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Multidrug-resistant *Salmonella* species from chicken meat sold at Surabaya Traditional Markets, Indonesia

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Abstract. *Fanissa F, Effendi MH, Tyasningsih W, Ugbo EN. 2022. Multidrug-resistant* Salmonella *species from chicken meat sold at Surabaya Traditional Markets, Indonesia. Biodiversitas 23: 2823-2829. Salmonella* species is one of the major infectious pathogens associated with poultry birds, which also has an impact on public health. Antimicrobials are used as growth promoters or treatment of diseases thus; this encourages the persistent spread of antimicrobial resistance (AMR). This study aimed to identify multidrug-resistant *Salmonella* species from traditional chicken meat sold at Surabaya markets. A total of 150 chicken meat swab samples were collected from five different traditional markets and identified using microbiological standard methods. The Kirby-Bauer disk diffusion method identified multidrug-resistant *Salmonella* species on five different antibiotics discs. A low prevalence of *Salmonella* species was detected with a frequency of 11.3% (17/150). Some of the *Salmonella* isolates showed strong resistance to trimethoprim-sulfamethoxazole, tetracycline, aztreonam, and chloramphenicol. Five (29.4%) out of seventeen were multidrug-resistant *Salmonella* species. The presence of multidrug-resistant *Salmonella* species in these traditional markets is worrisome, since, it can lead to an outbreak of *Salmonellosis* as a result of the consumption of contaminated chicken meat. Therefore, the detection of multidrug-resistant *Salmonella* species is significant in understanding their prevalence and development of AMR. More strong awareness education and monitoring programs are recommended to mitigate the persistent spread of antimicrobial resistance.

Keywords: Antimicrobial resistance profile, chicken meat, public health, *Salmonella species*, traditional market

INTRODUCTION

Foodborne diseases have become one of the global problems (Adley and Ryan 2016) that impact food safety and public health (Dewey-Mattia et al. 2018). Foodborne diseases directly affect the production line to consumer (Adley and Ryan 2016; Wibisono et al. 2020) which causes considerable morbidity and mortality worldwide (Finger et al. 2019; Havelaar et al. 2015). Foodborne diseases caused by microorganisms include various zoonotic diseases (Li et al. 2020; Yanestria et al. 2019) that can be spread through food products of animal origin (Ejo et al. 2016), particularly chicken products (Sabry et al. 2020; Wibisono et al. 2021).

Chicken meat is one of the most consumed animal products (Sin et al. 2020) and is known to act as a medium for the spread of the foodborne disease *Salmonellosis* (Obe et al. 2020; Wibisono et al. 2021) through, the contamination (Perin et al. 2020) along the production chain (Finger et al. 2019). *Salmonellosis* has become one of the major foodborne diseases with 93 million cases of infection and 155,000 to 230,000 deaths in the world annually in humans (Firouzabadi et al. 2020) (Rortana et al. 2021), due to *Salmonella species* infection (Dümen et al. 2015; Thung et al. 2016).

The importance of *Salmonella* spp. has related to the emergence of multidrug resistance (MDR) (Adiguzel et al. 2021) to animal and human treatments (Siriken et al. 2020) which spread through the food of animal origin (Brown et al. 2017), including commercial chicken meat (Thung et al. 2016). The need for safe and quality animal protein is a demand from consumer needs. Many foods of animal origin are contaminated with *Salmonella* sp., such as ducks (Loisa et al. 2016), livestock (Kurniawati et al. 2016), chicken (Wibisono et al. 2021), and aquatic animals (Yanestria et al. 2019). Pathogenic microorganisms in the food chain are transmitted to humans through a variety of foods including chicken and chicken products (Wibisono et al. 2020).

Despite the awareness of the risk of *Salmonellosis* from handling raw poultry, the community generally does not realize that *Salmonella* spp. can also spread between chickens and humans (Behravesh et al. 2014). Multidrug resistance (MDR) isolates *Salmonella* spp. have posed a major challenge to the clinical management of infection due to the absence of effective and affordable alternative antibiotics (Kariuki et al. 2015). *Salmonella* spp. with increased resistance to clinically important antimicrobials (Yang et al. 2020) such as β-lactams (Park et al. 2017), ciprofloxacin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole (Sun et al. 2021) have become an important concern for public health and food safety (Yin et al. 2016; Yang et al. 2019; Perin et al. 2020).

Public consumption of chicken meat that was sold in traditional markets is still high. Wrong handling methods and inadequate application of sanitation standards by sellers have caused the chicken meat to be very susceptible to contamination by antimicrobial resistance *Salmonella species* which can pose problems for public health and food safety. The possible contamination of chicken meats by antimicrobial resistance *Salmonella* species makes this study very important. Thus, this study is geared towards identifying multidrug-resistant *Salmonella* spp. from chicken meat sold at Surabaya traditional markets.

MATERIALS AND METHODS

Study design and sampling

A total of 150 chicken meat swab samples, 30 each were obtained from chicken meat sellers at five different traditional markets (Pucang Anom, South Keputran, Wonokromo, Manukan Kulon, and Pabean) at Surabaya, Indonesia, between October 2021 to January 2022. The sampling sites were selected based on the list of chicken markets provided by the Department of Veterinary Services, Indonesia. Farm selection was performed by the multistage random selection method. Chicken meat swabs were collected aseptically using sterile swabs with Ames transport media (Al-Ansari et al. 2021) and placed in each vacutainer swab tube containing labeled BPW media. Sampling was carried out in the morning from 05:00 to 07:00 am. The sampling location is shown in Table 1.

Isolation and identification of *Salmonella* species

Pre-enrichment was performed by inoculating the chicken meat swabs into 5ml buffered peptone water (BPW; Oxoid, Basingstoke, UK), and incubating at 37ºC for 24 h (Sharma et al. 2019; Tarabees et al. 2017). For each culture, 1 mL of BPW was inoculated into 10mL in Tetrathionate Broth (TTB, Merck, German) and incubated at 37℃/24h for a selective enrichment. One loopful of the culture was streaked onto *Salmonella*-*Shigella* Agar (Sigma Aldrich, German) in duplicate and incubated at 37℃/24h. After 24 h, the plates were examined for the presence of suspected *Salmonella* spp. Putative positive samples produced colorless colonies with black centers on the SSA plates (SNI 2008). Further biochemical and Gram staining was carried out (Andesfha et al. 2019).

Antibiotic susceptibility testing

Antimicrobial susceptibilities for all *Salmonella* spp. isolates were determined by the disk diffusion Kirby-Bauer method, on Muller-Hinton agar (Merck, German) and standardized by the Clinical and Laboratory Standards Insitute (CLSI 2018). All of the *Salmonella* spp. were tested against 5 different antibiotics: aztreonam (ATM) 30 µg, tetracycline (TET) 30 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (CHL) 30 µg, and trimethoprim-sulfamethoxazole (SXT) 25 µg (Oxoid, UK). Antimicrobials were selected based on their use in treating human *Salmonellosis* (Tarabees et al. 2017).

Briefly, 0.5 McFarland bacterial suspensions were prepared and plated on the agar surface. Five paper discs were placed onto each agar plate using a dispenser. The plates were incubated at 37ºC for 18 h. The resulting zones of inhibition were measured in millimeters using a vernier caliper and the measurements were rounded off to the nearest whole number. The antimicrobial sensitivity profiles of the isolates were determined following the zone of the inhibition diameter breakpoints and interpretative categories (susceptible, intermediate, or resistant) (CLSI 2018).

RESULTS AND DISCUSSION

Isolation and identification of *Salmonella* spp.

Out of the 150, chicken meat swab samples analyzed, only seventeen (11.4%) were found positive for *Salmonella* spp.. Typical colonies and Gram staining appearance of *Salmonella* isolates is presented in Figure 1.

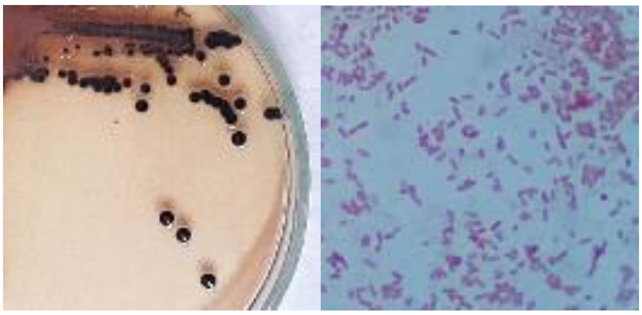
The highest prevalence of *Salmonella* isolates was found in the Wonokromo market, South Surabaya with the frequency of 26.6% (8/30); 5.3% (8/150), followed by Pabean and Manukan Kulon markets in North and West Surabaya with 13.3% (4/30); 2.6% (4/150) respectively. Keputran Selatan market in Central Surabaya had the least prevalence as presented in Table 2.

Antimicrobial resistance *Salmonella* spp.

The antimicrobial susceptibility test showed that all 17 *Salmonella* spp. isolates had the highest resistance to Tetracycline (82%, 14/17), followed by Trimethoprim-Sulfamethoxazole (59%, 10/17), Chloramphenicol (29%, 5/17), Ciprofloxacin (11.8%, 2/17) and Aztreonam (5.9%, 1/17). Summary of antimicrobial susceptibility test against *Salmonella* spp. presented in Table 3.

Table 1. Sampling locations

Market name	Regions	Total samples
Pucang Anom	East Surabaya	30
South Keputran	Central Surabaya	30
Wonokromo	South Surabaya	30
Pabean	North Surabaya	30
Manukan Kulon	West Surabaya	30



A B

Figure 1. A. *Salmonella* spp. colonies in SS agar; and B. with Gram staining

Table 2. The prevalence of *Salmonella* spp. isolates in Surabaya Traditional Markets, Indonesia

Regions	Market name	Prevalence
East Surabaya	Pucang Anom	0%
Central Surabaya	Keputran Selatan	0.7% (1/150)
South Surabaya	Wonokromo	5.3% (8/150)
West Surabaya	Manukan Kulon	2.6% (4/150
North Surabaya	Pabean	2.6% (4/150)

Table 3. *Salmonella* spp. antimicrobial resistance pattern

Antimicrobials agent	*Salmonella* spp. antimicrobial resistance pattern		
	R	I	S
Aztreonam	5.8% (1/17)	-	94% (16/17)
Tetracycline	82% (14/17)	5.8% (1/17)	11.8% (2/17)
Ciprofloxacin	11.8% (2/17)	35.2% (6/17)	53% (9/17)
Chloramphenicol	29% (5/17)	4% (4/17)	47% (8/17)
Trimethoprim-Sulfamethoxazole	59% (10/17)	11.8% (2/17)	29% (5/17)

Table 4. Multidrug-resistant *Salmonella* spp. isolates

Code	Markets origin	Region	Antimicrobial combination	n	%
B-15	Keputran Selatan	Central Suarabaya	ATM-TET-CHL-SXT	1	5.9
C-01	Wonokromo	South Surabaya	TET-CIP-SXT	1	5.9
C-08			TET-CHL-SXT	1	5.9
C-28				1	5.9
E-03	Pabean	North Surabaya	TET-CIP-CHL-SXT	1	5.9

Note: ATM: Aztreonam; CHL: Chloramphenicol; CIP: Ciprofloxacin; TET: Tetracycline; SXT: Trimethoprim-Sulfamethoxazole

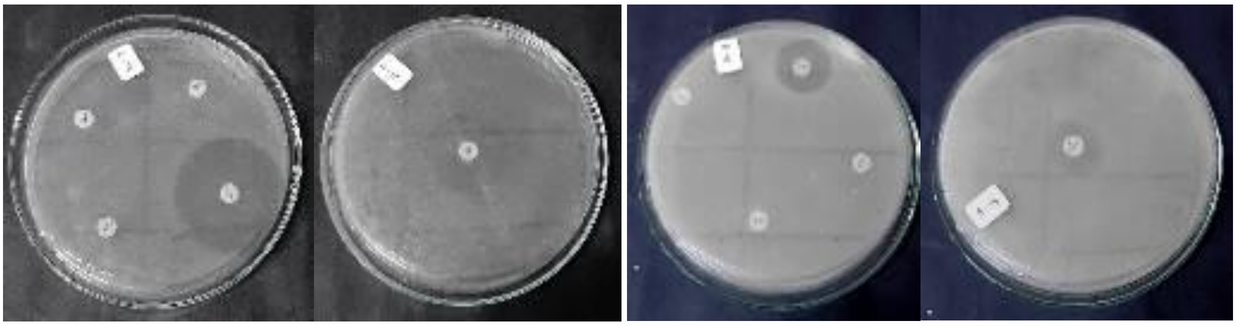


Figure 2. Multidrug-Resistant *Salmonella* spp. isolate, A. MDR 3 antibiotics, and B. MDR 4 antibiotics

Multidrug-Resistant *Salmonella* spp.

Five (29.4%) out of seventeen were multidrug resistance *Salmonella* spp. Multidrug resistance (MDR) occurred against three antimicrobial classes TET-CIP-SXT (5.9%, 1/17), TET-CHL-SXT (17.6%, 3/17), and four antimicrobial classes ATM-TET-CIP-CHL-SXT (5.9%, 1/17), TET-CIP-CHL-SXT (5.9%, 1/17). However, multidrug resistance *Salmonella* spp. was found to spread among three traditional markets; one isolates from the Keputran Selatan market in Central Surabaya, and three isolates came from Wonokromo market in South Surabaya, and one isolate from the Pabean market in North Surabaya as presented in Figure 2 and Table 4.

Discussion

To this day, foodborne illness remains a significant health problem all around the world. *Salmonella* spp. remains the second most common cause of human infection and food poisoning related to contaminated food (Ćwiek et al. 2020; Wibisono et al. 2020) of animal origin (Asif et al. 2017; Park et al. 2017). The prevalence of *Salmonella* spp. in this study was detected to be 11.3%. *Salmonella* spp. can be in domestic animals such as chickens (Gu et al. 2020). Therefore, continuous supervision and monitoring of chicken products, chicken, is very important to prevent the spread of *Salmonellosis* and to maintain public health (Choi et al. 2015). Poultry purchased must come from farm management that has a good hygiene and biosecurity system. Incoming poultry must have a high health status and must be purchased from a reliable supplier that has quality-assured breeding and hatching facilities (Harijani et al. 2020; Rahmahani et al. 2020). *Salmonella* spp. can be transmitted through chicken farms through vehicles, workers, clothing, footwear, equipment, water, food, garbage, insects, rodents, wild birds, pets, tools, and many other factors. Preventing the entry of *Salmonella* spp. into the farm can be done by limiting people entering the farm, wearing protective clothing, and wearing boots that have been disinfected. In addition, workers must know the basic hygiene principles, such as keeping hands and feet clean. The success of carrying out disinfection of chicken farms needs to be tested by taking samples on floors, walls, drinking water, eating places, and the environment, it is hoped that there will be public awareness of the dangers of antimicrobial resistance if the approach to antibiotic use (Immerseel et al. 2009; Permatasari et al. 2020).

Increased antimicrobial resistance in *Salmonella* spp. *species* from chicken meat in traditional markets (Chuah et al. 2018) occurred as a result of cross-contamination between chicken meat, the environment, and workers (Wang et al. 2019; Effendi et al. 2021) which led to an increase in multidrug-resistant isolates of *Salmonella* spp. at the final stage of processing (Carramiñana et al. 2004). Every year, antimicrobial resistance in *Salmonella* spp. is reported in retail chicken meat products (Gad et al. 2018) where sulfonamides and tetracyclines were antimicrobials with the highest detection frequency, followed by quinolones and β-lactams (Ben et al. 2019).

In this study, the highest resistance detected against antimicrobial tetracycline was 82% (14/17) and sulfamethoxazole-trimethoprim 59% (10/17) isolates were found in chicken meat samples from traditional markets in Central, South, and West Surabaya. Five (29.4%) out of seventeen were multidrug resistance *Salmonella* species. The highest resistance of *Salmonella* spp. against sulfamethoxazole-trimethoprim and tetracycline indicated that these antimicrobials were frequently used in production level animals (Carramiñana et al. 2004; Eguale 2018) causing selective pressure on *Salmonella* sp. (Lopes et al. 2016) and spread by contamination at the evisceration stage (Carramiñana et al. 2004) and is in line with the observation of our study.

Tetracycline is one of the most widely used antimicrobials in human and veterinary medicine in livestock worldwide (Mukherjee et al. 2019; Xu et al. 2020). Its excessive use for therapy and prophylaxis in animals contributes to the increase in resistance to *Salmonella* sp. (Gargano et al. 2021) as noted in this present study. Sulfonamides class antimicrobials have long been used in agriculture, aquaculture, animal, and human medicine (Mąka et al. 2015). Made it accumulate as environmental contaminants, originating from livestock wastewater (Pavelquesi et al. 2021) or animal waste is one of the factors contributing to increased resistance to *Salmonella* sp. (Peng et al. 2018).

Fluoroquinolones are recognized as standard antimicrobials for the treatment of invasive *Salmonellosis* in humans (Peruzy et al. 2020). Ciprofloxacin can survive in the body and can cause the development of *Salmonella* sp. resistant (Panzenhagen et al. 2016), which are excreted in urine or feces together with prophage and the bacteria *Salmonella* sp.which can pollute the environment (Bearson and Brunelle 2015). The use of chloramphenicol is known to have been banned in food-producing animals (Mechesso et al. 2020).

Resistance against chloramphenicol can be caused by long-term and frequent use of chloramphenicol at broiler production levels or through horizontal transmission (Sin et al. 2020). Resistance to chloramphenicol in *Salmonella* spp. is generally caused by cross-resistance with other antimicrobials due to their use at the level of production or human medicine (Mattiello et al. 2015). The emergence of antimicrobial resistance of *Salmonella* spp. against β-lactam antimicrobials has become a significant challenge for human medicine (Sabry et al. 2020), especially against Aztreonam (Xu et al. 2021). In several studies, *Salmonella* spp. that does not result in ESBL has also been reported worldwide (Ifeanyi et al. 2014). The presence of *Salmonella* isolates that do not produce ESBLs are known to be more susceptible to various other commercially used clinical antimicrobials (Menezes et al. 2010).

When *Salmonella* sp. becomes resistant to antimicrobials, its presence can pose a greater risk to human health (Zhu et al. 2017). This occurs as a result of failed treatment with potential antimicrobials, reduced treatment options, and increased disease severity (Doménech et al. 2015). Multidrug resistance isolates *Salmonella* spp. brought about by contamination in chicken meat can contribute to the spread of its resistance ability in the human gut microbiome (Al-Ansari et al. 2021). Their release into the environment can cause contamination (Daghrir and Drogui 2013), for various ecosystems (Ngangom et al. 2019) and create favorable selection pressures for the survival of resistance isolates (White et al. 2001), and contribute to the spread of multidrug-resistant *Salmonella* spp. in humans and food of animal origin (Mukherjee et al. 2019). The emergence of MDR *Salmonella* species has now become the focus of attention of various researchers. MDR show bacterial resistance to three or more class of antibiotics (Magiorakos et al. 2012). Resistance to these antibiotics can occur due to the pattern of continuous use of antibiotics in the chicken farms such as both treatment and feed additive and growth promoter. Continuous use of antibiotics is a trigger factor for high rates of antibiotic resistance (Rahman et al. 2018).

Overcoming these challenges will require efforts from various sectors to successfully control the spread and emergence of MDR in *Salmonella* sp. from broiler chickens. In addition, chicken and chicken products are frequently associated with outbreaks of *Salmonellosis* and therefore, are generally recognized as a major source of disease spread (Saravanan et al. 2015). *Salmonella* prevention and control can be achieved by adopting the Public Health Importance principles of Hazard Analysis Critical Control Point (HACCP) (World Organisation for Animal Health 2019). Actions must include active surveillance to monitor the emergence and spread of MDR of *Salmonella* sp. Infection prevention and control should also be optimized to limit further spread. Raising awareness is important to limit the inappropriate use of antibiotics, and evaluating the program of using antibiotics in the livestock industry, including on chicken farms, must regulate the sustainable use of antimicrobials strictly and appropriately (Zowawi 2016; Widodo et al. 2020).

In conclusion, these studies indicated the presence of *Salmonella* sp. in chicken meats sold at traditional markets in Surabaya, Indonesia, though at a lower rate with a frequency index of 11.4%. Multidrug resistance was observed in five isolates (29.4%) out of seventeen isolates studied. The presence of multidrug-resistant *Salmonella* sp. in these traditional markets is worrisome since it can lead to an outbreak of *Salmonellosis* as a result of the consumption of contaminated chicken meat. There is a need for constant and proper monitoring of chicken meat sold in open supermarkets by public health offices and also the use of antimicrobial agents on chicken farms by veterinarians.

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