Biota: Jurnal Ilmiah Ilmu-Ilmu Hayati, Vol. 9(2): 145-154, Juni 2024
p-ISSN 2527-3221, e-ISSN 2527-323X, https://ojs.uajy.ac.id/index.php/biota/issue/view/453
DOI: 10.24002/biota.v9i2.7814

Pattern of Antibiotic Resistance in *Salmonella sp.* Bacteria Contaminating Fresh Faeces of Laying Hens in Kediri District, West Lombok Regency

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Abstract

Antibiotic resistance has become a common problem in poultry farming in Indonesia. One of them is caused by improper use of antibiotics. This study aims to determine the pattern of resistance of *Salmonella sp.* isolated from laying hens to antibiotics. The samples used in this study were thirty samples of fresh faeces of laying hens from Kediri District, West Lombok Regency. The samples were isolated and identified through Gram staining and biochemical tests (indole, methyl red-Voges Proskauer, triple sugar iron agar, citrate, and urease), resulting in ten positive samples of *Salmonella sp.* The study continued with antibiotic resistance testing using disc diffusion or Kirby-Bauer methods. The antibiotics used in the study were aztreonam, ciprofloxacin, streptomycin, chloramphenicol, and tetracycline. The results showed that *Salmonella sp.* has been resistant to some antibiotics. The highest resistance levels are streptomycin and tetracycline at 50% (5/10), while the lowest is ciprofloxacin at 10% (1/10). Antibiotics that are still sensitive are aztreonam 90% (9/10) and chloramphenicol 100% (10/10). The findings of this study conclude that there is a pattern of antibiotic resistance in laying hens farms that can have a negative impact on human and animal health.

Keywords: Antimicrobial resistance, Lombok, Poultry, *Salmonella sp.*

Submitted :28 August 2023 ; Revised : 25 March 2023 ; Accepted : 1 April 2024

Introduction

Salmonella sp. is a bacterium contaminating poultry and is usually treated with antibiotics. It is a Gram-negative bacterium that is pathogenic causing food-borne diseases around the world. Diseases caused by *Salmonella sp.* is known as *foodborne disease*, which occurs when a person consumes food or drinks contaminated by these bacteria (Siyam & Cahyati, 2018). *Salmonella sp.* infection in animals and humans can cause salmonellosis, a condition that disrupts the gastrointestinal tract and, in some cases, can result in death (Suwandono *et al*., 2005; Sartika *et al*., 2016).

Antibiotics are chemical compounds obtained from microorganisms and to damage or kill microbes that have toxic properties (Hardiyanti, 2020). Antibiotics work through the inhibition of cell metabolism, cell wall synthesis, cell integrity, protein synthesis, and bacterial cell nucleic acid synthesis (Sandika & Suwandi, 2017). Antibiotics in livestock are used as preventive, treatment, and *Antibiotic Growth Promoter* (AGP) (Carter & Wise, 2004; Syafitri, 2023). Excessive use of antibiotics cause side effects and increase the risk of bacterial resistance (Purwidyaningrum *et al*., 2019).

Antibiotic resistance is the ability of bacteria to adapt and survive the effects of antibiotic treatment (Niasono *et al*., 2019). According to Agustin and Kholik's study in 2019, resistance testing for antibiotics was carried out on 27 samples of *Salmonella sp.* in North Lombok. The results showed different levels of resistance, namely 100% to Penicillin G, 95% to Ciprofloxacin, 54% to Sulfamethazole/Trimetoctrine, and 50% resistance to Ampicillin and Erthromycin. On the other hand, antibiotic resistance testing was

How to Cite : Ramadani, F. A., Riwu, K. H. P., Kholik, K., Mbura, Y. V. Y. & Zakarias, H. V. (2024). Pattern of Antibiotic Resistance in Salmonella sp. Bacteria Contaminating Fresh Faeces of Laying Hens in Kediri Districs, West Lombok Regency. Jurnal Ilmiah Ilmu-Ilmu Hayati 9(2): 145-154.

also carried out on 8 samples of *Salmonella sp*. bacteria on laying hens in Sesaot, West Lombok Regency. The results showed different levels of resistance, namely 37.5% to Tetracycline, 25% to Penicillin G, and 75% to Oxytetracycline (April *et al*., 2022).

Antibiotic resistance has a significant impact, including making treatment more difficult and requiring higher health costs. This impact is because treatment with antibiotics is no longer effective in curing the patient's disease (Beukes, 2011; April *et al*., 2022). Improper use of antibiotics significantly contributes to the occurrence of antibiotic resistance (Yulia *et al*., 2019). The transmission of pathogens between humans and animals highlights the role of antibiotic management and symbolizes the concept of "One Health" (Riwu *et al*., 2020). Humans, animals, and the environment are interrelated, so the issue of AMR resistance between them must be equally targeted (Laxminarayan *et al.*, 2013; Riwu *et al.,* 2022). Antibiotic resistance has also an impact on the surrounding community, especially chicken farmers who care for animals in cages adjacent to settlements (Agustin and Kholik, 2019). The purpose of this study was to determine the pattern of antibiotic resistance in *Salmonella sp*. bacteria sourced from fresh faeces of laying hens in Kediri District, West Lombok Regency.

Research Methods

This research method utilizes a laboratory exploratory approach with random sampling. The samples used are fresh faeces from laying hens originating from the Kediri District, West Lombok Regency. Fecal sampling is conducted because *Salmonella sp*. is typically found living and multiplying in the digestive tracts of animals and humans. *Salmonella sp.* that exits the digestive system can spread widely in the blood, bile, urine, environmental materials, and typically in faeces (Safitri *et al*., 2019). The number of samples (n) is determined based on Frederer's formula (1963), resulting in a total of 30 samples. Samples are collected in the morning from three coops (coops A, B, and C) on one farm, with 10 samples taken from each coop. Subsequently, isolation is conducted in the laboratory on the same day.

Sampling Method

The collection of laying hens faeces samples is conducted aseptically at the sampling site. The collection of faeces samples is carried out using sterile gloves on both hands, and the collected faeces are fresh (Riwu, 2022). The obtained faeces samples are placed into pre-labeled faeces containers. The transportation process of all obtained faeces specimen samples involves placing them into a cool box, which is also equipped with 21. Subsequently, the samples are directly sent to the laboratory for isolation.

Method of Isolation and Identification of *Salmonella sp.*

The testing conducted to isolate *Salmonella sp*. from fresh faeces of laying hens utilized *Salmonella Shigella Agar* (SSA) media. *Salmonella sp.* colonies on the media can be identified by their blackish color (Maritsa *et al*., 2017), while colonies with pink and cream-white colors are also characteristic of *Salmonella sp*. (Annisa *et al*., 2020). Some types of *Salmonella sp.* can produce black circles in the center of colonies as a result of H_2S gas production (Sari *et al*., 2018). Each sample undergoes three purification stages on *Salmonella Shigella Agar* (SSA) media before the identification process is carried out. Replication is performed three times to ensure the growth of pure colonies suspected to be *Salmonella sp*.

Salmonella sp. is identified through Gram staining and a series of biochemical tests, including indole, methyl red-voges proskauer, *Triple Sugar Iron Agar* (TSIA), citrate, and urease tests. In Gram staining, *Salmonella sp*. belongs to Gram-negative bacteria with rod-shaped morphology and pink color, due to thin peptidoglycan layers and high permeability levels (Amri *et al*., 2017). The indole test shows negative results, indicated by the absence of a red ring on the media surface (Safitri *et al*., 2019).

Susceptibility Testing

Salmonella sp. was taken using a sterile ose needle and then suspended in a tube containing 5 ml of 0.9% NaCl solution. The suspension was then compared with Mc Farland's turbidity of 0.5 (Wulansari *et al*., 2021).

Antibiotic resistance tests are performed using disc diffusion or Kirby-Bauer methods. First, a bacterial colony suspension is made, and the turbidity is adjusted to the standard of 0.5 Mc Farland which is equivalent to 1 x 10^8 CFU/ml. After that, a sterile *cotton swab* is dipped in bacterial suspension and applied to *Muller Hinton Agar* (MHA) media. The media is allowed to stand for 30 minutes. After that, the antibiotic disc is taken using sterile tweezers and placed on the surface of the substrate. Then, the media is incubated for 24 hours at a temperature of 37 °C. The diameter of the inhibitory zone formed was measured using a caliper (Rahmaniar *et al*., 2019). This test is done by doing three repetitions. The results of each inhibitory zone were compared with CLSI (*Clinical Laboratory Standard Institute*) standards (Hamida *et al*., 2019).

A resistance test is a test carried out to determine the sensitivity of bacteria to an antibiotic. Excessive or uncontrolled use of antibiotics can make some bacteria resistant to antibiotics (Mardiah, 2017). The antibiotics used for resistance testing in this study were ciprofloxacin (5µg), streptomycin (10 µg), tetracyclin (30 µg), aztreonam (30 µg), and chloramphenicol (30 µg).

Based on the provisions of the *Clinical Laboratory Standard Institute* (CLSI), the interpretation of the inhibitory zone measurement for the antibiotic is ciprofloxacin (5µg) with an intermediate of 21-30 mm, sensitive ≥31 mm, and resistant diameter of ≤20 mm. Streptomycin antibiotics (10 µg) have intermediates of 12-14 mm, sensitive ≥15 mm, and resistant ≤11 mm. Tetracyclin (30 µg) has an inhibitory zone with intermediates of 12-14 mm, sensitive ≥15 mm, and resistant ≤11 mm.

Furthermore, aztreonam (30 µg) has an intermediate of 18-20 mm, sensitive ≥21 mm, and a resistant diameter of ≤17 mm. Finally, chloramphenicol antibiotics (30 µg) have intermediates of 13-17 mm, sensitive ≥18 mm, and resistant diameters of ≤12 mm (CLSI, 2020).

Result and Discussion

Out of 30 fresh faeces samples collected from laying hens, 10 tested positive for *Salmonella sp*., with 50% originating from coop A, 10% from coop B, and 40% from coop C. Subsequently, the 10 *Salmonella sp*. positive samples underwent resistance testing, revealing 5 samples resistant to streptomycin and tetracycline antibiotics, and 1 sample resistant to ciprofloxacin antibiotic (Table 1).

Table 1. *Salmonella sp. resistance test results* against antibiotics based on the diameter of the inhibition zone.

Code	Aztreonam (ATM)	Ciprofloxacin (CIP)	Streptomycin (S)	Chloramphenicol (C)	Tetracyclin (TE)
5A	S	I	R	S	R
7A	S	R	S	S	I
8A	S	I	S	S	I
9A	S	I	S	S	S
10A	S	I	S	S	I
3B	I	I	I	S	I
1C	S	S	R	S	R
2C	S	I	R	S	R
3C	S	I	R	S	R
4C	S	I	R	S	R

Information:
Code A, B, C : Coops A, B, and C
Code 1-10 : Samples 1-10
S : Sensitive
I : Intermediate
R : Resistant

Table 2. Prevalence of *resistance of Salmonella sp.* against antibiotics

Antibiotic	**Sensitive (%)**	**Intermediates (%)**	**Resistant (%)**
Aztreonam	90%	10%	0%
Ciprofloxacin	10%	80%	10%
Streptomycin	40%	10%	50%
Chloramphenicol	100%	0%	0%
Tetracyclin	10%	40%	50%

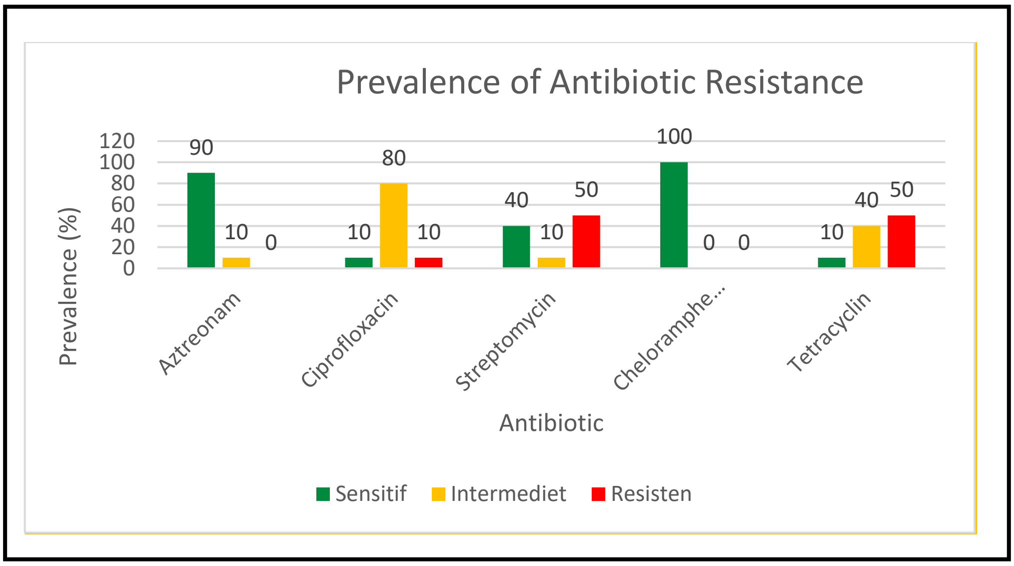

Figure 1. Diagram of the prevalence rate of *Salmonella sp.* against antibiotics.

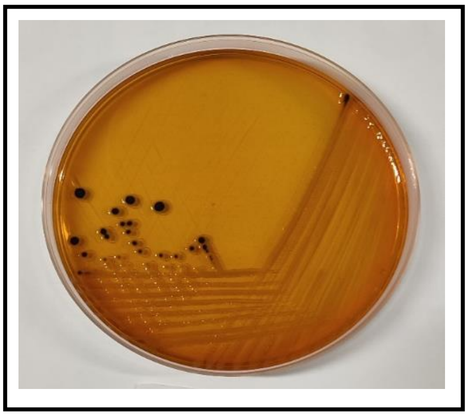

Figure 2. Salmonella sp. bacteria on Salmonella Shigela Agar (SSA*)* media

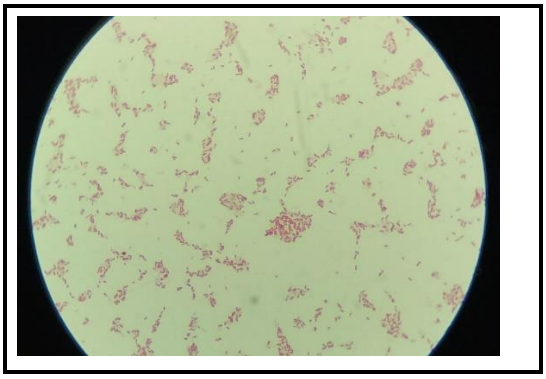

Figure 3. Results of microscopic examination of *Salmonella sp.* with 1000X magnification.

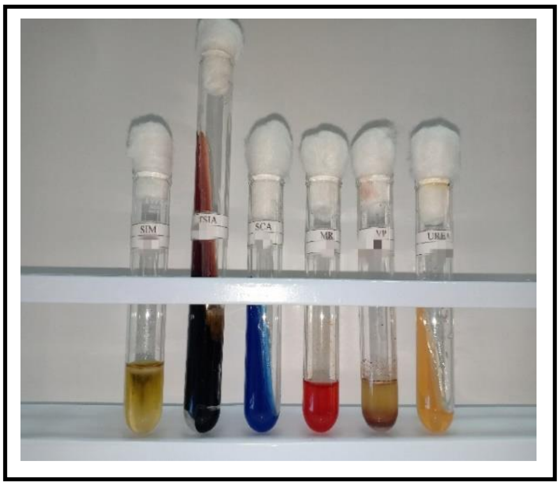

Figure 4. Biochemical test to identify *Salmonella sp*. (left to right: Indol, *Triple Sugar Iron Agar* (TSIA), Citrate, *Methyl-Red, Voges-Proskauer,* Urea)

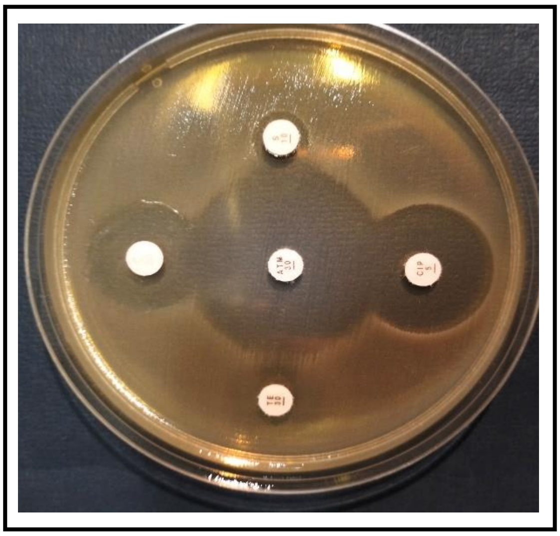

Figure 5. Test of *resistance of Salmonella sp* to antibiotics on *Muller Hinton Agar* (MHA) media

Antibiotic resistance testing results on *Salmonella Shigella Agar* (SSA) media using 10 isolates of *Salmonella sp.* can be seen in Tables 1 and 2. Based on the table, as many as 1 isolate (10%) entered the sensitive interpretation, 8 isolate (80%) intermediates, and 1 isolate (10%) samples were resistant to ciprofloxacin. Ciprofloxacin is a fluoroquinolone antibiotic that works by inhibiting the synthesis of proteins and nucleic acids of bacteria (Indrayani & Putra, 2019; Ningsih *et al*., 2021). According to Maka & Popowska (2016), almost all studies have tested the antibiotic ciprofloxacin to be more effective against *Salmonella sp*. and most investigations show no resistance to this antibiotic, only strains isolated from chicken samples collected in China show relatively high resistance to ciprofloxacin (42.1%) (Syafitri *et al*., 2023).

The highest level of resistance of *Salmonella sp.* isolates is against tetracyclin and streptomycin antibiotics. The level of resistance to tetracycline was 5 isolates (50%), 4 isolates (40%) intermediates, and 1 isolate (10%) sensitive to *Salmonella sp.* These results are in accordance with research data conducted by Siriken *et al*. (2015) which showed that a very high level of resistance was found in tetracycline antibiotics (>89.28%). Tetracycline resistance occurs due to changes in the permeability of bacterial cell walls, in resistant cells, the drug cannot be actively transported into the cell or will disappear quickly, so the minimum inhibitory concentration cannot be maintained (Utami, 2011; April *et al*., 2022). According to Miol *et al*. (2016), tetracycline is one of the most widely used antimicrobial drugs for treatment in livestock. This high level of resistance is because tetracycline is the most frequently used antibiotic and its use is not appropriate for dosage (Syafitri *et al*., 2023).

In streptomycin antibiotics as many as 5 isolates (50%) are resistant, 1 isolate (10%) is intermediate, 4 isolates (40%) are still sensitive. Streptomycin is an antibiotic that belongs to the Aminoglycoside group. This antibiotic is obtained from soil fungi such as *S. griceus* and some other *Streptomyces* species. Streptomycin is often used in the control of diseases caused by bacteria and fungi in animals (Mamatha *et al*., 2014). Streptomycin works by inhibiting bacterial protein synthesis by binding to the 30S and 16S ribosomal subunits of bacterial RNA. The existence of this relationship can interfere with the formation of amino acids by mRNA, so that the amino acid sequence in bacterial polypeptides is not appropriate, causing the formation of peptides that do not function or are toxic in bacterial cells (Hardjosaputra, 2008; Dwipayana *et al*., 2023). Hur *et al*. (2012) state that resistance to streptomycin antibiotics in *Salmonella sp.* is due to inactivation of the aminoglycoside adenyl transferase enzyme which cannot inhibit these bacteria.

Antibiotics that are still sensitive to *Salmonella sp.* In this study, chloramphenicol with 100% sensitivity and aztreonam with 90% sensitivity and 10% intermediates. This is because chloramphenicol and aztreonam are not antibiotics commonly used in laying hens farms in Kediri District, West Lombok Regency. The results of this study are in accordance with the surveillance results of chicken caecum samples against *E. coli* in Bali, West Nusa Tenggara, and East Nusa Tenggara which stated that chloramphenicol antibiotics are still classified as sensitive although there is a tendency to become resistant (Handayani *et al*., 2020). Chloramphenicol is a class of antibiotics that can inhibit bacterial growth but also kill bacteria (Aisha *et al*., 2018). The mechanism of action of chloramphenicol is to inhibit the binding of new amino acids to the peptide chain that appears, mainly because chloramphenicol inhibits peptidyl transferase. Chloramphenicol is primarily bacteriostatic and microbial growth will continue when the drug is discontinued (Estoepangestie *et al*., 2014).

Aztreonam is the first member of a new class of beta-lactam antibiotics, Monobaktam. Aztreonam has selective activity against aerobic Gram-negative bacteria and is inactive against Gram-positive bacteria. In in vitro tests, aztreonam has been shown to inhibit the growth of Enterobacteriaceae at low concentrations, except Enterobacter species, and is also active against *Pseudomonas aeruginosa*. The results of therapeutic trials showed that aztreonam was effective in treating Gram-negative infections, including urinary tract infections, lower respiratory tract infections, and uncomplicated septicemia. Aztreonam has also been shown to be effective in treating pseudomonas infections in most patients, except in patients with cystic fibrosis. With a different antibacterial spectrum from other antibiotics, aztreonam becomes a useful alternative to third-generation aminoglycosides or cephalosporins in the treatment of Gram-negative positive infections in patients (Biedenbach *et al*., 2015). The results of this study found that *Salmonella sp.* is resistant to ciprofloxacin (10%), streptomycin (50%), and tetracycline (50%), while antibiotics that are still sensitive are aztreonam (90%) and chloramphenicol (100%).

Coop B has a lower contamination level compared to coops A and C. This is partly due to the sanitation level of the coop. Maintaining coop cleanliness is crucial as it directly impacts the health of the chickens. Poor sanitation can lead to frequent chicken diseases, as stated by Rudiyansyah *et al*. (2015), emphasizing the significant role of sanitation in coop environmental conditions. Dirty coops, contaminated feed or water sources, and inadequate sunlight exposure are favorable conditions for diseases to thrive. Common diseases associated with poor cleanliness include the presence of *Coliform* bacteria (Ramadani *et al*., 2023).

The presence of *Coliform* bacteria in feed or drinking water can harbor enteropathogenic or toxigenic microbes harmful to health (Lusandika *et al*., 2017). *Coliform* bacteria consist of two types: fecal and non-fecal. Fecal *Coliform* bacteria are found in human or animal faeces and inhabit the digestive tract, while non-fecal bacteria are found on the bodies of dead animals. Fecal *Coliform* bacteria are related to animal faeces, including those found in chicken faeces. Some fecal *Coliform* bacteria include *Salmonella typhimurium, Serratia liquefaciens, Actinobacillus sp., Klebsiella ozaenae*, and *Escherichia vulneris* (Wijaya, 2013). Temperature and humidity are factors that influence bacterial growth. Humidity also affects the moisture content of chicken litter, allowing bacteria and fungi to thrive (Najibulloh *et al*., 2020).

Transmission of salmonellosis in humans generally occurs through consumption of food originating from animals contaminated with *Salmonella sp.* When someone consumes contaminated food, *Salmonella sp*. bacteria can cause infection in the body, characterized by symptoms affecting the digestive tract such as diarrhea, nausea, vomiting, and fever. Salmonellosis is widespread in almost all major cities in Indonesia. It is estimated that between 60,000 to 1,300,000 cases of salmonellosis occur per year with approximately 20,000 deaths caused by this disease (Suwandono *et al*., 2005).

Antibiotic resistance has significant impacts, including making treatment more difficult and requiring higher healthcare costs. This is due to the fact that antibiotic usage is no longer effective in curing patients' illnesses (Beukes, 2011). The global spread of antibiotic resistance occurs due to irrational and uncontrolled antibiotic usage, which enables the emergence of bacteria resistant to a group of antibiotics (Noor and Poeloengan, 2005).

Conclusion

Based on the results of research isolates of *Salmonella sp.* from fresh faeces of laying hens, the bacteria have become resistant to the antibiotics ciprofloxacin, streptomycin, and tetracycline. While the antibiotics aztreonam and chloramphenicol are still sensitive to *Salmonella sp*. Antimicrobial resistance has serious implications for human health. Resistant bacteria can spread to humans through the consumption of eggs and chicken meat, or through contaminated environments. This can lead to difficulties in treating human infections, ultimately increasing healthcare costs. Moreover, antimicrobial resistance also raises the risk of zoonotic diseases and diminishes the overall effectiveness of antibiotics.

Acknowledgments

The author would like to thank the Faculty of Veterinary Medicine (FKH) Mandalika University of Education (UNDIKMA) for the support in the form of facilities and assistance from various parties who have supported the completion of this research.

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