American Journal of Biomedical and Life Sciences
2014; 2(6): 163-166
Published online January 07, 2015 (http://www.sciencepublishinggroup.com/j/ajbls)
doi: 10.11648/j.ajbls.20140206.15
ISSN: 2330-8818 (Print); ISSN: 2330-880X (Online)

Antimicrobial inhibition on zoonotic bacterial *Escherichia coli* O157: H7 as a cause of food borne disease

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To cite this article:
Lucia Winata Muslimin, Abdul Wahid Jamaluddin, Ressy Dwiyanti, Mochammad Hatta. Antimicrobial Inhibition on Zoonotic Bacterial *Escherichia coli* O157: H7 as a Cause of Food Borne Disease. *American Journal of Biomedical and Life Sciences.* Vol. 2, No. 6, 2014, pp.163-166. doi: 10.11648/j.ajbls.20140206.15

Abstract: This study aims to accelerate zoonosis control system, secure food safety and improve the environmental quality. Meat samples, swab and water were acquired from five regions in South Sulawesi, Indonesia. The samples were implanted to bacterial growth medium inside the ice-box and carried to the lab to be inoculated with Nutrient Agar, Eosin Methylen Blue Agar Sorbitol Mac Conkey Agar (SMAC). IMVIC test, Biochemical Test and pathogenic test with blood Agar from the suspected *Escherichia coli* O157H7 result, was followed by PCR test to genetically identify the bacteria. The result was then examined for sensitivity test with antibiotics: Imipenem, Tetracycline, Erytromycin, Levofloxacine, Amoxycilin, Chloramphenicol and Ciprofloxacine. Among 117 samples. 43 was assumed positive with culture method, 12 was assumed positive with PCR *E.coli* O157H7.The most sensitive antibiotics, Imipenem, Ciprofloxacine and levofloxacine.

Keywords: Antimicrobial Inhibition, Food Borne Disease, *E.coli*

1. Introduction

Zoonosis is an infectious disease which can be transmitted from vertebrae to human. One of the causal agent of zoonosis and also pathogenic is *Escherichia coli* O 157H7, which recently known as an emerging zoonosis. In 2013, the isolated agent from infected children and those from animal is found to be the same agent. *E.coli*O157:H7 infection that is pathogenic to human is those that cause enterohemoraghic to food borne diseases. In many developing countries, proper hygiene is not strictly implemented when animals are slaughtered and meat products become contaminated. Contaminated meat may contain Escherichia coli (E.coli) O157:H7 that could cause diseases in humans if these food products are consumed undercooked.[1].

This research aims to accelerate zoonosis control system, secure food safety and improve the environmental quality. In 2012, author had conducted examination to fresh vegetable in Makassar which resulted in positive *Escherichia coli* contamination[2].

2. Materials and Methods

2.1. Samples Collection

Samples were collected aseptically in sterilised glass bottles and plastic bags with icepack. All samples were immediately transported to the Molecular Biology and Immunology Laboratory for Infectious Diseases, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia for analysis. Upon arrival in the Laboratory, samples were analysed immediately. Meat samples, swab and water were acquired from five regions in South Sulawesi, Indonesia i.e. Makassar,, Palopo, Maros, Sinjai and Bone sub district, from which we conducted three type of assessment by culture method Test, Polymerase Chain Reaction (PCR) and Antimicrobial Susceptibility Test

The samples from traditional markets in five regions of South Sulawesi (Makassar, Maros, Bone, Palopo and Sinjai)were acquired with cotton buds sterilized along with 100 ml water sample of water, and then sealed with ice packs

and carried inside an ice box to the laboratory for future examination(or testing).

2.2. Conventional Bacterial Culture

The samples were implanted to bacterial growth medium inside the ice-box and carried to the lab to be inoculated with: (a) *Nutrient Agar* to measure the amount of bacteria; (b)Eosin Methylen Blue Agarto identify colonies with metallic sheen colors; and (c) Sorbitol Mac Conkey Agar (SMAC)to identifycolonythat does not fermentaze lactose and colorless colony. In addition, we conduct IMVIC test to assess the Indole, Methyl Red, Voges Proskauer, TSIA and Cirate, Biochemical Test, glucose, Lactose, Sucrose, Maltose, sorbitol and pathogenic test with blood Agar, where the samples were inoculated at 37^{o} C for twenty-four hours[3].

The identification process were conducted in two ways: macroscopically by observing the growing colony morphology along with the forming hemolysis zone, and microscopically by observing the bacteria's shape using Gram colouring to highlight the red coccoid.

The growing metallic colony from the EMBA medium were streaked into the Blood agar medium and incubated for twenty-four hours at 37^{o}C, as well. The *E.coli*O157H7's presence were indicated by the growing blurry hemolysis zone around the colony (gamma hemolysis). This growing colorless colony were also incubated on the SMAC.

2.3. Polymerase Chain Reaction (PCR)

Suspected *Escherichia coli* O157H7 result, was followed by PCR test to genetically identify the bacteriausing method protocol sample preparation for PCR DNA according to previously procedure[4]. Protocol PCR with primers *E. coli* O157: H7*rfbE* gene(AE005429) is Forward: GCGCGAATTCGTGCTTTTGATATTTTTCCGAGTACATT GG and Reverse: CGCGAATTCTTTATATCACGAAAACGTGAAATTGCTG AT with concentration of 0.5uM of each, 5 ul DNA template and 47 ul of distilled water (Ultrapure, Invitrogen Co, Japan) were added to a 0.2 micro centrifuge tube containing AmpliTaq Gold. Conditions forthermocycling were as follow: 95°C for 10 minutes, 40 cycles of amplification (94°C for 30 seconds followed by 60°C for 40 seconds and 72°C for 40 seconds) and 72°C for 10 minutes. Using 1.8% agarose gel containing ethidium bromide (Sigma, USA), 5 ul of PCR product were analyzed by electrophoresis at 100 V for 30 minutes.PCR Product length for *rfbE* geneis 239 base pairs (bp) for *E.coli*[5].

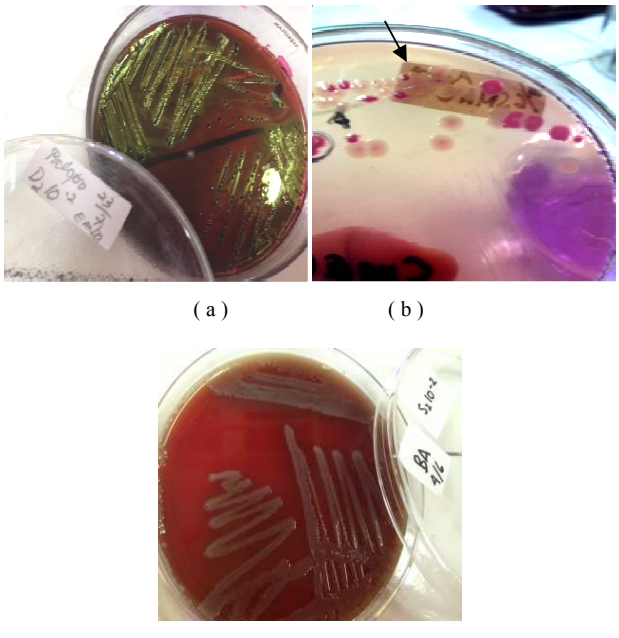
2.4. Antibiotic Susceptibility Test

Antibiotic susceptibility tests were performed on all isolates to determine their antibiotic-resistance profiles. Fresh overnight cultures were prepared and used for antibiotic sensitivity tests. An aliquot (100µL) of each isolate suspension equivalent to a 0.5% McFarland Standard was spread plated on Mueller Hinton agar (Oxoid, UK). Susceptibilities of the isolates to a panel of several different antibiotic discs (Oxoid, UK) were determined. Antibiotic discs were gently pressed onto the inoculated Mueller Hinton agar to ensure intimate contact with the surface and the plates were incubated aerobically at 37 °C for 18 h – 24 h. Inhibition zone diameters were measured and values obtained from the National Committee on Clinical Laboratory Standards were used to interpret the results obtained[6]. Bacteria isolates were then classified as resistant, intermediate resistant or susceptible to a particular antibiotic Imipenem, Tetracycline, Erytromycin, Levofloxacine, Amoxycilin, Chloramphenicol and Ciprofloxacine.

The sensitivity indication wasspecified by the forming translucent zone around the paper disc where its diameter was measured based on the disc productstandard.

3. Results

Among 117 samples. 43 was assumed positive with culture method,



(a) (b)

(c)

Figure 1. *Culture result of E coli.*

As can be seen from figure 1, the metalic colony shows different *E.Coli* characteristics .The metallic colony on the EMBA (a) were Gram colored, where the coccoidal bacteria were found negative *E.coli* O157 H7. The suspected *E.coli* colony were followed by inoculation on Sorbitol Mac Conkey agar medium (SMAC) (b), which resulted in colorless colony on Blood Agar medium, the colony was not blurry or has Y (gamma) hemolysis characteristic . Pathogenic test of *E.coli* O157:H7 with blood agar shows Y hemolysis (c).

On Triple Sugar Iron Agar (TSIA) medium, the sample was showing acid-acid, on urea, citrate, Voges-Proskauer test negative .On Methyl Red, Glucose, Lactose, Sucrose, Maltose shows positive result. One of the *E.coli* O157 H7 characteristic is sorbitol negative .The positive culture was

followed by PCR test to genetically identify the bacteria.

The PCR results revealed that 12 samples ~~was~~ were assumed positive with DNA specific for *E.coli* O157:H7 (with positive control on *E.coli* ATCC 35150), in which 9.75 % positive of *E.coli* O157:H7pathogen bacteria (Figure 2).

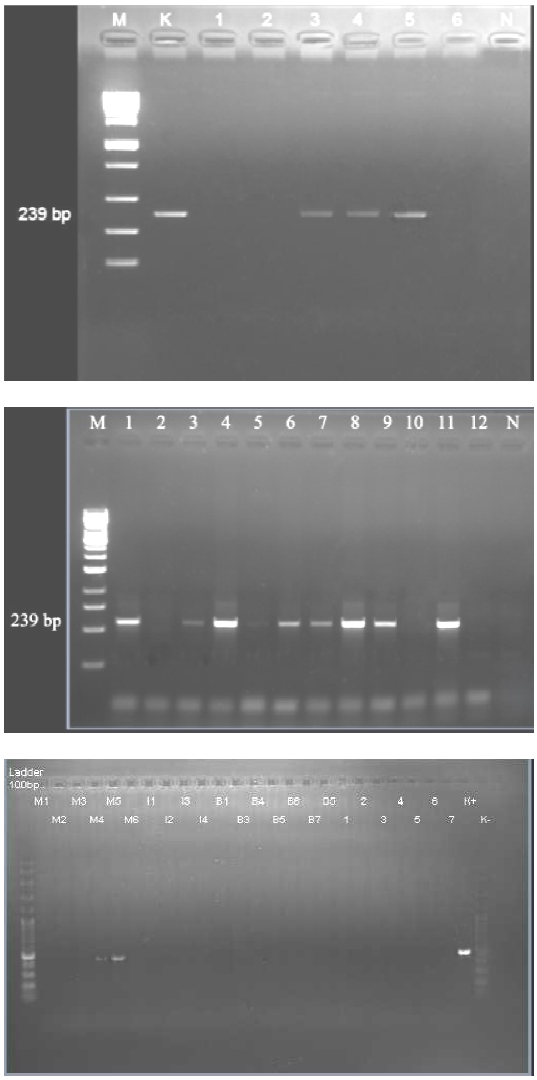


Figure 2. *PCR results for amplification of DNA specific of E.coli.*

4. Discussion

Escherichia coli (E. coli) are bacteria found in the environment, foods, and intestines of people and animals and as zoonosis infectious disease.

The recent burst of food poisoning cases from a single Federico's restaurant west of Phoenix has eclipsed the Farm Rich frozen food outbreak as the biggest toxic E. coli outbreak of 2013 and is among the largest domestic *E. coli* outbreaks of the past few years, according to Centers for Disease Control and Prevention (CDC)[7,8]. Public health officials have had to go back to 2011 to find an *E. coli* outbreak with nearly as many confirmed case patients as the 68 people counted as victims in the current spread of *E. coli* illnesses in Arizona[9]. During the past month, a strain of entero haemorrhagic *E. coli* (EHEC) has infected more than 2,400 people in 13 countries across Europe[10]

The some recently study concerning with multi-drug resistant strain of *E.coli*, type O157:H7, has been documented in some areas[11]. However, based on our biochemical test, the *E.coli* isolate that we obtained from the milk was not O157:H7 type due to the presence of negative result in sorbitol McConkey agar test. The bacterium *Escherichia coli* O157:H7 is a worldwide threat to public health and has been implicated in many outbreaks of hemorrhagic colitis, some of which included fatalities caused by hemolytic uraemic syndrome. Close to 75,000 cases of O157:H7 infection are now estimated to occur annually in the United States [12]. The low infectious dose and high virulence of E. coli O157:H7 make infections severe and life-threatening, particularly for young children, the elderly, and those with weakened immune systems. The main reservoir for E. coli O157:H7 is the intestinal tracts of healthy cattle. Individual cattle are transiently colonized and shed E. coli O157:H7 in their feces. The sources of E. coli O157:H7, which colonizes cattle, are not well understood, and little is known about the ecology of E. coli O157:H7 in the environment. Additionally, the high variability in the prevalence of E. coli O157:H7 among cattle suggests the possibility of a reservoir of E. coli O157:H7 external to cattle. However, other than the detection of E. coli O157:H7 in non-bovine animals, including sheep, horses, dogs, and wild birds [13]. Enterohemorrhagic Escherichia coli (EHEC) of O157:H7 serotype is identified serologically by its somatic O157 and flagellar H7 antigens. In routine clinical analysis, fecal specimens are plated onto sorbitol MacConkey agar and non-sorbitol-fermenting colonies are tested serologically for the O157 antigen. Only isolates that react with anti-O157 serum are serotyped further for the H7 antigen and assayed for virulence factors. Analysis for O157:H7 in foods is done by a similar protocol[14].

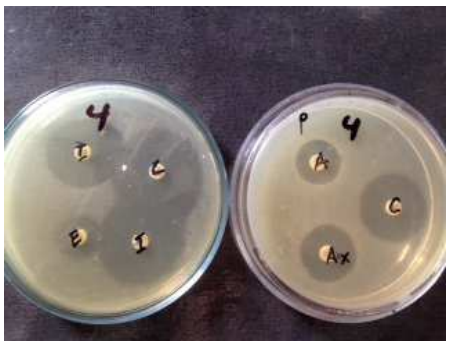


Figure 3. *Disc diffusion results of several antibiotics to E.coli.*

I = Immipenem
C1 =Cyprofloxacine
C = Chloramphinicol
T = Tetracycline
Ax = Amoxyciline
A = Amphyciline
E =Erytromicine

From sensitivity test with antibiotics, those that are the

most sensitive to *E.coli* O157:H7 (in order with mm unit) are Immipenem40.8 (S), Ciprofloxacine 39.9 (S), Levofloxacine 37.8 (S), Choramphinocol 27.2 (S), Tertacycline25.2 (I), Amoxycillin20.8 (I), Amphycillin18.8 (I)and Erytromicine16 (I).

In conclusion, the presence of pathogenic *E.coli* O157H7 bacteria in meat and the environment is highly significant (9.75%). The most sensitive antibiotics, Imipenem, mechanically prevent the cell membrane synthesis, while Ciprofloxacine and levofloxacine works by blocking the acid-nucleic bacteria synthesis.

Acknowledgements

This work was supported by LP2M Hasanuddin University, No 16187/UN4.42/PL09/2014. Thanks to Mrs. Meyby E.P Lempang for her assistant

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