(0)	88(7.01)
8	50(8.31)	23(7.99)	4(3.54)	3(4.84)	11(8.46)	3(8.11)	1(4.17)	95(7.56)
9	76(12.62)	21(7.29)	14(12.39)	5(8.06)	5(3.85)	4(10.81)	3(12.5)	128(10.19)
10	18(2.99)	3(1.04)	3(2.65)	1(1.61)	0(0)	0(0)	0(0)	25(1.99)
Total	602(100)	288(100)	113(100)	62(100)	130(100)	37(100)	24(100)	1256(100)

* Others include sushi (n=6), cake & bread (n=11), milk (n=2), beverage (n=4) and processed algae (n=14).

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Table 3. Results of AST for four *mcr-1* positive *Salmonella* isolates

Antimicrobial class	Antimicrobial agent	AST results (R/I/S)*			
		2020s302	2020s327	2020s329	2020s542
Penicillins	AMP	R	R	R	R
β-Lactam combination agents	SAM	I	R	R	R
Cephems	FEP	R	R	R	I
	CAZ	S	S	R	S
	CRO	R	R	R	R
	FOX	S	S	S	S
	CTX	R	R	R	R
Monobactams	ATM	S	I	R	R
Carbapenems	ETP	S	S	S	S
	IMP	S	S	S	S
	MEM	S	S	S	S
Lipopeptides	CT	R	R	R	R
	PB	R	R	I	R
Aminoglycosides	GEN	S	R	R	I
	AK	S	S	S	S
Tetracyclines	TET	S	R	R	R
	DC	S	R	R	R
	TGC	S	S	S	S
Quinolones and fluoroquinolones	CIP	S	R	R	I
	NAL	S	R	R	S
Folate pathway antagonists	SXT	S	R	S	R
	SMX	S	R	R	R
	TMP	S	S	S	R
Phenicols	CHL	S	R	R	R
	FFC	S	R	R	R
Nitrofurans	NIT	S	S	S	S
AMR profiles (number of antimicrobial class)		AMP-FEP-CRO-CTX-CT-PB (3)	AMP-SAM-FEP-CRO-CTX-CT-PB-GEN-TET-DC-CIP-NAL-SXT-SMX-CHL-FFC (9)	AMP-SAM-FEP-CAZ-CRO-CTX-ATM-CT-GEN-TET-DC-CIP-NAL-SMX-CHL-FFC (10)	AMP-SAM-CRO-CTX-ATM-CT-PB-TET-DC-SXT-SMX-TMP-CHL-FFC (8)

*Note: R, resistant; I, intermediate; S, susceptible

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TABLE 4 Chromosome and plasmid sequence information for four *Salmonella* isolates harboring *mcr-1* gene.

Strain/Plasmid	Description	Serotype	MLST type	Region	Food source	Size (bp)	G+C content	Plasmid replicon type (Inc group) [b]
2020s302	chromosome	Bredeney	241	Zhejiang	Chicken	4,746,813	52.2%	N/A
p2020s302[a]	plasmid					64,501	42.5%	IncI2
2020s327	chromosome	Schwarzengrund	241	Zhejiang	Chicken	4,590,316	52.0%	N/A
p2020s327-1[a]	plasmid					263,461	46.0%	IncHI2A
p2020s327-2	plasmid					36,368	52.6%	IncR
2020s329	chromosome	Kentucky	198	Zhejiang	Beef	4,821,018	52.2%	N/A
p2020s329-1	plasmid					84,611	50.0%	IncI1-I(Alpha)
p2020s329-2[a]	plasmid					209,722	46.5%	IncHI2A
2020s542	chromosome	Newport	45	Hubei	Chicken	4,625,084	52.2%	N/A
p2020s542-1	plasmid					258,726	46.9%	IncHI2A
p2020s542-2	plasmid					85,305	50.1%	IncI1-I(Alpha)
p2020s542-3[a]	plasmid					60,961	42.4%	IncI2
p2020s542-4	plasmid					30,166	52.9%	N/A

[a] These plasmids contain *mcr-1* gene;

[b] NA indicates the plasmid replicon-typing was not applicable for the chromosome. p2020s542-4 was not predicted to have an Inc group using PlasmidFinder.

Fig 1 Antimicrobial resistance rates and MDR rates of *Salmonella* recovered from different regions

Fig 2 Genetic environments related to *mcr-1* gene in different bacterial plasmids. The figure was generated by Easyfig (v2.2.5). Plasmids marked with "pCFSA" were carried by the *mcr-1* positive *Salmonella* isolates in our previous research **[1, 2]**, and plasmid pWW012 belonged to a *Salmonella* isoltae in our previous research from our lab (Accession number: CP022169) **[3]**, while plasmids pHNSHP45 and pHNSHP45-2 (Accession number: KP347127 and KU341381) belonged to *Escherichia coli* strain SHP45, which is the first isolate reported harbouring *mcr-1* gene **[4]**. Replicon types are showed into two groups for all plasmids. Confirmed and putative open reading frames (ORFs) are indicated by block arrows and their orientations with different colours, and arrow size is proportional to the predicted ORF length. *mcr-1* gene is indicated by a red arrow, while genes encoding mobile elements (Insertion sequence, IS) are indicated by blue arrows. Regions of homology between the plasmids ranging from 67% to 100% are indicated by the graded shaded regions between sequences.

[1] Hu Y, Fanning S, Gan X et al. *Salmonella* harbouring the *mcr-1* gene isolated from food in China between 2012 and 2016. J Antimicrob Chemother, 2019; 74: 826–8.

[2] Hu Y, Nguyen SV, Liu C, et al. Complete Genome and Plasmid Sequences of Seven Isolates of *Salmonella enterica* subsp. *enterica* Harboring the *mcr-1* Gene Obtained from Food in China. Microbiol Resour Announc. 2019;8(31):e00114-19.

[3] Wang W, Baloch Z, Zou M et al. Complete genomic analysis of a *Salmonella enterica* serovar Typhimurium isolate cultured from ready-to-eat pork in China carrying one large plasmid containing *mcr-1*. Front Microbiol, 2018; 9: 616.

[4] Liu YY, Wang Y, Walsh TR et al. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 2016; 16: 161–8.