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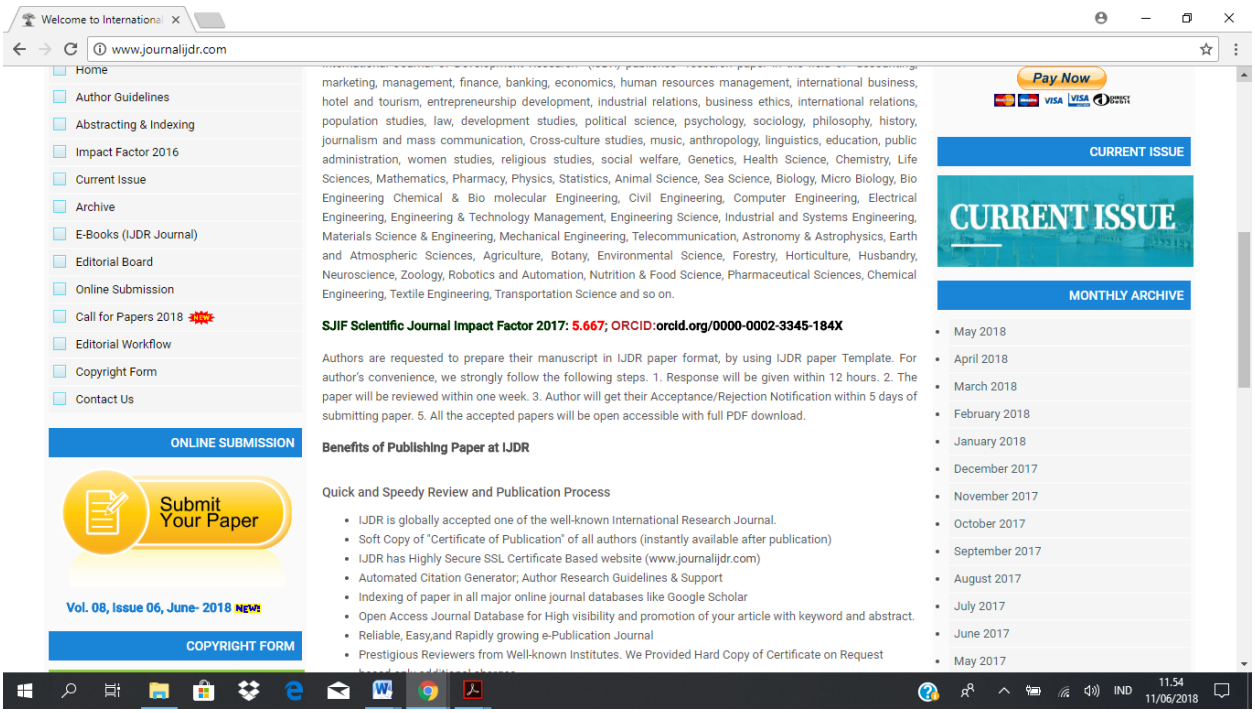
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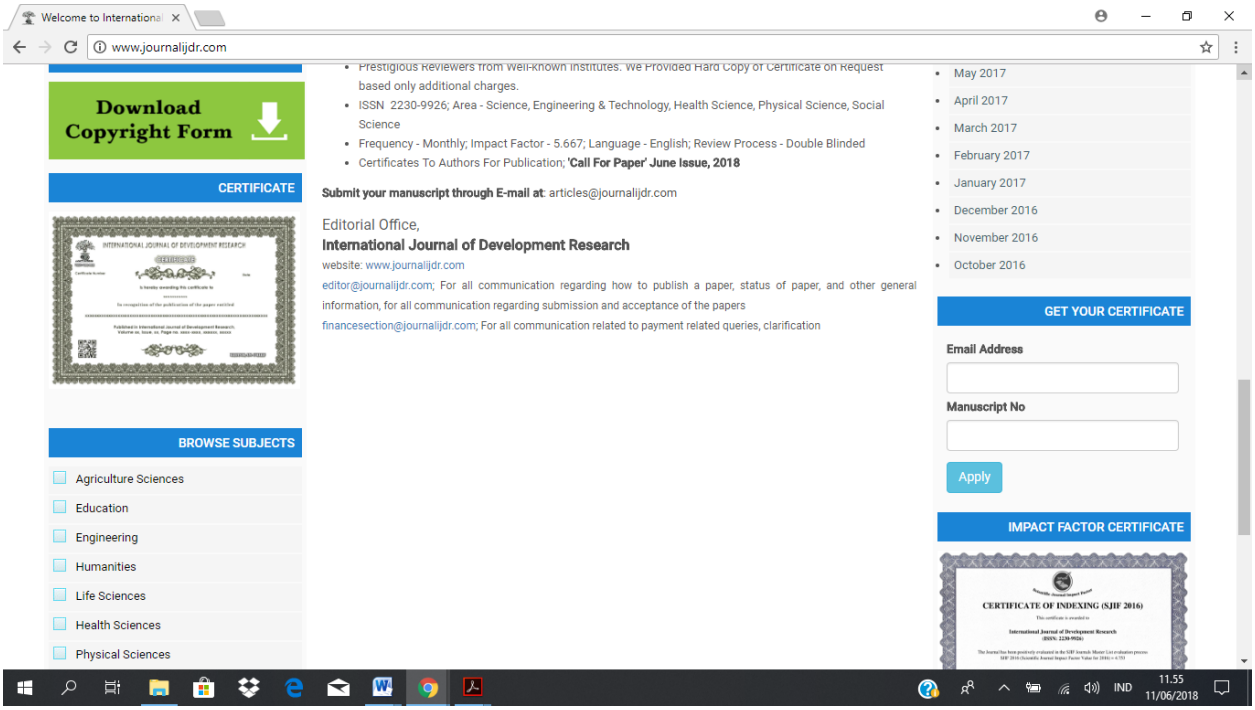
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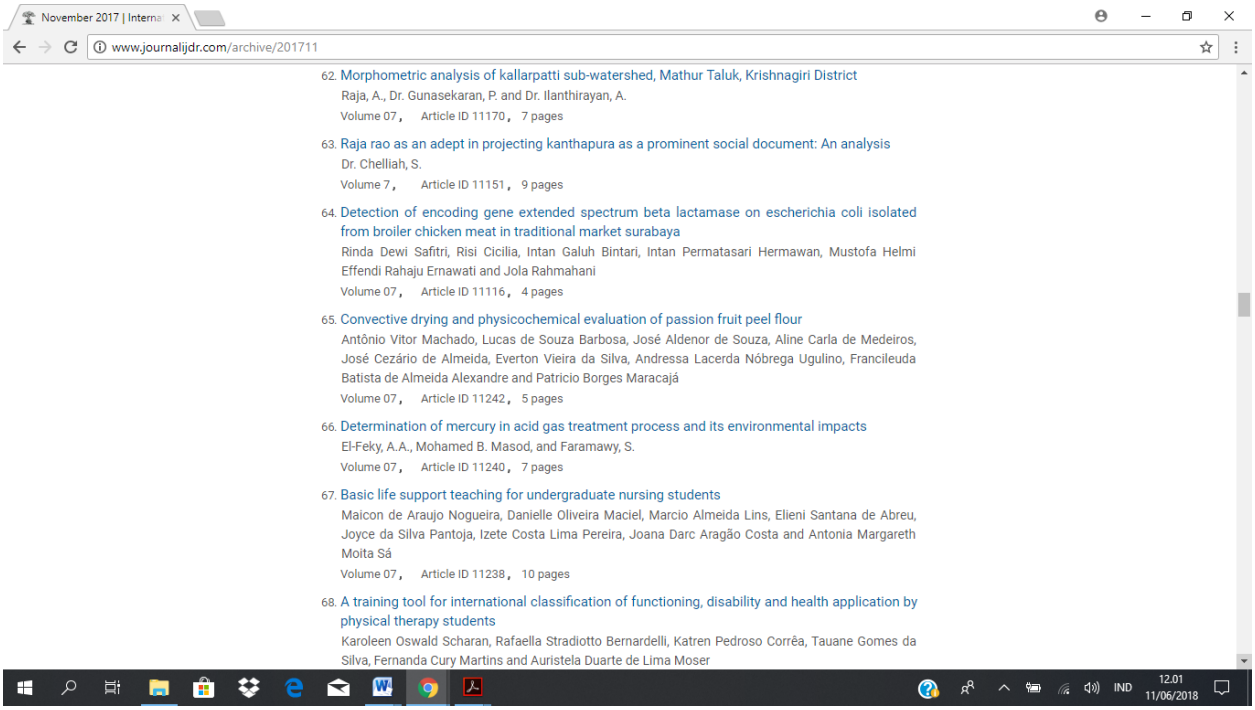

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

DETECTION OF ENCODING GENE EXTENDED SPECTRUM BETA LACTAMASE ON *ESCHERICHIA COLI* ISOLATED FROM BROILER CHICKEN MEAT IN TRADITIONAL MARKET SURABAYA

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ABSTRACT

This study aims to isolate, to identify, and to seek out fragments of encoding gene Extended Spectrum β-Lactamase on *Escherichia coli* isolated from swab surface of broiler chicken meat in a number of traditional markets in Surabaya. The result shows that 31 out of 50 samples positively contain *Escherichia coli*, shown through EMBA isolation media and identified using indole test. Sensitivity test shows that 100% of the isolates are resistant to Ampicilin, 48.4% are resistant to Cephazoline, 13% are resistant to Ceftazidime, 9.6% are resistant to Cefotaxime, 6.4% are resistant to Ceftriaxone and 87.2% are resistant to Tetracycline. 8 out of 8 (100%) samples of *E. coli* resistant show the presence of band towards bla_{TEM} gene of 861basepair (bp).

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INTRODUCTION

The phenomenon of antibiotic resistance is a health problem occurring all over the world with various adverse effects which can degrade the quality of health service. At least 23,000 out of 2 million people die each year resulting from bacterial infections which are resistant to more than one type of antibiotics. One of the bacteria which often carries such resistant property is Extended Spectrum Beta Lactamase (ESBL) bacteria produced by Enterobacteriaceae, especially *Escherichia coli* (Nathisuwan *et al*., 2001). The increasing occurrence of E. coli resistance, especially Extended Spectrum β-lactamase (ESBL) constitutes a serious issue (Rocha-Gracia *et al*., 2014). This is due to the fact that one of the transmission pathways is through animal source food contaminated by resistant bacteria.

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Chicken is one of animal source food which is prone to bacterial contamination for it contains an ideal substrate supporting the growth of contaminant bacteria, such as E. coli. E. coli bacteria are among the most common contaminant bacteria to contaminate meat (Norrung *et al*., 2009). bla_{TEM} which are resistant to antibiotics in food-producing animals, such as chickens, are of particular concern, since E. coli is able to transmit resistant genes to human populations (Molbak, 2004). Molecular analysis on resistant genes and cellular elements of antibiotic resistant shows that identical elements are found in bacteria which contaminate both animals and humans. It signifies the role of raw food as a medium in the contamination of resistant bacteria and resistant genes on human being through the food chain (Teuber, 2001; Odwar *et al*., 2015). Of the above explanation, study on detection of encoding gene of Extended Spectrum Beta-lactamase in *Escherichia coli* isolated from broiler chicken meat taken from traditional market in Surabaya is considered to be essential to conduct.

METHODOLOGY

SAMPLING

The samples were taken from five traditional markets in Surabaya; they are Pabean market (North Surabaya), Wonokromo market (South Surabaya), Keputran market (Central Surabaya), Pacar Keling market (East Surabaya), and Manukan market (West Surabaya). 10 samples were taken from each market meaning that the total sample in this study is 50 samples. The sample was taken by swab surface of chicken meat using sterile cotton bud and is inserted into a tube containing 1% peptone water. It is then immediately brought to the laboratory using cool box containing dry ice inside.

ISOLATION AND IDENTIFICATION

Swab samples in the medium of 1% peptone water were removed to Brilliant Green Bile Broth (BGBB) (E. Merck, Darmstadt, Germany) and were incubated at 37°C for 24 hours. Positive results in the medium of BGBB were then grown on the medium of Eosin Methylene Blue Agar (EMBA) (E. Merck, Darmstadt, Germany) in streak manner and were incubated at 37°C for 18 – 24 hours. A typical colony of *Escherichia coli* on the media of EMBA is indicated in metallic green.

BACTERIA SENSITIVITY TEST ON ANTIBIOTICS

Testing of *Escherichia coli* resistant to disc antibiotics (OXOID, Basingstoke, United Kingdom), Ampicillin 10 µg (CT0003), Cefotaxime 30 µg (CT0166), Ceftazidime 30 µg (CT0412), Ceftriaxone 30 µg (CT0417), Cefazolin 30 µg (CT0011) and Tetracyclin 30 µg (CT0054), were carried out by disc diffusion method on the medium of Mueler Hinton Agar (MHA) (E. Merck, Darmstadt, Germany) . Interpretation of the result was known through measurement of inhibitory zone diameter formed in accordance with the recommendation of the Clinical and Laboratory Standards Institute (CLSI, 2016).

IDENTIFICATION OF ESBL GENE WITH POLYMERASE CHAIN REACTION

The initial procedure in conducting PCR technique in this study was the DNA extraction. DNA extraction of *Escherichia coli* used boiling method. PCR amplification of the bla_{TEM} gene with 20 µl PCR reaction consisted of: 12.5 µL PCR Master Mix, 1 µL primary forward and 1 µL reverse primer, 5 µL DNA template and 0.5 µL nuclease free water. The mixture of PCR reagent was then added in the thermocycler. Primer used and the condition of PCR are presented in Table 1.

Tabel 1. Primer and thermal cycling condition that were used for amplification of *bla*TEM gene

Target gene	Primer used	Thermal Cycling Condition
*bla*TEM	F : 5' GTATCCGC TCATGGAGA CAATAACCCTG-3'	95°C 15 min → 30x [95°C 1 min, 58°C 1 min, 72°C 1 min] → 72°C 10 min → 4°C ∞
	R : 5'-CCAATGCTT AATCAGTGGAGGCACC-3'	

RESULT

The isolation and identification result on 50 swab samples of broiler chicken meat obtained from 5 traditional market in Surabaya shows that 31 samples (62%) indicates the occurrence of color change from translucent green to cloudy green in the medium of BGBB as well as the presence of gas in the durham tube. A metallic green colony with a black colony in the central part as well as indole ring formation is present in the media of Sulfid Indol Motility (SIM) with the addition of Kovach reagent. A total of 31 positive samples of *Escherichia coli* coli from isolation and identification underwent sensitivity test on antibiotics of Ampicilin, Cefotaxime, Ceftriaxone, Ceftazidime, Cephazoline, and Tetracylin. The sensitivity test indicates 100% E. coli isolates are resistant to Ampicilin, 48.4% to Cephazoline, 13% to Ceftazidime, 9.6% to Cefotaxime, 6.4% to Ceftriaxone, and 87.2% to Tetracycline. The illustration of sensitivity test result on antibiotics is presented in Figure 1.

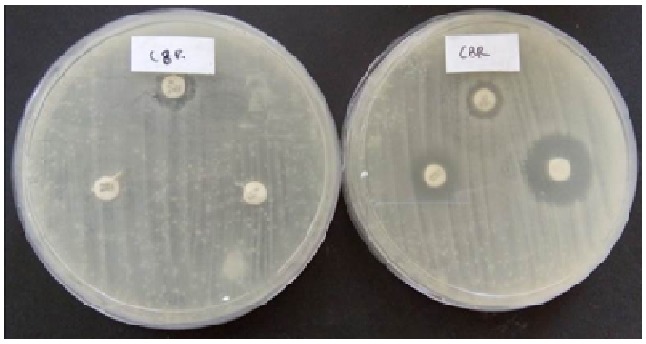

Figure 1. The illustration of sensitivity test on *Escherichia coli* isolates towards antibiotics.

Samples which are resistant to antibiotics in the sensitivity test were then undergone Polymerase Chain Reaction (PCR) in order to find the fragment of the encoding gene of Extended Spectrum Beta-lactamase (ESBL). 8 out of 8 E. coli resistant samples show the presence of DNA band from bla_{TEM} gene of 861bp. The electrophoresis result of PCR products can be seen in Figure 2.

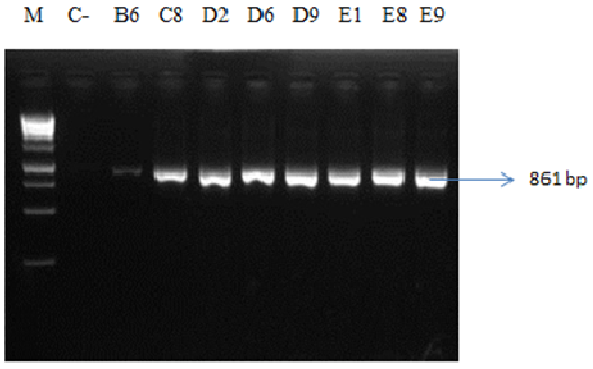

Figure 2. The electrophoresis result of PCR products indicates bla_{TEM} gene with band of 861bp

DISCUSSION

This study has successfully isolated *Escherichia coli* by 62% (31 out of 50) samples taken from swab surface of broiler chicken meat in 5 traditional markets in the area of Surabaya. This finding is similar to the study by Bhoomika *et al*. (2016) which detects E. coli in chicken meat in Chhattisgrash, India of 66.32%. The level of E. coli contamination in chicken meat reported by Odwar *et al*. (2014) is also considerably high at 78%.

Nevertheless, lower level of E. coli contamination is reported by Indana (2015) at 32.15%. High level of contamination of E. coli in chicken meat is a result of the surface contact with chicken carcass which is not water-resistant, chicken are placed mixed with other commodities, kiosk hygiene is not well-maintained, personal hygiene of the seller who does not wear gloves and apron, the presence of alive chicken in the place where chicken meat is sold, market hygiene and sanitary which remains poor due to the pile of garbage, the absence of adequate washing facilities, cutting board made of wood, and the absence of cold chain principle because the chicken meats are not stored at cold temperature (Mukti *et al*., 2017).

In the bacterial sensitivity test on antibiotics using Kirby Bauer method, the level of resistance towards Ampicilin is significantly high of 100%, while the level of resistance on Cephalosporin group of antibiotics: Cephazoline (48.4%), Ceftazidime (13%), Cefotaxime (9.6%), Ceftriaxone (6.4%). It is also obtained high level of resistance on Non-Beta Lactam antibiotics: Tetracycline (87.2%). The result of molecular detection on 8 out of 8 *Escherichia coli* isolates using Polymerase Chain Reaction indicates the band resulting from primer amplification of bla_{TEM} gene of 861 bp. bla_{TEM} gene is one of the ESBL enzyme-encoding genes which indicates that genotypically, samples of chicken meat isolated from the traditional markets in Surabaya are *Escherichia coli*-producing ESBL reservoirs. It is in accordance with the study by Overdevest *et al*. (2011) stating that there is contamination of ESBL-producing E. coli on chicken meat of 76.8% with percentage of bla_{TEM} gene presence of 14%. Younis *et al*. (2017) also report that bla_{TEM} gene constitutes the main gene found in ESBL-producing *Escherichia coli* isolated from frozen chicken carcass of 37.5%. Study regarding ESBL contamination on chicken carcass by Reich *et al*. (2013) reports that of 88.6% of ESBL-producing isolates, 27% of which contains bla_{TEM}, and *Escherichia coli* is the most commonly found bacteria.

The presence of ESBL-producing bacteria in poultry-based food directly affects public health (Garcia-Graells *et al*., 2012) because E. coli becomes a possible means in spreading resistant genes on animals and human beings. Such bacteria are known to be able to exchange resistant genetic material among strains (Costa *et al*., 2013). Generally, resistant gene transfer can undergo through three mechanisms: transformation, conjugation, and transduction. The issue on antibiotic resistance comes largely from horizontal gene transfer between bacterial species. Such mechanisms are acknowledged to become more efficient for bacteria to adapt to environmental changes compared to random mutations. Transformation and conjugation (Marshall *et al*., 2009) are the most commonly emerging transfer route.

The transfer of antibiotic-resistant genes from commensal bacteria to pathogenic bacteria relies on the density of the donor and the recipient bacteria, the availability of transfer mechanisms, nutrients, and selective pressure. Broiler chickens as one of the food-producing animals have the potential as a reservoir for ESBL-producing bacteria. The ESBL-producing bacteria can transmit from animal to human and potentially cause zoonotic diseases. ESBL-producing bacterial infections from consuming food-producing animal can lead to limited option in the treatment of patients. Such conditions extend the treatment period, increase the cost of treatment, increase the occurrence of the diseases, and death (Khosbayar *et al*., 2013).

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