IOP Conf. Series: Materials Science and Engineering **434** (2018) 012151 doi:10.1088/1757-899X/434/1/012151

# Detection *Escherichia Coli O157* contamination in soymilk from traditional market at Denpasar City

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**Abstract**. *Escherichia coli* are normal flora in human. Food and beverage contained *Escherichia coli* is a feces contamination indicator. One of pathogen *Escherichia coli* strain is serotype O157. *Escherichia coli* O157 causes bloody diarrhea and *Hemolytic Uremic Syndrome*. Reservoir can be derived from animal, human and water. In this study we used 80 sample of soy milk sold in traditional market at Denpasar City. The aim of this study is to identify *Escherichia coli* O157 and isolate *Escherichia coli* O157 containing gen *shiga like toxin-2*. *Mac Conkey* was used to differentiate between *Escherichia coli* and coliform that appeared in pink colour colonies. The colonies were obtained for Gram staining which was then examined by biochemical test (imvic). Positive *Escherichia coli* identified by imvic test, bred on SMAC and observed colonies. Colourless colonies identified as presumptive *Escherichia coli* O157 and to support identification was done single path test and latex agglutination test. The result showed that were 32 out of 80 soymilk sample contained *Escherichia coli*. Presumptive *Escherichia coli* O157 were found of 4 out of 32 isolate *Escherichia coli* (12,5%). All presumptive sample of *Escherichia coli* O157 were examined by the PCR procedures which were not contained of gen *shiga like toxin-2*. Contamination *Escherichia coli* O157 in soymilk showed poor hygienic processed soymilk include use to water contamination.

## 1. Introduction

Soymilk is beverage that's sold in traditional market and modern market. Packaging soymilk is labelled or non-labelled. Quality of soymilk has not been known in Denpasar Community. Soymilk contains high protein, low cholesterol and lactose, reduce bone loss and menopause symptom [1]. Cheap price of soybean encourage home production of soymilk [2]. Workers and equipment's that are used to prepare soymilk is contaminated source [3]. Soymilk can easily be a route transmitting foodborne [2]. *Foodborne disease* is disease caused by bacteria contaminated resulting from food and beverage. One of symptoms is diarrhea. One of bacterias causing diarrhea is *Escherichia coli* [4]. Highest diarrhea case in Denpasar occurred in 2003 and it is still to be the top ten disease in Denpasar [5].According to the World Health Organization ( WHO), Diarrhegenic *Escherichia coli* in around the world can be identified *Enterophatogenic Escherichia coli* (EPEC), *Enterohaemorhagic Escherichia coli* (EHEC) or *Shiga Toxin-secreting Escherichia coli* (STEC), *Enteroinvasive Escherichia coli* (EIEC), *Enterotoxigenic Escherichia coli* (ETEC), *Diarrhea Haemolitic Associated Escherichia coli* (DHEC), *Enteroaggregative Escherichia coli* (EAggEC), *Enteroadherent Escherichia coli* (EAEC), *Distending Cytolethal Toxin - coli Escherichia coli* (CDTEC) and *Diffuse adherent Escherichia coli* (DAEC) [6].

A large number outbreak of EHEC or STEC is associated to the consumption of raw seafood products, traditional dairy products, unpasteurized milk, contamination food from pollution sources, such as feces contamination, water contamination, equipment contamination, and undercooked foods [7].

EHEC is able to produce *shiga toxin* divide into *shiga like toxin-1* and *shiga like toxin-2* [8]. *Shiga like toxin-2* caused bloody diarrhea until Haemolytic Uremic Syndrome and *Shiga like toxin-1* caused diarrhea [9]. Quantitative and qualitative differences in disease - related STEC, classification STEC is divided into *E. coli* O157 and *non* - O157 [10].

The discovery of *E. coli* O157: H7 that causes 20,000 increased morbidity and 250 deaths each year in the United States in 1996. In 1994, the CDC reported that 30 outbreaks occurred because of the bacteria *Escherichia coli* pathogens [11]. Several studies Shiga *toxin – producing E coli* O157 in Indonesia have been conducted on animals and their products. In the study of Sartika *et al* found a positive *E. coli* O157: H7 in 14 samples from 19 dairy products of cows [12]. According to a research by Adeleke *et al*, soy milk has identified bacteria *Escherichia coli* and *Staphylococcus aureus* in all samples of soy milk and Agboke *et al*, 17 samples of soy milk products obtained ten samples that have gram negative bacteria and ten samples contained five samples were positive *Escherichia coli*. However, to study *E.coli* O157 *Shiga toxin* - producing soy milk is not known. Author did preliminary test in January 2013 and he found 7 samples of *E.coli* positive of 9 samples and 3 samples positive *E.coli* O157 of 7 samples positive *E. coli*. Based on the above consideration, research to microbiology quality of soymilk is noticed from *E.coli* contaminant and *shiga like toxin-2* gene in contaminated soymilk was found [2,11,12].

## 2. Method

This research is observational descriptive research to know *Escherichia coli* O157 contaminated and detected of *shiga like toxin-2 gene* from contaminated soymilk that is sold in traditional market. Population is liquid soymilk in labelled packaged and non-labelled packaged is sold in traditional market. Eighty soymilk samples were collected from traditional market both labelled and unlabelled. Samples were simultaneously streaked on Tryptone Soya Broth, on Mac Conkey, Gram staining, Biochemical test (Imvic), colony streaked on Sorbitol Mac Conkey, *Single-path* O157 test and confirmation test using agglutination latex. Detection of gene *Shiga like toxin-2* was carried out by the *Polymerase Chain Reaction*. Inclusion criteria are: liquid soymilk that is sold in traditional market both labelled package and unlabelled package. Exclusion criteria are: soymilk package that is damaged or soymilk that have expired date.

### *2.1. Isolation Escherichia coli and Escherichia coli O157*

Mac Conkey is used to differentiate *Escherichia coli* and *coliform* that appeared pink colour colonies. Those colonies were obtained for Gram staining then examined by biochemical test (imvic). Positive *Escherichia coli* identified by imvic test, straked on SMAC and observed colonies. Colourless colonies are identified as presumptive *Escherichia coli* O157 and to support identification was done single-path test and latex agglutination test [8,13].

### *2.2. Isolation of DNA templates*

All *E. coli* O157 isolates were grown in Nutrient Broth at 37˚C. Isolation of DNA template was kit Qiagen's procedures used to *QIAMP®DNA Mini Kit Cat No.51304, Lot No.142326957* with *QIAMP Mini Spin Column Lot. No 142320326*. A total of 3 ml *E. coli* O157 culture aliquots were centrifuged at 7500 rpm for 10 minute and supernatant was thrown. They pellet was added by 180µl of buffer ATL. Add 20µl proteinase K then mixed by vortexing for 15 second and add 200µl lysis buffer (AL buffer) then mixed again by vortexing for 15 second. Incubated in water bath at 56˚C for 10 minute, then added 200µl ethanol (96-100%) to the sample and mixed again by vortexing for 15 second. *Lysate E.coli* is used to binding DNA. *Lysate* remove drop into filter micro tube QIAamp Mini Spin Column and centrifuge at 8000 rpm for 1 minute and thrown filtrate. Filter contains DNA remove into new eppendorf

tube 2 ml then washing DNA. DNA filtrate added 500µl wash buffer (buffer AW1), wait for 5 minute then centrifuged at 8000 rpm for 1 minute. Discard the filtrate and placed QIAmp mini spin column to the new tube eppendorf 2ml. Add 500 µl wash buffer (AW2 buffer) and wait for 5 minute then centrifuge at 8000 rpm for 1 minute. Centrifuge again at 14.000 rpm for 3 minute. Placed the column in a clean 1,5 ml micro-centrifuge tube, add 50µl buffer AE, incubated at room temperature (15-25˚C) for 5 minute and then centrifuge at 8000 rpm for 1 minute.

*2.3. PCR amplification of shiga like toxin-2 gene*

PCR is used to PCR reagent Ferments Lot No.00098413 ( Dream TaqTM Green PCR Master Mix with composition: Master Mix Green buffer obtain green buffer, d ATP, dCTP, dGTP and d TTP each 0,4 mM and 4 mM MgCl2. Master mix 17,0 µl added 1,5µl Template DNA, 1,0µl primer Stx-2 forward, 1,0µl primer stx-2 reverse, added DW until volume 27,0µl. Centrifuge PCR tube at 2000 rpm for 5 minute then remove into *Thermocycler Eppendorf Mastercycler Personal MJ Mini* with condition : initial denaturation 94˚C for 7 minute, 35 cycles of 94˚C for 1 minute, 56˚C for 35 second, and extension at 72˚ for 1 minute, and post extension 72˚C for 5 minute. Specific primary according to Suardana is as follows: Stx-2 (F) 5-GCCATTAGCTCATCGGGATA-3 and Stx-2 (R) 5-CGAATGCTCAGTCTGACAGG-3 by measure of PCR product 1587bp. Amplification products were analyzed by electrophoresis in 1% agarose gels which was stained 3µl Ethidium Bromide [14].

## 3. Result

The result of 80 soymilk samples in traditional market shows that 34 samples positive *E.coli* on Mac conkey. 34 samples positive *E.coli* were Gram staining and all samples showed Gram negative bacteria (figure 2). 32 samples of 34 samples suspect *E.coli* were positive in Biochemical test (Imvic) to confirmed *Escherichia coli*. Four isolates of 32 isolate *E.coli* were found positive on Sorbitol Mac Conkey with colourless colony. Then they were confirmed by agglutination *latex* test and *single path* O157 test. The result of *agglutination latex* and *single-path* O157 is all sample positive *E.coli*. Agglutination latex test shows agglutination description as sand.

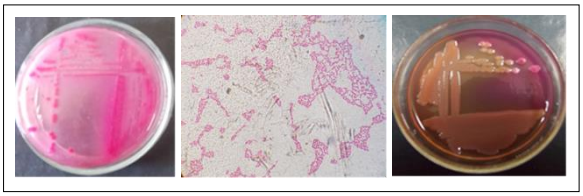

**Figure 1**. Microbiologi test; Mac Conkey, Gram and SMAC.

The result of E. coli contaminated soymilk in traditional market 32 samples of 80 samples (40%) in which 15% are in labelled package and 25% non-labelled package. E.coli O157 contaminated soymilk, 4 samples of 80 samples (5%) in which 1,25% are in labelled soymilk and 3,75% in non-labelled package. The absence Escherichia coli in North Denpasar sub-district and South Denpasar sub-district. E. coli O157 contaminated soymilk at West Denpasar is 14,28% in which 4,76% labelled package and 9,52% non-labelled package. E. coli O157 contaminated soymilk at North Denpasar is 7,14% non-labelled package (Table 1).

**Table 1.** Identification of Escherichia coli O157 from Escherichia coli isolate in soymilk from Traditional Market at Denpasar City.

| | Total Sample | *E. coli* | | *E.coli* O157 | |
|---|---|---|---|---|---|
| | | *n* | % | *n* | % |
| Labelled Package | 29 | 12 | 15 | 1 | 1,25 |
| Unlabelled package | 51 | 20 | 25 | 3 | 3,75 |
| Total | 80 | 32 | 40 | 4 | 5 |

*Result of PCR method

d was not showing tape overview. It means negative gene shiga like toxin in Escherichia coli O157 that were found in soymilk. It can be compared with positive control that have PCR product 1587bp.

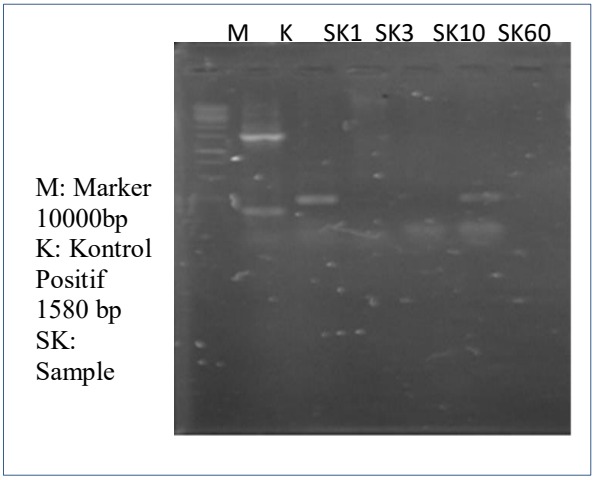


**Figure 2**. Polymerase Chain Reaction.

## 4. Discussion

Contamination bacteria of soymilk are caused by contamination in processing, tools, and water. The presence *Escherichia coli* and *Escherichia coli* 157 in soymilk shown poor processing soymilk and poor hygienity [15]. Observation at soymilk sellers shows that workers did not wash hand, did not used mask, gloves and that used dirty tools. Store place of soymilk use unsterile bottle or recycle bottle. Observation result in appropriate with research in which poor higyenity processing soymilk is *Escherichia coli* contamination source [2-3]. Animal feces and human feces contamination in water, and worker's hand are high risk of *Escherichia coli* O157 contamination [16].

PCR method is used to known the presence of *Shiga like toxin-2 gene*. The result is negative *Shia like toxin-2* of isolate *Escherichia coli* O157 from soymilk. *Escherichia coli* O157 can produce *shiga like toxin -1, shiga like toxin-2* or both. Negative *shiga like toxin-2* of isolate *Escherichia coli* O157 is also found by Suardana *et al* about detection *stx-1* and *stx-2* in cows feces and beef [17,18]. The same result negative *stx-2* of isolate *E.coli* O157 by Abdulmawjood *et al*, 4 samples negative *stx-2* of 32 isolate *E coli* O157 found were [19].

*Escherichia coli* O157 isolate does not always produce *stx-2*. Possible, *E.coli* O157 carries only *stx-1*, only *stx-2* or both. In this research, *E.coli* O157 isolate has chance to produce *stx-1* although the result is negative *stx-2*. Based on theory and literature, *Escherichia coli* O157 did not contain *stx-2* caused by *Escherichia coli* that was not infected with bacteriophage or bacteriophage infect *Escherichia coli* did not contain *stx-2* so that it way out expressed with *Escherichia coli* O157 [17-19].

## 5. Conclusion

*Escherichia coli* O157 contaminated by soymilk is 5% and *Escherichia coli* contaminated by soymilk is 40%. *Escherichia coli* O157 isolate of result identification did not contain *shiga like toxin-2*.

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