Jurnal Riset Veteriner Indonesia
Journal of the Indonesian Veterinary Research
P-ISSN: 2614-0187, E-ISSN:2615-2835
Volume 2 No. 1 (January 2018), pp. 35-40
journal.unhas.ac.id/index.php/jrvi/

Detection of *Proteus mirabilis* as foodborne disease bacteria in carcass of broiler chickens (*Gallus domesticus*)

Abdul Wahid Jamaluddin[1]*, Lucia Muslimin[1], Muhammad Natsir Djide[2]
[1]Study Program of Veterinary Medicine, Faculty of Medicine, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia
[2]Faculty of Pharmacy, Hasanuddin University, Jl. Perintis Kemerdekaan Km. 10, Makassar 90245, Indonesia

*Corresponding author: Abdul Wahid Jamaluddin (abdulwahidjamaluddin@unhas.ac.id)

Abstract

Poor hygiene of traditional market often resulted in bacterial contamination of chicken meat including Proteus mirabilis which is also serve as a foodborne disease in human. Proteus mirabilis is pathogenic to humans because it can produces the urease which results in urinary tract infections (UTI). This research aimed to determine the presence of Proteus mirabilis bacteria in chicken meat sold in some traditional markets in the city of Makassar. The samples used in this study (24 samples) were obtained from 6 Traditional Markets in Makassar. Colony observation, Gram staining and biochemical test were used to identify Proteus mirabilis in the chicken carcass. Positive samples were confirmed by Polymerase Chain Reaction (PCR). The result of this study revealed that 12.5% (3/24) were positively contaminated with Proteus mirabilis. The conclusion of this study was that chicken meat sold in the traditional market of Makassar has already been contaminated with Proteus mirabilis
Keywords: *Proteus mirabilis, foodborne disease, urinary tract infection, carcass chicken, traditional market*

Introduction

Foodborne disease is caused by consuming food or water contaminated (Sukron, 2011) and this is a part of zoonosis. Zoonosisis an infectious disease which can be transmitted from animals or products of animal origin to humans (Muslimin et al., 2014). Research conducted by Amare et al. (2013) revealed that 66 out of 290 hens were positive of *Proteus mirabilis* with an infected yolk sac found in the postmortem examination. *Proteus mirabilis*is pathogenic to human because it can lead to urinary tract infection (UTI), which is the most common clinical manifestations of infection *Proteus sp.* (Gonzalez et al., 2014).

Antibiotic resistance to *Proteus mirabilis*, isolated from chicken in China resulted as follows: Tetracycline (100%), sulfamethoxazole (80%), chloramphenicol (66%), nalidixic acid (66%), ampicillin (36%), cefotaxime (34%), ceftiofur (22%), amoxicillin and clavulanic acid (36% 16%) (Wong et al., 2013). Another case in India is semi-resistant/ moderately susceptible to antibiotics from *Proteus mirabilis* isolates derived from beef are Penisilin G, erythromycin, amoxiclav, Cefoxitin and Cotrimoxazole (Gupta et al., 2014). On the other hand,the use of

antibiotics now used to prevent and treat infections caused by bacteria in humans and livestock is now a great concern for their resistance resulting in failure of the treatment of infections diseases caused by pathogenic bacteria in humans and increased costs of treatment (Noor et al. 2006). This research aimed to determine the presence of *Proteus mirabilis* bacteria in chicken meat sold in some traditional markets in the city of Makassar.

Materials and Methods

Twenty-four chicken meat samples were taken randomly from six traditional markets in Makassar. Total Plate Count (TPC) test was conducted by inserting 1 gr of sample into a sterile plastic and then crushed with a mortar. Samples were added by aquadest 9 ml aseptically, then inserted into the tube and labeled.

The 10^{-1} dilution sample described above was put into 3 ml Tryptone Soya Broth (TSB) for isolation, then incubated for 24-48 hours at 37°C. Bacterial suspension in TSB which has been incubated was scratched into the Mac Conkey Agar (MCA) and then incubated for 18-24 hours at 37°C. Bacterial colony from MCA was taken and stained by Gram staining, followed by biochemical tests. *Proteus mirabilis* ATCC 43 071 was used as positive control. Biochemicaltest included TSIA, SIM, MR / VP, citrate, urea, catalase test, and 4 tests such as glucose, lactose, sucrose,and mannitol. Samples that have a similar biochemical profile to control *Proteus mirabilis* ATCC 43 071 were then processed with PCR.

The primer used in this study was designed to identify the coding DNA sequence of the *Proteus mirabilis* gene as 5-CCGGAAC AGAAGTTGTC GCTGGA -3' for forward and 5-GGCT CTCC TACC GACT TGATC-3' for reverse (PT. Genetic Science Indonesia, Jakarta, Indonesia) with a length of 532 bp.The PCR reaction was conducted under the following condition; denaturation cycle at 95°C for 15 minthen 94 ºC about 1 min, annealing at 63 ° C for 30 sec, extension at 72 ° C for 1 min followed by 40 cycles and final extension at72 ºC for 7 min and 12 ° C ± 30 min for storage. The size of the DNA fragments PCR amplification product *Proteus mirabilis* was compared to the size of the DNA marker (Marker) to know the size ofthe target DNA (bp). Positive result was indicated by the band at 532bp size.

Results and Discussion

Based on the data in Table 1, there were 4 samples from 3 markets exceeded the maximum limit of microbial contamination in chicken meat determined by BSN ($1x10^{-6}$ CFU / g). This is possibly due to the traditional market conditions of selling chicken meat openly and the location of markets that are on the busy roadside, possibly increased the microbial contamination. Based on the profile of bacterial colonies on MacConkey agar, Gram staining and biochemical tests on 24 samples / colorless colonies on MacConkey from 6 traditional markets in the city of Makassar, there are 8 samples (33%) were positively identified with *Proteus sp* (Table 2).

Table 1. Results of Chicken Meat TPC at 6 Makassar City Traditional Market

No	Place of sampling	Number samples Of	Sample Code	TPC CFU/g
1.	Market D	4	D1	$4,6x10^5$
			D2	$4,2x10^5$
			D3	$4,4x10^5$
			D4	$3,3x10^5$
2.	Market S	4	S1	$1,4x10^5$
			S2	$1,7x10^5$
			S3	$1,6x10^5$
			S4	$2,2x10^5$
3.	Market B	4	B1	$2,4x10^5$
			B2	$2,8x10^5$
			B3	$1,4x10^5$
			B4	$1,3x10^5$
4.	Market TL	4	TL1	$11x10^5$
			TL2	$11,2x10^5$
			TL3	$11,4x10^5$
			TL4	$10,8x10^5$
5.	Market PT	4	PT1	$19x10^5$
			PT2	$21x10^5$
			PT3	$18,3x10^5$
			PT4	$22,5x10^5$
6.	Market MC	4	MC1	$18,3x10^5$
			MC2	$22,5x10^5$
			MC3	$20x10^5$
			MC4	$18x10^5$

* Limit of Microbial Contamination (BMCM)> $1x10^{-6}$ CFU / g (BSN. 2009)

Proteus sp such as Proteus mirabilis was colorless on Mac Conkey agar because this bacteria did not ferment the lactose. *Proteus sp* which is a gram-negative bacteria are red (by safranin color given on the last stage) when stained by Gram staining due to its thin peptidoglycan wall, so it is easily removed when washed with alcohol and was not be able to retain the purple color of crystal violet as described in Figure 1 and 2.

The result of biochemical test of *Proteus mirabilis* in TSIA showed a slant pink and butt black appearance due to the ability of this bacteria to ferment glucose and alkaline and produce H_2S. In Urea test, *Proteus mirabilis* has ability to produce urease and this enzyme will break down urea marked color change from yellow to medium pink as in figure 3 and 4. Eight samples (33%) were positively identified with *Proteus sp* then proceed to PCR analysis.

Eight *Proteus sp*-positive samples were confirmed by PCR. The result of PCR revealed only 3 out of 8 samples showed the band at 532bp. The positive control of *Proteus mirabilis* showed band at the same length. Dwiyitno (2010) explained that the PCR technique enables the identification of microorganisms specifically and rapidly in different types of food products/food samples.

Table 2. Results of biochemical tests on 24 samples of six traditional market town of Makassar

No	Sample code	Gram color	TSI A	MRVP		SIM	C	U	G	L	S	M	note
				MR	VP								
	ATCC 43071	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
1.	D1	(-)	(-)	(-)	(-)	(-) Motility (-) H2S +	-	-	-	-	-	-	
	D2	(-)	(-)	(-)	(-)	(-) Motility (-) H2S +	+	+	+	-	-	-	
	D3	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	D4	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
2.	S1	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	S2	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	S3	(-)	-	+	(-)	(-)	+	+	-	-	-	-	
	S4	(-)	-	+	(-)	(-)	+	-	-	-	-	-	
3.	B1	(-)	-	-	(-)	(-)	-	+	-	-	-	-	
	B2	(-)	+	+	(-)	(-)Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	B3	(-)	-	-	(-)	(-)	-	+	-	-	-	-	
	B4	(-)	-	-	(-)	(-)	+	-	-	-	-	-	
4.	TL1	(-)	-	-	(-)	(-)	+	-	+	+	+	+	
	TL2	(-)	-	-	(-)	(-)	+	-	-	-	-	-	
	TL3	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
	TL4	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
5.	PT1	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
	PT2	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
	PT3	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	PT4	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
6.	MC1	(-)	-	-	(-)	(-)	-	-	-	-	-	-	
	MC2	(-)	+	+	(-)	(-) Motility + H2S +	+	+	+	-	-	-	Further tests PCR
	MC3	(-)	+	+	(-)	(-) Motility + H2S +	-	-	+	-	-	-	
	MC4	(-)	-	-	(-)	(-)	-	-	-	-	-	-	

Note:
TSIA = *Triple Sugar Iron Agar*
SIM = *Sulfur Indol Motility*
MR = *Metil Red*
VP = *Voges Proskauer*
G = Glucose
L = Lactose
S = Sucrose
M = Mannitol
C = cytrate
U = Urea

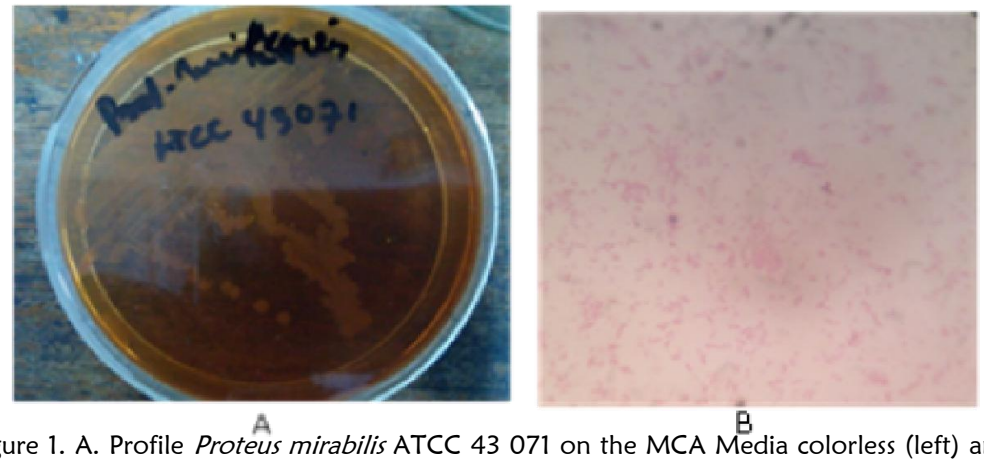

Figure 1. A. Profile *Proteus mirabilis* ATCC 43 071 on the MCA Media colorless (left) and B. Results of gram (-) staining 1000x magnification (right).

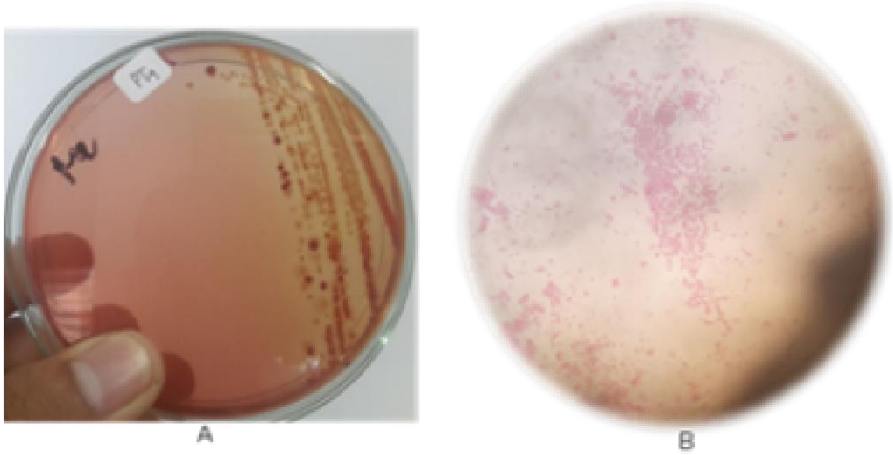

Figure 2. A. Profile colony isolates pt4 the MCA Media colorless (left) and B. Results of gram (-) staining. 1000x magnification (right).

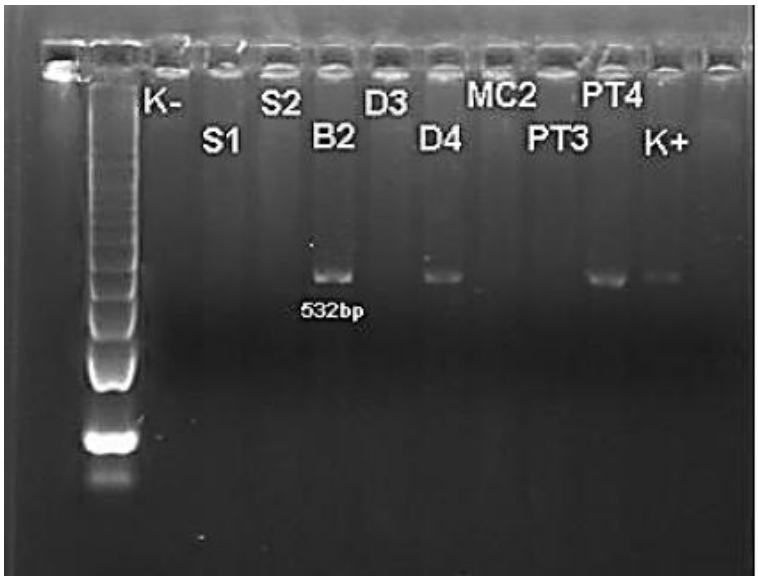

Figure 3. PCR analysis results from 8 samples of the chicken carcass (K- = H2O, K+ = *Proteus mirabilis* ATCC 43 071)

The detected *Proteus mirabilis* contamination of the PCR method suggested that consumers have to be aware of chicken meat sold in traditional markets in Makassar. It is also necessary to improve hygiene and sanitation of the traditional market to prevent the adverse effects of *Proteus mirabilis*.

Conclusion

The results of this present study suggested that *Proteus mirabilis*was found in 3 out of 24 samples of chicken meat. Chicken meat sold in the traditional market of Makassar has already been contaminated with *Proteus mirabilis.*

Acknowledgment

The Authors would like to thank the Scholarship for Directorate General of ScholarshipHigher Education (DIKTI) for Beasiswa Unggulan 2012. The author states there is no conflict of interest with the parties concerned in this research.

References

Amare, A., Amin, AM., Shiferaw, A., Nazir, S., and Negussie, H. 2013. Yolk Sac Infection (Omphalitis) in Kombolcha PoultryFarm,. American-Eurasian Journal of Scientific Research 8 (1): 10-14

BSN [Badan Standar Nasional]. 2009. SNI 7388: Batas Cemaran Mikroorganisme dalam Pangan asal Hewan. Badan Standar Nasional,Jakarta

Dwiyitno. 2010. Identifikasi Bakteri Pathogen Pada Produk Perikanan Dengan Teknik Molekuler. Jurnal Squalen 5(2):67-78

Geneaid Biotech ltd 2014. *GENAIDInstructionManualVer.02.12.14.gSYNCDNAExtractionKit*. www.genaid.com.

Gupta, RK., Ali, S., Shoket, H., Mishra, VK. 2014. PCR-RFLP Differentiation of Multidrug-Resistant Proteussp. Strains from raw beef. Current ResearchinMicrobiologyand Biotechnology 2 (4): 426-430.

Gonzalez, G., Bronze, MS., Boydet,DR .2014. http://emedicine.medscape.com/article/ 226434-overview#showall,diakses 23/11/14.

Muslimin, LW., Jamaluddin, AW., Dwiyanti, R., Hatta, M. 2014. Antimicrobial inhibition on zoonotic bacterial Escherichia coli O157: H7 as a cause of foodborne disease. American Journal of biomedical and life Sciences; 2(6): 163-166.

Noor, SM., Poeloengan. 2006. Pemakaian Antibiotika Pada Ternak dan Dampaknya pada Kesehatan Manusia. Lokakarya Nasional Keamanan Pangan Produk Peternakan.

Sukron, S., 2011. Pengetahuan dan sikap Mahasiswa Fakultas Kedokteran Hewan Institut Pertanian Bogor terhadap Foodborne disease. Fakultas Kedokteran Hewan institut Pertanian Bogor.

Wong, MH., Wan, HY., Chen, S. 2013. Characterization of multidrug-resistant Proteus mirabilis isolated from chicken carcasses. PubMed Feb;10(2):177-81.