



## The Activity of *Salmonella Typhii* in Milkfish (*Chanos chanos*) with Invitro.

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### Abstract

Milkfish is a seafood commodity that is very popular with the community. However, milkfish can also be a source of spreading bacteria if it is not managed hygienically and safely. The objective of this experiment was to activate *Salmonella typhii* in milkfish using the invitro test. This research was called out in Microbiology Laboratory at the Faculty of Medical, Hasanuddin University, Makassar, Indonesia. The result of this experiment indicated that *Salmonella typhii* gave a positive result for the Biochemical test with TSIA media, SIM media, MRVP media, citrate, urea, Glucose, Sucrose, Lactose, Mannitol at three samples of milkfish out of ten samples taken randomly at the fish market and fish auction in Makassar, the contamination level of *Salmonella typhii* bacteria in all observed samples was moderate with a percentage level of 30%. Next test antibacterial and antibiotic tests were carried out with MHA medium and antibiotics, and the antibiotic Ciprofloxacin has a high degree of sensitivity test.

### Article History

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### Keyword

*Salmonella typhii*,

*Invitro*,

*TSIA*,

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### Introduction

Salmonellosis is a disease caused by *Salmonella* bacterial infection in humans and animals that attacks the gastrointestinal tract, which includes the stomach, small intestine and large intestine or colon (Zelpina and Noor, 2020). Bacteria that are often found in food and drink contaminated one of which is *Salmonella* sp. Usually, infection of *Salmonella* sp causes salmonellosis (Lestari et al, 2017). Salmonellosis is zoonotic, meaning that this disease can be transmitted from animals to humans (Dharmojojo, 2001).

*Salmonella* that plays the most role is *S.typhi* because it is still a major health problem causing typhoid fever in developing countries. Bacteria with a level of danger that can cause death (Ariyanti and Supar, 2017). Typhoid fever is generally found in developing and low-income countries, as well as countries with tropical climates, WHO estimates that there are around 16 million cases annually worldwide and around 600,000 people die (WHO, 2018). Data information from GBD for Indonesia itself according to gender in 2019, the value



obtained for men is 187.06 DALYs per 100,000 while for women it is 122.99 DALYs per 100,000 (Global Burden of Disease, 2020). Specifically for South Sulawesi Province, a value of 147.88 DALYs per 100,000 was obtained in 2019 (Ainil, M. 2021). Transmission of bacteria in foods, there are 39% that are positively contaminated by *Salmonella typhi*. This can be influenced by several factors, namely food processing, selling environmental hygiene, contaminated water, and flies as disease vectors (Putri et al, 2019)

Foodborne disease is a disease caused by microorganisms and is a poison found in food that then enters through the mouth and is digested in the stomach inside the body (Muna et al, 2020). Fish is foodstuff with a high protein source, containing essential amino acids needed by the body. Fish contains high nutrients but is easily decomposed, caused by the presence of microbes found in fish (Melawati, et al, 2019).

Milkfish can also be a source of spreading bacteria if not managed hygienically and safely. The multiplication of bacteria in the body of fish is not only caused by bacteria naturally found in fish (in the stomach contents, gills, and skin) but also by bacteria originating from other sources that contaminate the fish, including pathogenic bacteria, such as *Salmonella typhii* (Samiadi, 2021).

*Salmonella* species encompasses a group of common rod shape, gram-negative rods that are motile, 1.0 to 3.0  $\mu\text{m}$  long, and 0.8 to 1.0  $\mu\text{m}$  wide (Darmawan et al, 2020). *Salmonella* will produce pink bars on Gram stain on microscopic examination. *Salmonella* can ferment glucose, produce gas, but do not ferment lactose and sucrose. Generally, *Salmonella* sp. produces  $\text{H}_2\text{S}$ , is resistant to certain chemicals, and grows at 15-41°C with a pH of 6-8. The formation of gas comes from the reaction of acids which are retained based on media (Ulya, 2020). On media containing high protein, *Salmonella* sp. Grow quickly and can even grow as many as 90,000 cells in just 6 hours (Yunus, et al. 2017). In a culture so that the colonies are large, 2-8 millimeters in diameter, slightly convex round, clear, smooth, on BAP media do not cause hemolysis, on Mac Concey media *Salmonella* sp. Does not ferment lactose (NLF), the consistency is smooth *Salmonella* sp. survive in water that is frozen for a long time, these bacteria are resistant to certain chemicals (eg brilliant green, sodium tetrathionate, sodium deoxycholate) that inhibit the growth of other enteric bacteria, but these compounds are useful to be added to the isolation medium *Salmonella* sp. on the stool sample (Irianto, 2018). Typhoid fever is one of the endemic diseases in Indonesia, so it must be given serious attention because it can be a threat to public health. Moreover, there has been an increase in relapse cases and there is resistance to the drugs used, which will make it more difficult for various efforts to be made related to this disease (Depkes, 2006). *Salmonella* incubation time lasts 7-14 days depending on the number of bacteria, virulence, and immune response (Hardianto, 2019)

One way to prevent bacterial contamination is to wash your hands (Taliya, 2021). Contaminated microbes in food are the result of direct or indirect contamination with pollutant sources (Amiruddin et al, 2017). Cross contamination can also occur if the

processing and preparation process does not meet the requirements, such as food that is not clean and undercooked (Yuniatun, et al. 2017).

The objective of this research was to look at the activity of contaminated *Salmonella typhii* in milkfish sold in unclean fish markets, besides that the length of the process from cutting until the milkfish is ready for consumption, is very possible for contamination of milkfish, including contamination at the time of slaughter, namely with the market or unhygienic fish auction places, tools used for cutting, water used for washing, workers' hands, contact with parts that are often contaminated such as scales, internal organs, gill covers, and others.

## Materials and Methods

### Experimental Location and Samples

This experiment was conducted in Microbiology Laboratory at the Faculty of Medical Hasanuddin University, Makassar, Indonesia, in June 2021. The population is all samples of milkfish scattered in markets and fish auctions in Makassar City. Samples were taken as ten samples with areas selected by Probability Sampling.

### Isolation and Identification with Specific Medium

Isolation and identification with McConkey Agar (MCA) or BHIB medium, SSA medium, and NA medium were incubated at 35oC for 24 hours.

### Biochemical Test for *Salmonella Typhii*

For the identification of *S.typhii*, specific biochemical tests were conducted according TSIA media, SIM media, MRVP media, citrate, glucose, sucrose, urea, Lactose, mannitol were incubated at 35oC for 24 hours.

### Gram Strain

Gram stain with crystal violet, iodine, safranin, and alcohol 70%.

### Antibacterial and Antibiotic Tests

Antibacterial test with MHA media (Mueller Hinton Agar) and antibiotics used such as Ceftriaxone, Sulfamethazole, Ampicillin, Ciprofloxacin, Chloramphenicol (Andi Munawir, 2021)

## Results and Discussion

Milkfish samples totaled ten samples by taking milkfish (*Chanos chanos*) samples at different locations in the Fish Market and Auction in Makassar City by providing sample codes A-J. The sample criteria examined were scales, gill covers, gills, fluids in the fish's mouth, and parts of the fish's intestines figure 1.



**Figure 1. Samples Milkfish (*Chanos Chanos*)**

Milkfish samples are put into BHIB (Brain Heart Infusion Broth) Medium, as seen in figure 2, where BHIB is a microorganism growth medium which is rich in nutrients which is suitable for various types of microorganisms including bacteria.



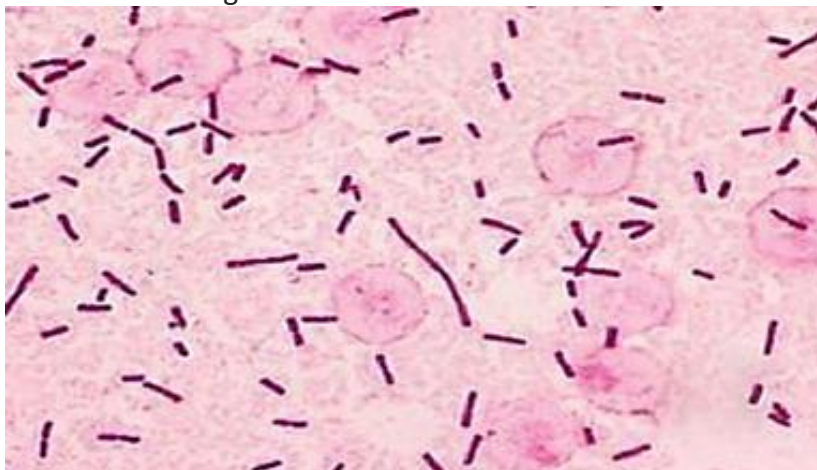
**Figure 2. Identification Test with BHIB Medium**

Test Selective medium SSA (*Salmonella Shigella Agar*) and NA (*Nutrient Agar*) is a selective medium for detecting and identifying *Salmonella* and *Shigella* bacteria at figure 3.



**Figure 3. Culture on SSA and NA Selective Medium**

*Salmonella typhii* belongs to the gram-negative bacteria in the form of rods as shown in the gram stain with Figure 4.



**Figure 4. Gram Stain at *S.typhii***

Biochemical test with TSIA media, SIM, MR-VP, Citrate, Glucose, Lactose, Sucrose, and Mannitol in Figure 5. Specific characteristics of *Salmonella typhii* in that the TSIA medium gives a reddish color change with a red slant which includes alkali and a red butt forming H<sub>2</sub>S gas, then the SIM medium changes color, and there is movement of motile bacteria characterized by the spread of bacteria with the spread of growth in the area around the ose puncture, then on the MR-VP medium there is a color change from yellow to reddish, then citrate test, urea test, lactose test, and the sucrose test did not change color, while the glucose and mannitol test gave a color change from red to yellow.



**Figure 5. Biochemistry Test with TSIA, MR-VP, Citrate, Urea, Glucose, Lactose, Mannitol, Sucrose**

In Table 1, samples coded C, G, and J were suspected of *Salmonella typhii*, then sample A was suspected of *Providencia stuartii* bacteria, then samples B, D, E, H, and I were suspected of *Proteus mirabilis* bacteria, with reference to Great Wohls (1980).

**Table 1. Table of Observation of Biochemical Test of Bacterial Samples**

No	Code	BHIB Media	Mac Conkey Agar (MCA) Media	SSA and NA Media	Gram Stain	TSIA		SIM				MRVP		Cit	Ur ea	G	L	S	M	Interpretation	
						Slant	Butt	H <sub>2</sub> S	Gas	Indol	Motil	H <sub>2</sub> S	MR								VP
1.	A	Cloudy	Colourless, Concave	Round, Big	Basil gram (-)	Alkali	Acid	-	-	+	+	-	+	-	+	-	+	-	-	-	<i>Providencia stuartii</i>
2.	B	Cloudy	Cloud red	Round, Big	Basil gram (-)	Acid	Acid	+	+	-	+	+	+	-	+	-	+	+	+	+	<i>Proteus mirabilis</i>
3.	C	Cloudy	Colourless, Convex	Round, Small	Basil gram (-)	Alkali	Acid	+	-	-	+	+	+	-	-	-	+	-	-	+	<i>Salmonella typhi</i>
4.	D	Cloudy	Cloud red	Round, Big	Basil gram (-)	Acid	Acid	+	+	-	+	+	+	-	+	-	+	+	+	+	<i>Proteus mirabilis</i>
5.	E	Cloudy	Cloud red	Round, Big	Basil gram (-)	Acid	Acid	+	+	-	+	+	+	-	+	-	+	+	+	+	<i>Proteus mirabilis</i>
6.	F	Cloudy	Convex red	Round, Small, Smooth	Basil gram (-)	Acid	Acid	-	-	+	+	-	+	-	-	-	+	+	+	+	<i>Escherechia coli</i>
7.	G	Cloudy	Colourless, Convex	Round, Small	Basil gram (-)	Alkali	Acid	+	-	-	+	+	+	-	-	-	+	-	-	+	<i>Salmonella typhi</i>
8.	H	Cloudy	Cloud red	Round, Big	Basil gram (-)	Acid	Acid	+	+	-	+	+	+	-	+	-	+	+	+	+	<i>Proteus mirabilis</i>
9.	I	Cloudy	Cloud red	Round, Big	Basil gram (-)	Acid	Acid	+	+	-	+	+	+	-	+	-	+	+	+	+	<i>Proteus mirabilis</i>
10.	J	Cloudy	Colourless, Convex	Round, Small	Basil gram (-)	Alkali	Acid	+	-	-	+	+	+	-	-	-	+	-	-	+	<i>Salmonella typhi</i>

In Figure 6, the measurement of inhibition using the antibiotics ceftriaxone, sulfamethoxazole, ampicillin, ciprofloxacin, chloramphenicol, and tetracycline, can be seen in Table 2 the most sensitive antibiotic against *Salmonella typhii* is ciprofloxacin (CIP) with a high inhibition value compared to with other antibiotics which refer to the measurement and inhibition of antibiotics with reference to Great Wohls (1980) or (Andre B, and Novita, 2022).

The level of contamination of *Salmonella typhii* bacteria in milkfish shows a percentage of 30%, which is included in the moderate level, this indicates that the level of contamination with this bacteria is still high.



**Figure 6. Antibacterial Test with Antibiotics Ceftriaxone, Sulfamethazole, Ampicillin, Ciprofloxacin, Chloramphenicol**

**Table 2. Antibacterial Test Using Certain Antibiotics**

		AMP	TE	SXT	C	CIP
1.	<i>Salmonella typhii</i>	17	19	16	18	21
2.	<i>Proteus mirabilis</i>	8,2	24,4	17,4	16,6	25,5
3.	<i>Providentia stuartii</i>	6,7	6,3	19,5	22	21
4.	<i>Escherechia coli</i>	6,9	20,4	22,3	25,5	11,9

Description :

AMP : Ampicilin

TE : Tetracyclin

SXT : Sulfamethazole

C : Chloromfenikol

## Conclusion

Samples of *Salmonella typhii* were obtained in 3 samples of the ten samples studied. The level of contamination of *Salmonella typhii* bacteria in all samples observed was moderate, with a percentage level of 30% of samples taken at fish markets and auctions in Makassar City. The antibiotic Ciprofloxacin has a high sensitivity test rate compared to the four antibiotics, namely Tetracycline (TE), Chloramphenicol (C), Ampicillin (AMP), and Sulfamethoxazole (SXT).

## References

- Ainil, M. 2021. Angka kejadian demam tifoid berdasarkan kajian serologis di RS Unhas Makassar, Puskesmas Tamalanrea tahun 2019-2020. Fakultas Kedokteran, UNHAS, Makassar.
- Amiruddin, R. R., Darniati., dan Ismail, 2017. Isolasi dan identifikasi *Salmonella* sp. pada ayam bakar di rumah makan kecamatan Syiah Kuala kota Banda Aceh. *Jimvet*, 01(3): 265-274.
- Andi Munawir, Anastasia A, 2021. Uji Aktivitas Bakteri *Salmonella typhii* pada sayuran Lalapan Kemangi (*Ocimum sanctum* L) secara invitro. *UMI Medical Journal* Vo 6, No 2, 31 Desember 2021, e-ISSN: 2685-7581.
- Andre Budi, Novita L. 2022. Pola Resistensi Bakteri *Salmonella typhii* terhadap antibiotik Ceftriaxone dan Ciprofloxacin. *Journal Health and Science, Gorontalo*, Vol 6 Nomer 1, April 2022, e-ISSN: 2656-9248.
- Ariyanti, T., dan Supar. 2017. Problematika salmonellosis pada manusia. Lokakarya Nasional Penyakit Zoonosis, Jakarta.
- Departemen Kesehatan Republik Indonesia, 2006, Pedoman Pengendalian Demam Tifoid. Menteri Kesehatan Republik Indonesia, Jakarta.
- Darmawan, A., Lucia, M., Sitti, A., dan Hapsari, M. (2020). Kontaminasi *Salmonella* sp. pada daging ayam broiler yang dijual di beberapa Pasar Tradisional di Makassar. *Indonesia Medicus Veterinus*, 9(2) : 168-176.
- Dharmojono, 2001, Limabelas Penyakit Menular dari Binatang ke Manusia. Milenia Populer, Jakarta.
- Global Burden of Disease, 2020. Global burden of 369 diseases and injuries in 204 countries and territories. *The Lancet*, 396(10258), pp. 1204–1222. Doi : 10.1016/S0140-6736(20)30925-9.
- Hardianto, D. 2019. Telaah metode diagnosis cepat dan pengobatan infeksi *Salmonella typhi*. *Jurnal bioteknologi dan biosains Indonesia*, 6(1): 149-158.
- Irianto, K., 2018, Epidemiologi Penyakit Menular dan Tidak Menular Panduan Klinis, Alfabeta, Bandung, Jawa Barat.
- Lestari, I Dewa A.M.D Dan Hendrayana, Made Agus, 2017. Identifikasi Dan Diagnosis Infeksi Bakteri *Salmonella typhi*. Skripsi. Fakultas Kedokteran : Universitas Udayana Denpasar.
- Melawati, B., Fakhurrizi, dan Abrar, M., 2019. Deteksi bakteri *Salmonella* sp pada ikan asin talang-talang (*Scomberoides tala*) di kecamatan Leupung kabupaten Aceh Besar. *Jimvet*, 3(3): 175-180
- Muna, Fauzul dan Khariri. 2020. Bakteri Patogen Penyebab Foodborne Disease. *Jurnal UIN Alaudin*. Makassar.
- Putri, Meiwa R., Tri Umiana S., Syazili Mustofa dan Ety Apriliana. 2019. Identifikasi Bakteri *Salmonella typhi* Pada Makanan Jajanan Gorengan Yang Dijual di Depan Sekolah Dasar Negeri Kecamatan Kedaton Kota Bandar Lampung. Universitas Lampung : *Jurnal Agromedicine*, Vol 6, No. 2.
- Samiadi, L.A. 2021. Salmonellosis. Artikel Kesehatan Pencernaan. <http://hellosehat.com>. Salmonellosis.id Diakses 15 Januari 2022 Pukul 11.00 WIT
- Taliya Putri Wardani, 2021. Identifikasi *Salmonella typhii* pada swab telapak tangan pedagang makanan di SD Kanisus Semanggi II Surakarta. Stikes Nasional, Surakarta



- Ulya, N. N., Fitri, I. dan Widyawati, I. D. 2020. Gambaran makroskopis dan mikroskopis bakteri *Salmonella typhi* dan *Salmonella paratyphi* pada penderita demam tifoid. Jurnal Sintesis, 1(2): 40-46.
- World Health Organization. 2018. Typhoid Fever. <http://www.WHO.int>. diakses pada tanggal 28 July 2021.
- Yuniatun, T., Purwantisari, S., dan Yuliawati, S. 2017. Hubungan hygiene sanitasi dengan kualitas mikrobiologis pada makanan gado-gado di Kecamatan Tembalan Kota Semarang. Jurnal Kesehatan Masyarakat, 5(4) : 491-499.
- Yunus, R., Mongan, R., dan Rosnani. 2017. Cemaran bakteri Gram negatif pada jajanan siomay di Kota Kendari. Medical Laboratory Tecnology Journal, 3(1) : 87-92.
- Zelpina, E., dan Noor, SM. 2020. Non-Typhoid *Salmonella* penyebab Foodborne disease: pencegahan dan penanggulangannya. Wartazoa, 30(4):221-229.