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Antibiotic Resistance Profiles of *Escherichia coli* O157:H7 in Cattle at South-Kuta, Badung Regency, Bali, Indonesia

[1]Oktivia Chandra Mustika, [2]Komang Januartha Putra Pinatih and [3]I Wayan Suardana

[1]Student of Faculty of Veterinary Medicine, Udayana University,
J.L. PB.Sudirman-Denpasar, Bali 80232, Indonesia
[2]Department of Microbiology, Faculty of Medicine, Udayana University,
Jl, PB, Sudirman-Denpasar, Bali 80232, Indonesia
[3]Department of Veterinary Public Health, Faculty of Veterinary Medicine, Udayana University,
Jl. PB. Sudirman Denpasar-Denpasar, Bali-80232,Tlp. (0361) 223791, 701808, Indonesia

Abstract: *Escherichia coli* O157:H7 is one of bacterium that known as a zoonotic agent. The spread of bacterium is usually through food contaminations and cattles are known as the main resevoir of this agent. The infections of *E. coli* O157:H7 can cause mild diarrhea to bloody diarrhea in young cattle and in adult are usually as a carriers. The humans infections by this agent can cause *hemorragic colitis* and *hemolytic uremic* syndrome (HUS). This study used five local isolates of *E. coli* O157:H7 *i.e*. FS.KS-5, FS.KS-17, FS.KS-36, FS.KS-44 and FS.KS-55 resulted from 60 faecal samples. The study was conducted by cultivating of isolate in sorbitol macConkey agar (SMAC), followed by testing on O157 latex aglutination and finally by testing on H7 antiserum. The sensitivity test of isolate againts various antibiotics was conducted using Kirby-Bauer methode. The isolates were tested on penicillin G, ampicillin, sulfamethoxazole and steptomycin. The results of study indicated all of isolates were resistant to penicillin G (100%). Moreover, sensitivity test to the ampicillin showed 80% resistant and 20% sensitive. Sensitivity test to the sulfamethoxazole showed 20% resistant, 40% intermediate and 40 % sensitive, as well as to the antibiotic streptomycin were 20% resistant, 20% intermediate and 60% sensitive. The results also showed three out of five isolates of *E. coli* O157:H7 (60%) namely FS.KS-17, FS.KS-44 and FS.KS-55 were resistant against two types of antibiotics (penicillin G and ampicillin). On the other hand, FS.KS-5 has shown resistant to four antibiotics. Therefore, it is necessary more studies in the future in order to track the evolution of this type of resistance among isolates.

Key words: Antibiotics · Cattle · *Escherichia coli* O157:H7 · Sensitivity Test · South Kuta

INTRODUCTION

Escherichia coli commonly presents in both human and animal digestive tracts as a normal flora, but one of their strains *i.e E. coli* O157:H7 is known as a virulent strain in humans. Cattles and sheep are known as the main resevoir of these agent [1, 2]. Some kinds of food can act as the transmission of *E. coli* O157:H7 infection. The infection is generally caused by the consumptions of vegetables, beef and milk. *E. coli* O157:H7 can cause mild diarrhea to bloody diarrhea in young cattles, but in adult cattles are usually as carriers [2, 3]. The humans infections by this agent can cause hemorragic colitis and hemolytic uremic syndrome (HUS) [4].

The antibiotics that generally used to treat the VTEC infections are ampicillin, karbenisillin, sepalotin, chloramphenicol, gentamicin, kanamycin, nalidix acid, norfloxacin, tetracycline, ticarcillin, tobramycin, trimethoprim and sulfamethoxazole [5, 6]. According to the previously survey that conducted by researcher, the author found the group of penicillin (ampicillin), aminoglycosides (gentamicin) and macrolides (erythromycin) were often used to treat the bacterial infections of cattles in South Kuta District.

Corresponding Author: I Wayan Suardana, Department of Veterinary Public Health, Faculty of Veterinary Medicine, Udayana University, Jl. PB. Sudirman Denpasar, Bali 80232, Indonesia.
Tel: (+62 361) 223791, 701808.

Based on the problem above, the study of the sensitivity test of *E. coli* O157:H7 isolated from feses of Bali cattles in South-Kuta District againts antibiotics penicillin G, ampicillin, sulfamethoxazole and streptomycin are needed to be revealed.

MATERIALS AND METHODS

Isolates Included in this Study: This study used five local isolates of *E. coli* O157:H7 resulted from isolation of 60 faecal samples namely FS.KS-5, FS.KS-17, FS.KS-36, FS.KS-44 and FS.KS-55 which were isolated from South Kuta District, Badung regency, provincee of Bali.

Cultivation of Isolates: Positive isolates of *Escherichia coli* O157:H7 isolated from feses of Bali cattles in South Kuta District were cultivated in sorbitol macConkey agar (SMAC) to determine whether the isolates were indeed to strain of *E. coli* O157. The suspected colonies of *E. coli* O157 are characterized by the form rounded colonies with varying sizes, colourless or negative sorbitol [7, 8]

The positive isolates were re-confirmed using *E. coli* O157 latex aglutination test (Oxoid DR620 M) by reacting a loop of suspected colonies of *E. coli* O157 from SMAC medium with a drop of O157 latex and a drop of aquades before homogenized. Positive reaction is characterized by the formation of precipitation within one minute of observation [9].

Flagella H7 test were conducted by using H7 antisera (Difco™*E. coli* Antisera). Isolates were firstly cultivated in motility medium for twice. Positive result was characterized by the spread of it's growth in the pucture. Furthermore, isolates were cultivated in five ml of the brain heart infusion (BHI) medium and they were inactivated by formalin 40% as many as 0,3 ml formalin in 100 ml of BHI, afterward it was refered as an antigen. Continuosly, the antigens tested with H7 antisera that which was diluted using a ratio of 1: 500. Serological test was performed by reacting of 50 ml antigen with 50 ml of H7 antisera before incubation into a waterbath at 50°C for 24 hours. Positive results were characterized by the formation of precipitation on the base plate [10].

Antimicrobial Susceptibility Test: The susceptibility test of *E.coli* O157:H7 against various antibiotics was done using disk diffusion method according to Kirby-Bauer. Antimicrobial susceptibility test following disk diffusion method mentioned by National Committee for Clinical Laboratory Standard (NCCLS) [11] was applied to determine the susceptibility of bacteria against Penicillin G 10 unit, Ampicillin 10 µg, Sulfamethoxazol 25 µg, Streptomcin 10 µg and aquades steril as a negative control. The fresh bacterial culture 48 hour old were inoculated in 0,85% NaCl suspension to the level of turbidity equivalent to 0,5 McFarland standards (equivalent to 1 x 10^8 to 4 x 10^8 CFU/ml) and sided with sterile cotton swab onto a Mueller-Hinton agar (Oxoid). Antibiotic discs were applied after dying the paltes for 3-5 minute and the plate were incubated in microaerophilic atmosphere at 42°C for 48 hours. The zone of inhibition around the disc were measured using digital caliper. The size of the inhibition zone was compared with the disc manufacture's standard and classified as sensitive (S), intermediate (I) or resistant (R) to the given drug [12]

RESULTS

Cultivation of *Escherichia Coli* O157:H7: The cultivation five isolates of *E. coli* O157:H7, namely FS.KS-5, FS.KS-17, FS.KS-36, FS.KS-44 and FS.KS-55, showed that five isolates were confirmed as isolates of *E. coli* O157:H7. The test on the sorbitol MacConkey agar (SMAC) test showed clear/colourless colonies or negative sorbitol (not fermented sorbitol). Furthermore, the confirmation test using the *E. coli* O157 latex test showed the agglutination as well as in the test using H7 antisera. Therefore both tests have shown positive results. The results were appropriate with the characteristics of *E. coli* O157:H7 as well as previously study [9, 10].

The susceptibility test of *Escherichia coli* O157:H7 against antibiotics Penicillin G, ampicillin, Sulfamethoxazole and Streptomycin.

The susceptibility test of five isolates of *E. coli* O157:H7 against antibiotic penicillin G, ampicillin, sulfamethoxazole and streptomycin was presented some varied patterns of sensitivity. The killing zone of of *E. coli* O157:H7 to various antibiotics on the sensitivity test was shown in Figure 1 and the results were presented in Table 1.

Exp: R: Resistance; S: Sensitive; I: Intermediete; 1: Penicilin G 10 Unit; 2:Ampicilin 10 µg; 3: Sulfamethoxazol 25 µg; 4: Streptomycin 10 µg; 5: Negative Control.

The data on Table 1 shows all of *E. coli* O157:H7 were resistant to the penicillin G (100%). Moreover, the susceptibility test to the ampicillin showed 80% of isolates were resistant and 20% sensitive.

Table 1: The results of sensitivity test of *E. coli* O157: H7 isolates against various antibiotics

No	Isolates	Repetition	Killing zone diameter (mm)				
			1	2	3	4	5
1	FS.KS. 5	1	10	14	0	11	0
		2	0	7	0	10	0
		3	0	0	0	10	0
		Mean	3,3	7	0	10,3	0
		Exp.	R	R	R	R	
2	FS.KS. 17	1	0	0	30	20	0
		2	0	0	30	20	0
		3	0	0	31	19	0
		Mean	0	0	30,3	19,6	0
		Exp.	R	R	S	S	
3	FS.KS. 36	1	8	18	24	15	0
		2	0	11	0	20	0
		3	0	18	24	15	0
		Mean	2,6	15,6	16	16,6	0
		Exp.	R	S	S	S	
4	FS.KS. 44	1	0	0	10	15	0
		2	0	0	15	15	0
		3	0	0	10	14	0
		Mean	0	0	11,6	14,6	0
		Exp.	R	R	I	I	
5	FS.KS. 55	1	0	10	15	20	0
		2	0	10	15	20	0
		3	0	9	15	19	0
		Mean	0	9,6	15	19,6	0
		Exp.	R	R	I	S	

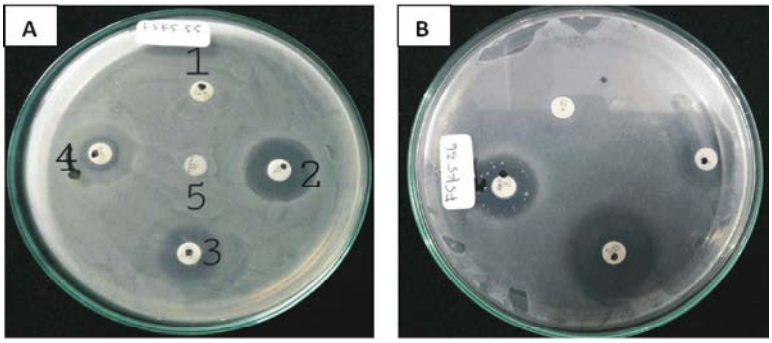

Fig. 1: The sensitivity test of *E. coli* O157:H7 local isolates on Mueller Hinton agar. The figure showed a clear zone around paper disc of each antibiotic. 1: penicilin G 10 unit; 2: streptomycin 10 µg; 3: sulfamethoxazol 25 µg; 4: ampicilin 10 µg; 5: negative control. A: isolates FS.KS-55; B: FS.KS-36.

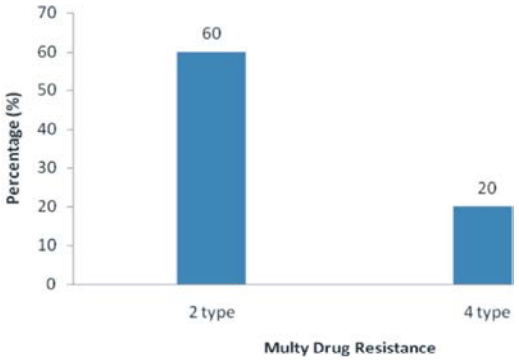

Fig. 2: Diagram of multiple drug resistance of *E. coli* O157:H7 against various antibiotics

The susceptibility test to the sulfamethoxazole showed 20% resistant, 40% intermediate and 40 % sensitive, as well as to the antibiotic streptomycin showed 20% resistant, 20% intermediate and 60% sensitive. Furthermore, the study also showed multiple drug resistance of some isolates which presented in Figure 2.

The data at Figure 2 showed three out of five isolates of *E. coli* O157:H7 (60%) were resistant against two types of antibiotics (penicillin G and ampicillin) namely FS.KS-17, FS.KS-44 and FS.KS-55. On the other hand, FS.KS-5 has shown resistant to four antibiotics. The study indicated there was multiple drug resistance of isolates *E. coli* O157:H7 to antibiotics.

DISCUSSION

The highly resistance of *E. coli* O157:H7 isolates against various antibiotics at South Kuta (Table 1) and the finding of multiple drug resistance (Figure 2) is in accordance with the opinion of some researchers previously. The use of antibiotics are not only used in veterinary indication for therapy and prevention of bacterial infections, but may also be added continuously to animal feeds to promote growth, increase feed efficacy and decrease waste production. The antibiotics used for this purpose are commonly called feed savers, antimicrobial growth promoters or performance enhancers [13].

The use of antimicrobials in medicine (clinical and veterinary), coupled with their application in initial husbandry (often at a sub therapeutic levels), is regarded as a potential driving force for the selection of antimicrobial resistant bacteria [14, 15].

The highly resistance of *E. coli* O157:H7 at South-Kuta was in line to previously research in Spain. A total of 722 Shiga toxin-producing *Escherichia coli* (STEC) isolates recover from humans, cattle, ovine and food during the period from 1992 to 1999 in Spain were examined to determine antimicrobial resistance profiles. Fifty-eight (41%) out of 141 STEC O157:H7 strains showed resistance to at least one of the 26 antimicrobial agents tested. STEC O157:H7 showed a higher percentage of resistant strains recovered from bovine (53%) and beef meat (57%) than from human (23%) and ovine (20%) sources. Sulfisoxazole (36%) had the most common antimicrobial resistance, followed by tetracycline (32%), streptomycin (29%), ampicillin (10%), trimethoprim (8%), cotrimoxazole (8%), chloramphenicol (7%), kanamycin (7%), piperacillin (6%) and neomycin (5%). The multiple resistance pattern most often observed was that of streptomycin, sulfisoxazole and tetracycline [16]. The other research also found the high prevalence and widespread distribution of multi-resistant *E. coli* O157:H7. The research in Nigeria found all of *E. coli* O157:H7 isolates were resistant to one or multiple antibiotics. Tetracycline resistance was the highest in 91.4% of the isolates, while 72.9% resistance to nitrofurantoin and chloramphenicol, 65.7% to cefuroxime, 44.3% resistance to cotrimozole, 35.7% resistance to naladixic acid and 11.4% resistance to gentamicin [17].

Pennicillin G and ampicillin resistance of *E. coli* O157:H7 were the highest found in South-Kuta because they were more commonly available for use as growth promotion and routine chemoprophylaxis among livestock especially for Bali cattle. Penicillin G and ampicillin are beta-lactam antibiotics, where *E. coli* O157 is known as Gram-negative bacteria which has betalatamase enzyme. It is an enzyme that can inactivate beta-lactam antibiotics.

CONCLUSION AND RECOMENDATIONS

Susceptibility test of *E. coli* O157:H7 isolated from feces of Bali cattle in South-Kuta District indicated all isolates were resistant to penicillin G (100%) and most of them also indicated resistant to ampiclilin. Furthermore, the results of study showed three out of five isolates (60%) were resistant against two types of antibiotics (penicillin G and ampicillin) and one isolate was resistant against four antibiotics. More studies should be carried out in the future in order to track the evolution of this type of resistance.

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REFERENCES

1. Blanco, J., M. Blanco, J.E. Blanco, A. Mora, E.A. Gonzalez, M.I. Bernardez, M.P. Alonso, A. Coira, A. Rodriguez, J. Rey *et al*., 2003. Verotoxin-producing in Spain: prevalence, serotypes and virulence genes of O157:H7 and non-O157 VTEC in ruminants, raw beef products and humans. Exp Biol Med (Maywood), 228(4): 345-351.
2. Rey, J., S. Sanchez, J.E. Blanco, J. Hermoso de Mendoza, M. Hermoso de Mendoza, A Garcia., C. Gil, N. Tejero, R. Rubio and J.M. Alonso, 2006. Prevalence, serotypes and virulence genes of Shiga toxin-producing *Escherichia coli* isolated from ovine and caprine milk and other dairy products in Spain. Int J Food Microbiol, 107(2): 212-217.
3. Heuvelink A.E., J.T. Zwartkruis-Nahuis, R.R. Beumer and E. de Boer, 1999. Occurrence and survival of verocytotoxin-producing *Escherichia coli* O157 in meats obtained from retail outlets in The Netherlands. J Food Prot, 62(10): 1115-1122.
4. Nataro, J.P. and J.B. Kaper, 1998. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev, 11(1): 142-201.

5. McKee, R., R.H. Madden and A. Gilmour 2003. Occurrence of verocytotoxin-producing *Escherichia coli* in dairy and meat processing environments. J Food Prot, 66(9): 1576-1580.
6. Beier, R.C., T.L. Poole, D.M. Brichta-Harhay, R.C. Anderson, K.M. Bischoff, C.A. Hernandez, J.L. Bono, T.M. Arthur, T.G. Nagaraja, T.L. Crippen *et al*., 2013. Disinfectant and antibiotic susceptibility profiles of *Escherichia coli* O157:H7 strains from cattle carcasses, feces and hides and ground beef from the United States. J. Food Prot., 76(1): 6-17.
7. Suardana, I.W., B. Sumiarto and D.W. Lukman, 2007. Isolation and identification of *Escherichia coli* O157:H7 on beef at Badung Regency, Province of Bali. J. Vet., 8(1): 16-23.
8. March, S.B. and S. Ratnam, 1986. Sorbitol-MacConkey medium for detection of *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol., 23(5): 869-872.
9. March, S.B. and S. Ratnam, 1989. Latex agglutination test for detection of *Escherichia coli* serotype O157. J. Clin. Microbiol., 27(7): 1675-1677.
10. Farmer J.J. and B.R. Davis, 1985. H7 antiserum-sorbitol fermentation medium: a single tube screening medium for detecting *Escherichia coli* O157:H7 associated with hemorrhagic colitis. J. Clin. Microbiol., 22(4): 620-625.
11. National Committee for Clinical Laboratory Standards.: NCCLS document. In. Villanova, PA: National Committee for Clinical Laboratory Standards: v.
12. Nigatu, S., A. Mequanent, R. Tesfaye and L. Garedew, 2015. Prevalence and Drug Sensitivity Pattern of Campylobacter jejuni Isolated from Cattle and Poultry in and Around Gondar Town, Ethiopia. Global Veterinaria, 14(1): 43-47.
13. Van Den Bogaard, A.E. and E.E. Stobberingh, 2000. Epidemiology of resistance to antibiotics. Links between animals and humans. Int. J. Antimicrob. Agents, 14(4): 327-335.
14. Walsh, C., G. Duffy, R. O'Mahony, S. Fanning, I.S. Blair and D.A. McDowell, 2006. Antimicrobial resistance in Irish isolates of verocytotoxigenic *Escherichia coli* (*E. coli*)--VTEC. Int. J. Food Microbiol., 109(3): 173-178.
15. Walsh, C. and S. Fanning, 2008. Antimicrobial resistance in foodborne pathogens--a cause for concern. Curr. Drug Targets, 9(9): 808-815.
16. Mora, A., J.E. Blanco, M. Blanco, M.P. Alonso, G. Dhabi, A. Echeita, E.A. Gonzalez, M.I. Bernardez and J. Blanco, 2005. Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbiol., 156(7): 793-806.
17. Olatoye and I. Olufemi, 2010. The Incidence and Antibiotics Susceptibility of *Escherichia coli* O157:H7 from Beef in Ibadan Municipal, Nigeria. African Journal of Biotechnology, 9(8): 1196-1199.