

2

by Reina .,

Submission date: 31-Mar-2020 05:14AM (UTC+0200)

Submission ID: 1285880720

File name: 2._JURNAL_INTERNASIONAL.pdf (1.03M)

Word count: 3866

Character count: 21430

Detection of *invA* gene of *Salmonella* from milkfish (*Chanos chanos*) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique

Sheila Marty Yanestria¹, Reina Puspita Rahmaniar², Freshinta Jellia Wibisono¹ and Mustofa Helmi Effendi³

1. Department of Veterinary Public Health, Faculty of Veterinary Medicine, Wijaya Kusuma University, Surabaya, Indonesia; 2. Department of Microbiology, Faculty of Veterinary Medicine, Wijaya Kusuma University, Surabaya, Indonesia; 3. Department of Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia.

Corresponding author: Mustofa Helmi Effendi, e-mail: mheffendi@yahoo.com

Co-authors: SMY: sheila.marty11.sm@gmail.com, RPR: pita.reina@gmail.com, FJW: freshinta.uwks@gmail.com

Received: 03-09-2018, Accepted: 07-12-2018, Published online: 29-01-2019

doi: [10.14202/vetworld.2019.170-175](https://doi.org/10.14202/vetworld.2019.170-175) How to cite this article: Yanestria SM, Rahmaniar RP, Wibisono FJ, Effendi MH (2019) Detection of *invA* gene of *Salmonella* from milkfish (*Chanos chanos*) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique, *Veterinary World*, 12(1): 170-175.

Abstract

Aim: The study aimed to detect the *invA* gene in *Salmonella* isolated from milkfish in the Sidoarjo wet fish market.

Materials and Methods: A total of 84 samples were prepared in enrichment media and isolated on the surface of *Salmonella* Shigella Agar. *Salmonella* growth produces transparent colonies with blackish color in the middle due to H₂S gas formation. Samples were identified as *Salmonella* based on macroscopic colony morphology. Presumptive *Salmonella* sp. was put on Bismuth Sulfite Agar media. *Salmonella* was determined based on the results of the biochemical test that has been carried out using Microbact identification kits from negative gram staining.

Results: The results of this study indicate that 32 of 84 samples (38.09%) were *Salmonella* bacteria. Furthermore, the *invA* gene detection was carried out using the polymerase chain reaction technique. Electrophoresis results showed four positive samples contained *invA* gene with a length of 284 bp.

Conclusion: Results in this study indicate that contamination of milkfish with *Salmonella* needs strict hygienic measures to prevent their transmission to human.

Keywords: human health, *invA* gene, milkfish, polymerase chain reaction, *Salmonella*.

Introduction

Indonesia is an agricultural country that has a large enough fishery potential. The amount of capture fisheries production in 2014 was 6.20 tons, an increase of 0.34 million tons from 2013 which was only 5.86 million tons. The amount of fish production from the aquaculture sector has also increased from year to year [1]. Some fish products contaminated with bacteria can cause a disease called foodborne disease. Foodborne disease or also called foodborne diarrheal disease is a zoonotic disease and is found throughout the world. Foodborne diarrheal disease is a disease transmitted by animal's carriers that are healthy to humans through food contamination [2].

One of the bacteria that cause foodborne disease is *Salmonella* and is called salmonellosis. This disease is endemic in almost all major cities in Indonesia, and the incidence of typhoid fever caused by *Salmonella typhi* continues to increase with the incidence of 350-810 cases per 100,000 population

with a mortality rate of [21]. Typhoid fever is found throughout the year [3]. There are several transmission routes for salmonellosis, but the majority of human infections are derived from the consumption of contaminated foods, especially those of animal origin. Most cases of salmonellosis in fish start from consuming raw fish meat without first processing and cross-contamination [4]. The US Food Drug Administration reports data that *Salmonella* is the most common contaminant bacteria found in fish and fish products [5]. Established conventional methods to detect and identify *Salmonella* are time-consuming and include selective enrichment and plating followed by biochemical tests [6].

Salmonella detection can be done in various ways, one of which is using the polymerase chain reaction (PCR) technique. This technique can be used on very complex sample substances such as food and is able to work quickly and specifically. Due to the advantages, PCR is suitable to detect the presence of *Salmonella*. Detection of *Salmonella* using PCR can be done in a short time with high accuracy so that appropriate handling can be done on food [7]. The *invA* gene from *Salmonella* contains unique DNA sequences and is proven to be a PCR target gene suitable for *Salmonella* detection [8].

In this research, samples from milkfish were tested for isolation of *Salmonella*, by culturing and

Copyright: Yanestria, et al. Open Access. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated.

biochemical method, and then, they were confirmed by *invA* gene-specific PCR methods.

Materials and Methods

Ethical approval

Fresh milkfish were used in this study; hence, ethical approval was not necessary. Milkfish samples were collected from Sidoarjo wet fish market.

Salmonella culture method

Milkfish samples are cut with a sterilized knife. Grind the meat and digestive tract of the fish in a mortar with a stamper, then take 1 g and put it in 5 ml of selenite broth, and incubate it for 24 h at 37°C.

Bacterial isolation is carried out by taking bacterial suspensions with inoculating loop which is first sterilized using Bunsen fire. The suspension is then implanted in *Salmonella* Shigella Agar (SSA) which has been labeled according to the sample using the streak method to obtain a separate colony which is then incubated for 24 h in a temperature of 35-37°C. The growth of *Salmonella* produces transparent or colorless colonies with blackish color in the middle due to H₂S gas formation [9]. Presumptive *Salmonella* is put on Bismuth Sulfite Agar (BSA) media. Bismuth sulfite is a selective medium for *Salmonella* isolation in the laboratory. BSA is a modification of Wilson and Blair Formula. Typhoid-causing organisms grow well in this medium, with the characteristics of black colonies. Gram-positive and other coliform bacteria are inhibited. These media are generally used for the detection of *Salmonella* species.

Microscopic examination and biochemical test

Positive samples on BSA media were then given Gram staining to determine the type of Gram bacteria that grew. Positive results on Gram examination show that bacteria in the form of long and medium rods, red, Gram-negative bacteria spread perfectly without forming long chains or groups [10]. After Gram staining, the bacteria were tested with biochemical tests [11]. The *Salmonella* was determined based on the results of the biochemical test that has been carried out using Microbact identification kits from OXOID.

DNA extraction

The initial step of PCR is DNA extraction of bacterial culture in *Salmonella* Shigella media to be extracted by boiling method. The boiling method was carried out for 10 min and centrifuged for 5 min at a speed of 6000 rpm. The supernatant is used for PCR amplification.

PCR amplification

Detection of *Salmonella* invasive encoding gene was carried out using the PCR test. The reagents for PCR amplification consisted of 2.5 µl DNA template, 5 units of GoTaq DNA polymerase, 1 × GoTaq PCR reaction buffer (containing 1.5 mM MgCl₂), 0.2 mM PCR nucleotide mix, and 0.6 µM DNA primers with a final volume of 50 µl. Specific primers used to detect *Salmonella* are primary forward (*invA*) GTG AAA TTA TCG CCA CGT TCG

GGC AA and primer reverse TCA TCG CAC CGT CAAAGG AAC C [12].

The PCR reagent mixture was then put in a thermocycler with an initial incubation of 94°C for 1 min, followed by 35 cycles consisting of 94°C for denaturation for 1 min, annealing at 58.3°C for 30 s, and 72°C elongation for 30 s followed by the final extension at 72°C for 7 min. Each of the 3 µl of amplification products was mixed with 3 µl of loading solution until well mixed.

Electrophoresis of PCR products

The results of PCR product amplification were electrophoresis in 1.5% agarose gel and stained with ethidium bromide. Markers are also inserted into the agarose gel well to determine the DNA size of the PCR product; then, electrophoresis is run for 40 min with a constant voltage of 100 v. Electrophoresis results were observed under ultraviolet light. The results obtained are DNA band patterns (DNA bands) which show different numbers and patterns. 100 bp DNA ladder was used as a marker.

Sensitivity and specificity of PCR [13]

PCR assay was evaluated in various concentration of DNA and various concentration of viable *Salmonella* Typhimurium for analyzing sensitivity of PCR. For sensitivity test based on DNA concentration, DNA extract was sequentially diluted ten-folds and based on the viable bacterial count, *S. Typhimurium* was sequentially diluted from 10⁹ to 1 colony-forming unit/mL, and DNA was extracted from each dilution. DNA extracted from *Staphylococcus aureus* was used as negative control, in each PCR run. The PCR products were visualized by electrophoresis and observed as described above. The minimum concentration of DNA giving a positive signal was recorded. The specificity of PCR was evaluated by comparing with six different *Salmonella* serovars and cross-tested with 5 non-*Salmonella* isolates, i.e., *Escherichia coli*, *Listeria monocytogenes*, *Proteus mirabilis*, *S. aureus*, and *Vibrio parahaemolyticus* [13].

Results and Discussion

Results of Salmonella culture and identification

Testing on SSA media is done by macroscopic observation. On macroscopic examination, *Salmonella* growth produces transparent or colorless colonies with blackish color in the middle due to H₂S gas formation (Figure-1). Macroscopic colony examination results on SSA media same on BSA media that showed 32 positive samples from 84 samples (38.09%) (Figure-2) and is shown in Table-1. These results were determined as *Salmonella* on Gram staining (Figure-3) and biochemical test (Figure-4).

Detection of encoding gene Salmonella

The results of detection of *Salmonella* invasive encoding genes performed using PCR tests shown in Figure-5, which shows positive *Salmonella invA* genes at 284 bp, and *Salmonella* which did not have *invA* genes (Figure-6).

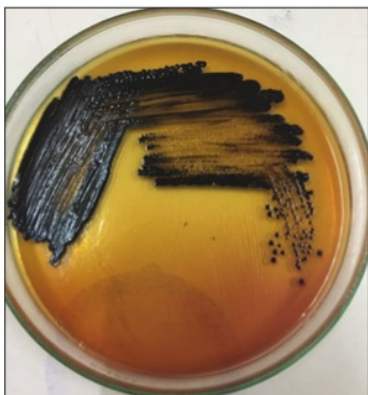


Figure-1: Result of *Salmonella* bacterial culture on *Salmonella Shigella* Agar media.



Figure-4: Positive *Salmonella* on biochemical test results using Microbact identification kits (arrows indicate positive *Salmonella*).



Figure-2: Result of *Salmonella* on Bismuth Sulfite Agar media.

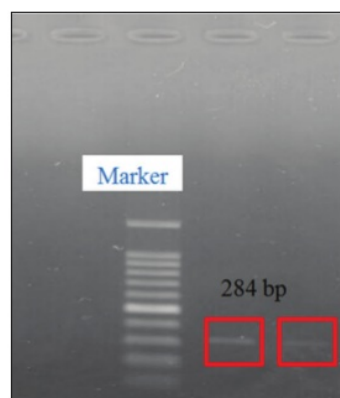


Figure-5: Results of detection of *invA* gene encoding *Salmonella* on milkfish.

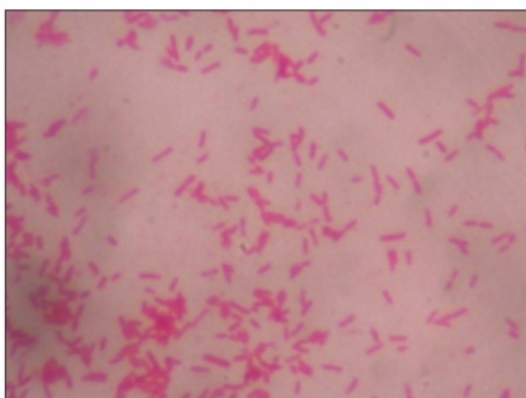


Figure-3: Microscopic examination result of *Salmonella* with negative Gram staining.

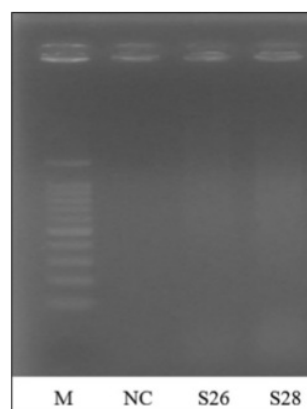


Figure-6: DNA visualization of *invA* gene *Salmonella* amplification products on a 1.5% agarose gel (tris-borate-ethylenediaminetetraacetic acid). Lane M=DNA marker 100 bp ladder, lane NC=*S. aureus* DNA as a negative control, lane S26; S28 positive *Salmonella* without positive result of *invA* gene *Salmonella*.

Salmonella infection is a major bacterial disease in fishes, poultry, and humans, causing significant economic loss and illnesses [10,14,15]. Out of 84 samples, we isolated 32/84 (38.09%) of *Salmonella* by conventional culture method, and out of 32 positive *Salmonella*, we confirmed by PCR 4/32 (12.5%).

Based on the results of PCR, there were 4 samples from 32 samples of *Salmonella* isolates which

detected an *invA* gene with a length of 284 bp, while 28 samples did not show the target band. The absence of target bands in 28 samples showed that the strains of *Salmonella* bacteria obtained were not invasive or might also use other invasive mechanisms [16]. The use of certain PCR methods from *InvA* gene in most diagnostic and research laboratories is possible, and through molecular identification techniques of *Salmonella*, this method is the simplest and cheaper method [6].

At present, *Salmonella* is detected by standards bacteriological, biochemical, and serological techniques. These techniques are generally time-consuming, tedious, and expensive [17]. This research also corroborates some of the previous work carried [48] using the PCR method, in which non-typhoidal *Salmonella* was isolated from animals, food, and samples of human excrement in developing countries [18]. The observed prevalence of invasive *Salmonella* genes from poultry and poultry products is different from some other studies, which report a higher prevalence [6]. This variation may be due to differences in research methodology; gene-specific involvement, sample type, sample size and hygiene practices on the farm, and geographical location. *Salmonella* is known to infect animal products by various species; however, one serovar may be dominant in location for many years before being replaced by another. The most common and clinically significant serovar containing the *invA* virulence gene that causes salmonellosis globally is *S. Typhimurium* and *Salmonella enteritidis* [19].

Molecular methods are increasingly important in detecting and typing *Salmonella*. Tests are published with the aim of replacing traditional serotyping with direct detection of various genes [20]. PCR examination technique with the *invA* gene is very sensitive and specific in detecting *Salmonella*. Detection of *Salmonella*-specific by PCR with primers for *invA* gene is fast, sensitive, and specific to detect *Salmonella* in many clinical samples [21]. This research supports the ability of this specific primer set to confirm isolates as *Salmonella*. The *invA* gene contains sequences specific to the genus *Salmonella* and is considered the international standard for its characterization [16].

The *invA* gene is indeed targeted for the diagnosis of *Salmonella* organisms at the genus level [22]. *Salmonella*-specific primers' ability to detect *Salmonella* species quickly and accurately in this study is mainly due to the primers sequence selected from the invasion of the *S. Typhimurium* gene as previously reported by Darwin and Miller [23]. This is also consistent with research conducted by Pererat and Murray [24] that the results of PCR on various serotypes of *Salmonella* showed positive results, while PCR results on non-*Salmonella* strains such as *E. coli*, *Klebsiella*, *Proteus*, and *Shigella* were negative for the *invA* gene.

Table-1: Results of *Salmonella* isolation and *invA* gene detection.

	Milkfish samples	<i>Salmonella</i>	<i>invA</i> gene
Total number	84	32	4

First step in the intracellular pathogenicity cycle of *Salmonella* is the invasion of intestinal epithelial cells, and this step is controlled by the *invA* gene. The *invA* gene encodes proteins in bacterial cell membranes that are needed for invasion into host epithelial cells [23,25]. This gene is located in pathogenicity island I or referred to as *Salmonella* Pathogenicity Island (SPI); the DNA region is related to the pathogenicity of *Salmonella enterica* and is owned by all serotypes [26]. SPI serves to add complex virulence by bacteria to the infected host [24].

Many invasion genes encoded in SPI I and their expression are activated by the *HilA* gene; transcription factors are also encoded in SPI I. In a study conducted by Murray and Lee [26] regarding the role of *Salmonella* invasion genes during infection in mice after intragastric inoculation, it was found that strains containing invasion genes that had mutations were found from intestinal tissue cells, and systemic tissue compared to strains that had not undergone previous mutation.

Galan *et al* [47,7] also identified the *invA* gene as the first gene consisting of at least two additional invasion genes that are *Salmonella* to enter culture epithelial cells, but now there is an *invA* gene that has mutations. *S. Typhimurium invA* mutants are less capable of invading culture epithelial cells. *InvA* mutants do not alter the normal structure of the microvilli of epithelial cells and also do not cause changes in the distribution of actin microfilaments from cells. *InvA* mutants are significantly inhibited in their ability to enter cells, no emergence of invasive gene bands can also be caused by mutant genes that are unable to replicate on the intestinal mucosal surface, it can also be caused due to susceptibility to bactericidal host factors such as phagocytes. The invasion gene has been found in *Salmonella* with its ability to kill phagocytes *in vitro*.

With PCR, a single gene product can be strengthened in a single PCR reaction and in this study is shown as a rapid method for identifying one gene related to invasion virulence. Such early detection can have major benefits in public health, especially for rapid diagnosis and finding the ideal vaccine, epidemiological investigations, and prophylactic strategies for Salmonellosis in Sidoarjo, Indonesia. In summary, the findings from this study showed the distribution of *invA* gene (12.5%) in milkfish sources (Table-1). This requires planning and implementing control programs to prevent and control infections for spreading to public health.

Conclusion

This study describes the prevalence of *Salmonella* contamination in the milkfish samples obtained from Sidoarjo wet fish market, Indonesia. The study found contamination of *Salmonella* in milkfish, indicating potential sources and routes of the *Salmonella* transmission in the fishery. Isolation of *Salmonella* carrying invasion gene (*InvA* gene) in this study could show poor sanitation of the environment in which milkfish are sold at wet fish market Sidoarjo, East Java Province, Indonesia.

Authors' Contributions

MHE supervised the project. SMY was a project leader and RPR carried out bacterial isolation and molecular work. FJW collected the samples. 38 authors contributed in the drafting and revision of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank the Ministry of Research, Technology and Higher Education of Indonesia for providing research funds with grant number 36/16/LPPM/UWKS/III/2018 for the research title: DETEKSI DAN KARAKTERISASI *Salmonella* spp. PADA IKAN BANDENG (*Chanos chanos*) DI TEMPAT PELELANGAN IKAN KABUPATEN SIDOARJO. 41

Competing Interests

The authors declare that they have no competing interests.

Publisher's Note

Veterinary World remains neutral with regard to jurisdictional claims in published institutional affiliation.

References

- Ministry of Maritime Affairs and Fisheries. (2015) A Report on Trends In Coastal Marine Resources And Fisheries Management in Indonesia.
- Kakatkhar, A., Gautam 29, Nagar, R.K., Karani, V. and Bandekar, J.R. (2010) Incidence of food-borne pathogens in freshwater fish from domestic markets of Mumbai. *Fish. Technol.*, 47(2): 195-200.
- Poeloengan, M., Komala, I. and Noor, S.M. (2014) Bahaya *Salmonella* Terhadap Kesehatan. Prosiding Lokakarya Nasional Penyakit Zoonosis, p216-224.
- Hassan, R., Tecle, S., Adcock, B., Kellis, M., Weiss, J., Saupe, A., Sorenson, A., Klos, R., Blankenship, J., Blessington, T., Whitlock, L., Carleton, H.A., Concepción 14, Ivedo, J., Tolar, B., Wise, M. and Neil, K.P. (2018) Multistate outbreak of *Salmonella* Paratyphi B variant L(+ tartrate(+)) and *Salmonella* Weltevreden infections linked to imported frozen raw Tuna – United States, 2015. *Epidemiol. Infect.*, 146(11): 1461-1467. 44
- Olgunoglu, I. A. (2012) In: Mahmoud, B.S. 43 editor. *Salmonella – A Dangerous Foodborne Pathogen*. Published by InTech, Janeza Trdine 9, 51000 Rijeka, Croatia. p 18-108.
- Shanmugasamy, M., Velayutham, T. and Rajeswar, J. (2011) *InvA* gene-specific PCR for detection of *Salmonella* from 20 lers. *Vet. World*, 4(12): 562-564.
- Prayoga, W., dan Wardani, A.K. (2015), Polymerase chain reaction for detection of *Salmonella* spp.: Literature review. 5 *Pangan Agroindu.*, 3(2): 483-488.
- Abdel-Aziz, N.M. (2016) Detection of *Salmonella* species in chicken carcasses using genus-specific primer belong to *invA* gene in Sohag city, Egypt. *Vet. World*, 9(10): 1125-1128. 12
- Ibrahim, B. and Sheshi, M. (2014) Isolation and identification of bacteria associated with fresh and smoked fish (*Clarias gariepinus*) in Minna metropolis, Niger state. 7 *geria. J. Appl. Environ. Micro.*, 2(3): 81-85.
- Nidaullah, H., Abirami, N., Shamila-Syuhada, A.K., Chuah, L.O., Nu 7, H., Tan, T.P., Zainal, A.F.W. and Rusul, G. (2017) Prevalence of *Salmonella* in poultry processing environments in wet markets in Penang and Perlis, 24 aysia. *Vet. World*, 10(3): 286-292.
- Todar, K. (2005) *Salmonella* and Salmonellosis. *Todar's Online Textbook of Bacteriology*. © 2005 Kenneth Todar University of Wisconsin-Madison Department of Bacteriology, USA.
- Upadhyay, B.P., Utrarachkij, F., Thongshoob, J., Mahakunkijcharoen, 17 Yongchinda, N., Suthienkul, O. and Khusmith, S. (2010) Detection of *Salmonella invA* gene in shrimp enrichment culture by polymerase chain reaction. 28 *theast Asian J. Trop. Med. Public Health*, 41(2): 426-435.
- Amalia, U., Dewanti-Hariyadi, R. and Poemomo, A. (2014) Rapid detection of *Salmonella* in shrimp by polymerase 3 ain reaction. *J. Teknol. Ind. Pangan*, 25(1): 78-82.
- Bibi, F., Qaisrani, S.N., Ahmad, A.N., Akhtar, M., Khan, B.N. and Ali, Z. (2015), Occurrence of *Salmonella* in freshwater fishes: A review. *J. Anim. Plant Sci.*, 25(2): 13-310.
- Whiley, H. and Ross, K. (2015) *Salmonella* and eggs: From production to plate. *Int. J. Environ. Res. Public Health*, 2(3): 2543-2556.
- Malorny, B., Hoorfar, J., Bunge, C. and Helmuth, R. (2003) Multicenter validation of the analytical accuracy of *Salmonella* PCR: Towards an international standard. *Appl. 1 viron. Microbiol.*, 69(1): 290-296.
- Hernandez, T., Sierra, A., Rodriguez-Alvarez, C., Torres, A., Arevalo, M.P., Calvo, M. and Arias, A. (2005) *Salmonella* Enterica serotypes isolated from imported frozen chicken meat in Canary Islands. *J. Food Prot.*, 68(12): 2702-2706.
- Suwit, C., Suvic 6, R., Fred, U., Pakpoom, T. and Prapas, P. (2014) Prevalence and antimicrobial resistance of *Salmonella* isolated from carcasses, processing facilities and the environment surrounding small scale poultry slaughterhouses in Thailand. *Southeast Asian J. Trop. Med. 33 ublic Health*, 45(6): 1392-1400.
- Rodriguez, J., Rondón, I. and Verjan, N. (2014) Serotypes of *Salmonella* in broiler carcasses marketed at Ibaguë, 9 lombia. *Braz. J. Poult. Sci.*, 17(4): 545-552.
- Kardos, G., Farkas, T., Antal, M., Nógrády, N. and Kiss, I. (2007) Novel PCR assay for identification of *Salmonella* Enterica serovar infantis. *Lett. Appl. Microbiol.*, 45(4): 414-425.
- Lampel, K.A., Orlandi, P.A. and Kornegay, L. (2000) Improved template preparation for PCR-based assay for detection of food-borne bacterial pathogens. *Appl. Environ. Microbiol.*, 66(10): 4539-4542.
- Abdeltawab, A.A.E., Ahmed, A.M., Aish 23 A.R., Fatma, H.I.E. and Mohammed, E.E.S.A. (2013) Detection of common (*invA*) gene in salmonellae isolated from poultry using polymerase chain reaction technique. *Benha Vet. 10 J. J.*, 25(2): 70-77.
- Darwin, K.H. and Miller, V.L. (1999) Molecular basis of the interaction of *Salmonella* with the intestinal mucosa. *Clin. Microbiol. 11 ev.*, 12(3): 405-428.
- Pererat, K. and Murray, A. (2008) Development of a PCR

- 27

56

ORIGINALITY REPORT

25%

SIMILARITY INDEX

17%

INTERNET SOURCES

16%

PUBLICATIONS

14%STUDENT PAPERS

PRIMARY SOURCES

1**isca.in**

Internet Source

1%**2****www.afnor-validation.org**

Internet Source

1%**3****journals.psmpublishers.org**

Internet Source

1%**4****idl-bnc.idrc.ca**

Internet Source

1%**5****Submitted to Ar School For Math & Sci High School**

Student Paper

1%**6****Chin-Chin Huang, Shao-Hung Wang, Li-Te Chin, Chang-Lin Huang, Li-Ting Sun, Chien-Shun Chiou, Pei-Chun Tu, Chishih Chu.****"Salmonella enterica serotype typhimurium and S. Stanley differ in genomic evolutionary patterns and early immune responses in human THP-1 cell line and CD14+ monocytes",
Comparative Immunology, Microbiology and****1%**

Infectious Diseases, 2019

Publication

7	udspace.udel.edu Internet Source	1%
8	Submitted to Universiti Malaysia Sarawak Student Paper	1%
9	tudoster.idea.unideb.hu Internet Source	1%
10	www.csw-journal.org Internet Source	1%
11	Submitted to Leeds Beckett University Student Paper	1%
12	Submitted to Universitas Bung Hatta Student Paper	1%
13	Submitted to University of Hong Kong Student Paper	1%
14	www.medscape.com Internet Source	1%
15	D. I. Stewart. "Stimulation of Microbially Mediated Chromate Reduction in Alkaline Soil-Water Systems", Geomicrobiology Journal, 10/2007 Publication	1%
16	Submitted to Universiti Malaysia Pahang Student Paper	1%

17	www.thaiscience.info Internet Source	1 %
18	Submitted to UC, Boulder Student Paper	1 %
19	Submitted to University of Duhok Student Paper	1 %
20	etheses.uin-malang.ac.id Internet Source	1 %
21	R.Y. Murphy, B.L. Beard, E.M. Martin, A.E. Keener, T. Osaili. "Predicting process lethality of Escherichia coli O157:H7, Salmonella, and Listeria monocytogenes in ground, formulated, and formed beef/turkey links cooked in an air impingement oven", Food Microbiology, 2004 Publication	1 %
22	royalsocietypublishing.org Internet Source	1 %
23	Submitted to Manipal University Student Paper	<1 %
24	M. Agbaje, R. H. Begum, M. A. Oyekunle, O. E. Ojo, O. T. Adenubi. "Evolution of Salmonella nomenclature: a critical note", Folia Microbiologica, 2011 Publication	<1 %

"Abstracts cont.", Clinical Microbiology and

25	Infection, 2004 Publication	<1 %
26	www.dovepress.com Internet Source	<1 %
27	Submitted to University of Stellenbosch, South Africa Student Paper	<1 %
28	Submitted to Michigan State University Student Paper	<1 %
29	Mrinal Samanta, Pushpa Choudhary. "Safety of Fish and Seafood", Elsevier BV, 2019 Publication	<1 %
30	Widya Paramita Lokapirnasari, Andreas Berny Yulianto, Djoko Legowo, Agustono. "The Effect of Spirulina as Feed Additive to Myocardial Necrosis and Leukocyte of Chicken with Avian Influenza (H5N1) Virus Infection", Procedia Chemistry, 2016 Publication	<1 %
31	Y.-W. Ku, S. P. McDonough, R. U. M. Palaniappan, C.-F. Chang, Y.-F. Chang. "Novel Attenuated Salmonella enterica Serovar Choleraesuis Strains as Live Vaccine Candidates Generated by Signature-Tagged Mutagenesis", Infection and Immunity, 2005 Publication	<1 %

32	Peter Korosec, Mira Silar, Renato Erzen, Mitja Kosnik. "The influence of antimicrobial therapy on the sensitivity of Legionella PCR", Scandinavian Journal of Infectious Diseases, 2009 Publication	<1 %
33	D Cortes Vélez, V Rodríguez, N Verjan García. "Phenotypic and Genotypic Antibiotic Resistance of Salmonella from Chicken Carcasses Marketed at Ibagué, Colombia", Revista Brasileira de Ciência Avícola, 2017 Publication	<1 %
34	d-nb.info Internet Source	<1 %
35	china.iopscience.iop.org Internet Source	<1 %
36	es.scribd.com Internet Source	<1 %
37	Submitted to Padjadjaran University Student Paper	<1 %
38	www.futuremedicine.com Internet Source	<1 %
39	M. A. Rahman, A. K. M. A. Rahman, M. A. Islam, M. M. Alam. "DETECTION OF MULTI-DRUG RESISTANT SALMONELLA FROM	<1 %

MILK AND MEAT IN BANGLADESH",
Bangladesh Journal of Veterinary Medicine,
2018

Publication

-
- | | | |
|----|--|------|
| 40 | files.aiscience.org
Internet Source | <1 % |
|----|--|------|
-
- | | | |
|----|--|------|
| 41 | bmcgenomics.biomedcentral.com
Internet Source | <1 % |
|----|--|------|
-
- | | | |
|----|---|------|
| 42 | Submitted to Napier University
Student Paper | <1 % |
|----|---|------|
-
- | | | |
|----|--|------|
| 43 | Submitted to International Islamic University
Malaysia
Student Paper | <1 % |
|----|--|------|
-
- | | | |
|----|---|------|
| 44 | Submitted to University of Venda
Student Paper | <1 % |
|----|---|------|
-
- | | | |
|----|---|------|
| 45 | www.jarvm.com
Internet Source | <1 % |
|----|---|------|
-
- | | | |
|----|---|------|
| 46 | www.thieme-connect.de
Internet Source | <1 % |
|----|---|------|
-
- | | | |
|----|--|------|
| 47 | Submitted to University of Sunderland
Student Paper | <1 % |
|----|--|------|
-
- | | | |
|----|--|------|
| 48 | R. Hassan, S. Tecle, B. Adcock, M. Kellis et al. "
Multistate outbreak of Paratyphi B variant L(+)
tartrate(+) and Weltevreden infections linked to
imported frozen raw tuna: USA, March–July | <1 % |
|----|--|------|

-
- | | | |
|-----------|---|----------------|
| 49 | biologia.savba.sk
Internet Source | <1 % |
|-----------|---|----------------|
-
- | | | |
|-----------|--|----------------|
| 50 | www.biolreprod.org
Internet Source | <1 % |
|-----------|--|----------------|
-
- | | | |
|-----------|--|----------------|
| 51 | Tariq khalil Abe, Balqees Hassan Ali. "Molecular Detection of Salmonella pullorum from Poultry Ceca and Vaccine Preparation", International Journal of Poultry Science, 2018
Publication | <1 % |
|-----------|--|----------------|
-
- | | | |
|-----------|--|----------------|
| 52 | N. Ziyate, B. Karraouan, A. Kadiri, S. Darkaoui, A. Soulaymani, B. Bouchrif. "Prevalence and antimicrobial resistance of Salmonella isolates in Moroccan laying hens farms", Journal of Applied Poultry Research, 2016
Publication | <1 % |
|-----------|--|----------------|
-
- | | | |
|-----------|--|----------------|
| 53 | orbit.dtu.dk
Internet Source | <1 % |
|-----------|--|----------------|
-
- | | | |
|-----------|--|----------------|
| 54 | Submitted to Armstrong Atlantic State University
Student Paper | <1 % |
|-----------|--|----------------|
-
- | | | |
|-----------|--|----------------|
| 55 | D RODRIGUEZLAZARO. "A rapid and direct real time PCR-based method for identification of Salmonella spp.", Journal of Microbiological Methods, 2003
Publication | <1 % |
|-----------|--|----------------|
-

56

C. Gahan. "Specific immune mechanisms of defence against foodborne pathogens", Food consumption and disease risk, 2006

Publication

<1 %

57

Karasova, D.. "Comparative analysis of Salmonella enterica serovar Enteritidis mutants with a vaccine potential", Vaccine, 20090820

Publication

<1 %

Exclude quotes Off

Exclude matches Off

Exclude bibliography Off