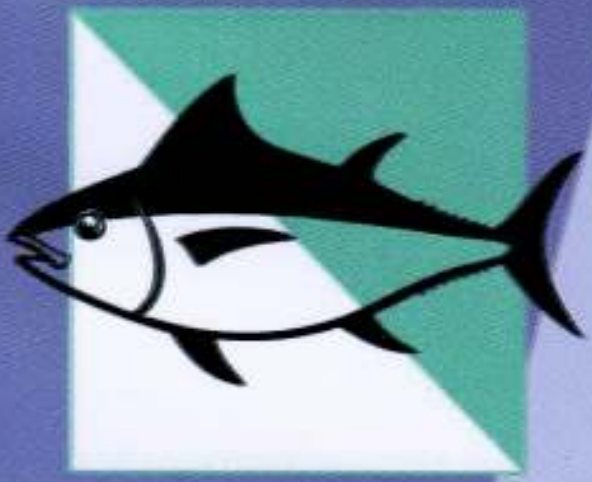
ISSN 2550-1283
VOLUME 1 NUMBER 1 – FEBRUARY 2017

Journal of VETERINARY AND ANIMAL SCIENCES



PUBLISHED BY
INSTITUTE FOR RESEARCH AND COMMUNITY SERVICES,
UDAYANA UNIVERSITY, BALI, INDONESIA

ISSN 2550-1283

JOURNAL OF VETERINARY AND ANIMAL SCIENCE

Published by
Institute for Research and Community Services
Udayana University

Secretariat
Institute for Research and Community Services Udayana University
Bukit Jimbaran, Badung, Bali, Indonesia
Phone: +62-361- 704622 / 703367
Email: jvas@unud.ac.id

ISSN 2550-1283

Journal of Veterinary and Animal Science

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ISSN 2550-1283

Journal of Veterinary Medicine and Animal Science
Vol. 1 Vol. 1, February 2017

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Original Article

Case Report

Antimicrobial Susceptibility Patterns And DNA Plasmid Profiles Of Escherichia Coli O157:H7 Isolated From Feces Of Chicken

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Abstract. Escherichia coli O157:H7 has been identified as one of the most devastating microorganism causing diseases in human. The infection by this bacteria causes a wide spectrum of clinical manifestations ranging from asymptomatic, diarrhea or bloody diarrhea, up to serious clinical conditions such as hemorrhagic colitis, and hemolytic uremic syndrome. Poultry is known as one of its reservoir besides cattle and pig. Moreover, the use of antibiotic in animal feed as growth promoters is common worldwide. Due to its potential threat to public health, sensitivity test against various antibiotics and studying the plasmid DNA profiles are necessary. The study was initiated by cultivation of isolates, followed by test of antimicrobial susceptibility against various antibiotics and then by analyzing the plasmid DNA profiles. Results of study showed that among of 7 E. coli O157:H7 local isolates originated from chicken feces, as many as 42.9%; 14.3%; 14.3%; and 14.3% were resistant to 2; 3; 4; and 5 various of antibiotics, respectively. Base on the type of antibiotic, 85.7%; 71.4%; and 42.9% showed resistance to Methicillin, Penicillin G, Doxycycline and Streptomycin. Plasmid DNA of each isolates showed characters variation too, ranging from 2 to 4 bands with molecular weight ranging from 19.457 bp; 15.137 bp, 5.749 bp, 4.118 bp, 3.500 bp, 2.957 bp and 2.024 bp. Results of study indicated that local isolates of E. coli O157:H7 isolated from chicken showed multiple antibiotic resistance against various antibiotics, as well as its plasmid DNA profiles.

Keywords: **Chicken, Escherichia coli O157:H7, multiple antibiotics resistance, plasmid DNA**

I. INTRODUCTION

In Indonesia, little is known about the information of E.coli O157:H7 as a zoonotic agent, in which human diseases caused by these bacteria that produce STEC ranges from mild diarrhea to hemorrhagic colitis and hemolytic uremic syndrome (HUS). Infection by these bacteria has fatally affects especially to children, the elderly, and immunocompromised patients with highly morbidity and mortality rate [1][2]. Furthermore, poultry is also known as a natural reservoir besides cattle and pig as major reservoirs of these bacteria [3].

On the order side, the use of antimicrobial as growth promoters is common worldwide including in poultry. It has been used intensively and even tend to be in accordance with the dose and the recommended times. These facts have been known contributed to the increasing of bacterial resistance [4][5][6]. The increasing of bacterial resistance to various types of antibiotics is a serious problem that must be addressed as a result of its effects towards public health [5].

Carter and Wise (2004) stated that the genetic elements of bacteria commonly found in chromosome (as a major genetic element), as well as in the plasmid. Plasmid DNA is a genetic element extra chromosomal that is stable and replicate independently. It has molecular weight from 1 to hundreds MDA [7]. Plasmid does not only carry the gene that is essential for metabolic activity, but also carry certain genes such as the gene that encode the ability to exchange genetic and resistant trait to chemicals, which is normally toxic to the bacteria itself [8]. Plasmid has an important role also in transferring genes that encode virulence factors from pathogenic strains to non-pathogenic strains [8]. Moreover, variations in R plasmids can carry multiple antibiotic resistance genes on a single plasmid. Krauss et al. (2003) emphasized that the resistance gene of E.coli O157:H7 is in the form of 90-kb (pO157). This study aimed to evaluate the antimicrobial susceptibility, and characterize DNA plasmids of E. coli O157:H7 strains isolated from poultry.

II. RESEARCH METHOD

Selection and Cultivation of Isolates

A total of 7 locally isolates i.e MK 19/8(4), MK 5/3(5), MK 41, MK 5/10(4), MK 5/3(8), MK 40 and MK35 originated from gastrointestinal of poultry were selected in this study, and E. coli O157:H7 ATCC 43894 was used as a control. All isolates were cultured on selective medium sorbitol MacConkey agar (SMAC) and incubated at 37^{O}C for 24 h. Positive results on SMAC was characterized by colorless colony. Isolates that were positive on SMAC medium were further tested using E. coli O157 latex agglutination test for confirmation. In order to ensure the isolate is E. coli O157:H7, the identification was further tested using H7 serotype test that characterized by the form of precipitation on the bottom of plate.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test for all E.coli O157:H7 isolates were performed by using Kirby Bauer method [9]. One ose of each isolates were grown in 5 ml of nutrient broth medium for 15 minutes while adjusted to the Max Farland turbidity standard No. 1/2 (3 x 108 cfu). Subsequently, the culture were plated on to Mueller Hinton agar with a cotton swab and left for 5 minutes before antibiotic disc was placed on top of it. Five antibiotics disk i.e. methicillin (5mg), penicillin G (10 units), trimethoprim sulfamethoxazole (25mg), doxycyclin hydroxide (30 mcg) and streptomycin (10 mcg) were plated on Mueller Hinton agar with sterile water was used as a negative control, then incubated at 37oC for 18-24 hours. Inhibition zone formed around an antibiotic disc were measured with calipers and then interpreted using the Clinical and Laboratory Standards Institute (CLSI) criteria [10].

Isolation of Plasmid DNA

Plasmid DNA was prepared using Gene Jet Plasmid Miniprep Kit according to the manufacture recommendation. Pellet cell was resuspended in 250 µl of the resuspension solution and completely vortex until no cell clumps remains. Add 250 µl of the lyses solution and mixed thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear. Furthermore, add 350 µl of the neutralization solution and then centrifuge at 12.000 x g for 5 minute. The supernatant was transferred to the spin column and re-centrifuge at 12.000 x g for 1 minute. Discard the flow-through and place the column back into the same collection tube. Add 500 µl of the wash solution, centrifuge 12.000 x g for 1 minute and discard the flow-through. Repeat the wash procedure using 500 µl of the wash solution. Discard the flow-through and centrifuge for additional 1 minute to remove residual wash solution. Transfer spin column into fresh 1.5 ml micro centrifuge tube, and add 50 µl of the elution buffer to elute the plasmid DNA. Incubate for 2 minute at room temperature and centrifuge 12.000 x g for 2 minute. Discard the column and store the purified plasmid DNA at -20oC. These were then used in electrophoresis that were performed in this study.

Electrophoresis of plasmid DNA

A 1% (w/v) agarose gel concentration was used in this study. Crude plasmid DNA extract solutions were subjected to electrophoresis using horizontal apparatus with a constant voltage 100 V for 60 minute. Gel was stained with ethidium bromide 5 µl/ml for 10 minute, and it was observed using UV trans-illuminator and photographed with a Polaroid camera.

Data analysis

The determination of antimicrobial susceptibility by the disk-diffusion method on Mueller-Hinton agar plates was analysis according to Clinical Standard Institute (2014), and plasmid DNA profiles were presented descriptively following comparison with a standard marker.

III. RESULTS AND ANALYSIS

Antimicrobial Susceptibility of the Isolates

The 7 isolates obtained from gastrointestinal tract of chicken were positive E. coli O157:H7 strain after the confirmation test. All isolates showed colorless colony on sorbitol MacConkey agar (SMAC) medium and formed precipitation after tested using latex agglutination test as well as control isolate ATCC 43894 (Fig.1).

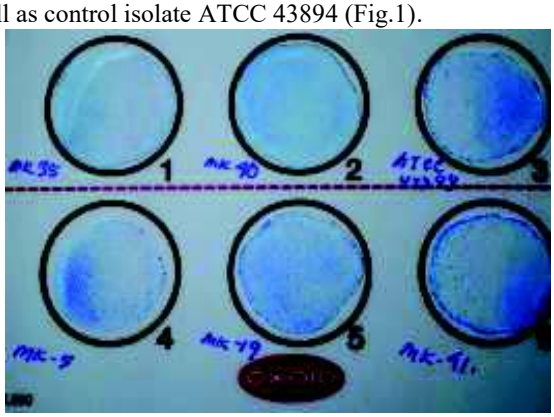

Fig 1. Positive reaction of isolates MK35, MK40, MK5, MK 19 and MK 41 on E. coli O157 latex agglutination test. Arrows indicate the presence of precipitation for MK 19 isolate.

The properties of antimicrobial susceptibility test of isolate MK5/10(4) on Muller Hinton medium was presented in Fig. 2 while the complete results from all isolates including E. coli O157:H7 controls were summarized in Table. 1.

Fig 2. Antimicrobial susceptibility test Escherichia coli O157:H7 on Muller Hinton Agar. 1: Methicillin (5 µg), 2: Penicillin G (10 unit), 3: Sulfamethoxazole Trimethoprim (25 µg), 4: Doxycycline hydrochloride (30 µg), 5: Streptomycin (10 µg), and 6: negative control.

TABLE 1.
INHIBITION ZONE OF THE SEVEN E.COLI O157:H7 ISOLATES FROM GASTROINTESTINAL TRACT OF CHICKEN TOWARDS VARIOUS ANTIBIOTICS.

No	Isolates	Repetition	Inhibition Zone (mm)					
			1	2	3	4	5	6
1.	ATCC25922 (Control)	1	0	8,5	21,5	16,0	12,6	0
		2	0	12,2	22,2	10,8	12,2	0
		Average	0	10,35	21,85	13,4	12,4	0
		Category	R	R	S	I	I	
2.	MK 19/8(4)	1	0	16,2	0	6,3	10,0	0
		2	0	17,1	0	6,0	13,3	0
		Average	0	16,65	0	6,15	11,65	0
		Category	R	S	R	R	I	
3.	MK 5/3(5)	1	0	6,0	19,1	14,1	12,2	0
		2	0	0	20,0	14,0	12,1	0
		Average	0	3,0	19,55	14,05	12,15	0
		Category	R	R	S	S	I	
4.	MK41	1	0	0	20,9	8,8	9,2	0
		2	0	0	22,7	9,8	12,5	0
		Average	0	0	21,8	9,3	10,85	0
		Category	R	R	S	R	R	
5.	MK5/10(4)	1	0	6,0	24,3	16,6	12,9	0
		2	0	0	24,5	16,5	13,0	0
		Average	0	3,0	24,4	16,55	12,95	0
		Category	R	R	S	S	I	
6.	MK5/3(8)	1	0	6,0	22,6	16,6	12,2	0
		2	0	8,5	22,0	14,4	11,7	0
		Average	0	7,25	22,30	15,5	11,95	0
		Category	R	R	S	S	I	
7.	MK40	1	13,1	21,7	23,4	14,2	0	0
		2	14,0	22,1	26,0	14,8	0	0
		Average	13,55	21,9	24,7	14,5	0	0
		Category	I	S	S	S	R	
8.	MK35	1	0	0	0	8,5	7,2	0
		2	0	0	0	0	7,0	0
		Average	0	0	0	4,25	7,10	0
		Category	R	R	R	R	R	

Annotation: R: resistant, S: sensitive, I: intermediate, 1: methicillin (5 µg), 2: penicillin G (10 unit), 3: trimethoprim sulfamethoxazole (25 µg), 4: doxycycline hydrochloride (30 µg), 5: streptomycin (10 µg), and 6: negative control

Table 1 showed that most of the E. coli O157:H7 locally isolates were resistant to more than one antimicrobials. Three out of 7 isolates (42.9%) were resistant to methicillin and penicillin G; one out of 7 (14.3%) isolate was resistant to methicillin, trimethoprim sulfamethoxazole , and doxycycline hydroxide, one out of 7 (14.3%) isolate was resistant against methicillin, penicillin G, doxycycline hydroxide, and streptomycin, and one out of 7 (14.3%) isolate showed resistance to all of the antimicrobials tested. Identification by the type of antimicrobials, 6 out of the 7 (85.7%) locally isolates were resistant to methicillin, 5 out of 7 (71.4%) isolates showed resistance to penicillin G, 2 out of 7 (28,6%) isolates resistance to trimethoprim sulfamethoxazole, 3 out of 7 (42.9%) isolates resistance to doxycycline hydroxide and streptomycin, respectively.

These results proofed the findings of Gallard et al, (2001), which revealed the multiple drug resistance of E. coli O157:H7 [11]. Brander et al. (1991) explained the existence of multiple drug resistance as a result of the use of drugs, especially antibiotics through food, beverages and intensively parenteral route although in accordance with the recommended dose and time. These facts would have some impact on the increase of bacterial resistance.

The use of antibiotics as feed additives (feed supplement) in poultry (chicken) is a fundamental problem that must be addressed in a sustainable manner, as it will have an impact on the occurrence of multiple drug resistance. Furthermore, Tabbu (2000) revealed that at the chicken farms in Indonesia, farmers often use antibiotics of the amino group of glycosides, peptides, aminocyclitol and tetracycline for controlling colibacillosis [12]. The use of these antibiotics are usually not controlled, so this conditions too contributed to the increase in multiple drug resistance in poultry.

Plasmid DNA profile

Plasmid DNA profiles of the 7 isolates of E.coli O157:H7 local isolates which were identified resistance to various antibiotic is shown in Fig. 3 and its representative properties was presented in Fig.4.

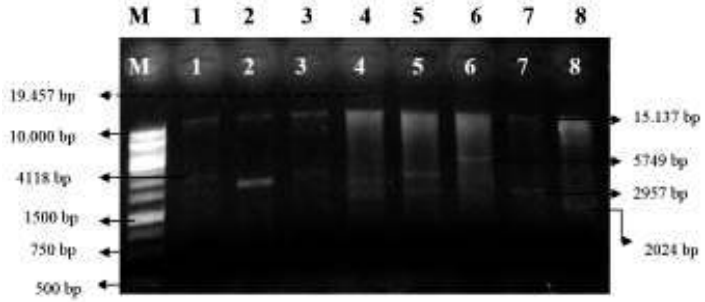

Fig 3. Plasmid DNA profiles of the 7 local isolates of E.coli O157:H7 and one control isolate on Agarose 1%. Line 1: positive control ATCC 43894, line 2: MK19/8(4), line 3: MK5/3(5), line 4: MK41; line 5: MK5/10(4), line 6: MK5/3(8), line 7: MK40, and line 8: MK35. M: Marker 1Kb DNA Ladder (Microzone Ltd).

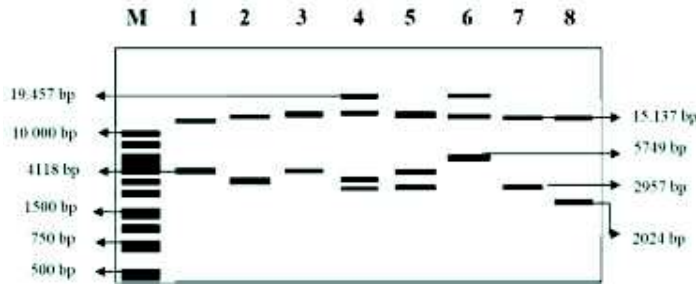

Fig. 4: Representative graph of plasmid DNA profiles of the 7 local isolates of E.coli O157:H7 and one control isolate on Agarose 1%. Line 1: positive control ATCC 43894, line 2: MK19/8(4), line 3: MK5/3(5), line 4: MK41; line 5: MK5/10(4), line 6: MK5/3(8), line 7: MK40, and line 8: MK35. M: Marker 1Kb DNA Ladder (Microzone Ltd).

Figures 3 and 4 showed the variations in the plasmid DNA profiles. Control isolate ATCC 43894 showed 2 variation bands 15.137 bp and 4118 bp, as well as MK5/3 (5). Moreover, MK19/8 (4) showed more variation of the plasmid DNA with 15137 bp and 3500 bp products. MK41 showed four different bands, namely 19.457 bp, 15.137 bp, 3500 bp and 2957 bp. MK5 /10 (4) showed three bands, namely 15 137 bp, 4118 bp and 2957 bp. Isolates MK5 / 3 (8) showed three variations of the band namely 19.457 bp, 15.137 bp and 5749 bp. Furthermore, MK40 and MK35 showed variety band in 15.137 bp and 2957 bp. The order isolate i.e. MK40 showed band in position 15.137 bp and 2024 bp for isolate MK35.

The variety of plasmid DNA band of each isolates certainly can be used as a primary opinion for further investigation in order to gain detail variation in plasmid DNA of the isolates. This opinion referred to Carter and Wise (2004) whom stated that, plasmid does not carry the gene that is essential for metabolic activity, but its carry certain genes such as the gene which encode the ability to reproduce genetic exchange and the resistance gene to chemicals, which is normally toxic to the bacteria itself.

It is believe that plasmid has an important role because it can transfer the genes that encode virulence properties of pathogenic strains to non-pathogenic strains. Furthermore, variations on the R plasmid can carry multiple antibiotic resistance genes on a single plasmid. Especially for E. coli O157, Krauss et al. (2003) mentioned that the plasmid of E. coli O157: H7 is known to have the resistance gene in the form of 90-kb (pO157) [13]. This fact is supported by previous studies by Makino et al. (1998) who has completed sequencing of the 93-kb plasmid known as pO157. This plasmid often found in clinical EHEC cases worldwide. Besides, it was also found 3.3 kb plasmid which is known as pOSAK1. This plasmid often found in cases of EHEC isolated in Japan. Makino study found that pO157 and pOSAK1 has the same gene plasmid DNA sequences for the F and R 100 factors that are known to have an important role in transferring drug resistance during DNA replication [14].

IV. CONCLUSION

The study showed that most of local strains of E. coli O157:H7 were resistant to various antibiotics, and had a tendency to be multiple drug resistance. The study also showed that almost all isolates were resistant to antibiotics commonly used in agriculture i.e. methicillin, penicillin G, and doxycycline hydrochloride and streptomycin. These results also in line with the profile of its plasmid DNA.

ACKNOWLEDGMENT

The authors would like to thank Prof. Dr. Supar, MS for his kindness to provide control isolates of E. coli O157:H7 ATCC 43894, and also wish to thank Udayana University Institute for Research and Community Service for supporting this project through Basic Research Grant contract No: 0161/023-04.2/XX/2010 date on December, 31st 2009.

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