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Rai MK, Carpinella C. 2006. Naturally Occurring Bioactive Compounds. Elsevier, Amsterdam.

Chapter in book:

Webb CO, Cannon CH, Davies SJ. 2008. Ecological organization, biogeography, and the phylogenetic structure of rainforest tree communities. In: Carson W, Schnitzer S (eds) Tropical Forest Community Ecology. Wiley-Blackwell, New York.

Abstract:

Assaeed AM. 2007. Seed production and dispersal of *Rhazya stricta***.** 50th annual symposium of the International Association for Vegetation Science, Swansea, UK, 23-27 July 2007.

Proceeding:

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Thesis, Dissertation:

Sugiyarto. 2004. Soil Macro-invertebrates Diversity and Inter-Cropping Plants Productivity in Agroforestry System based on Sengon. [Dissertation]. Universitas Brawijaya, Malang. [Indonesian]

Information from internet:

Balagadde FK, Song H, Ozaki J, Collins CH, Barnet M, Arnold FH, Quake SR, You L. 2008. A synthetic *Escherichia coli* predator-prey ecosystem. Mol Syst Biol 4: 187. www.molecularsystemsbiology.com

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Presence of multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) of *Escherichia coli* isolated from cloacal swab of broilers in several wet markets in Surabaya, Indonesia

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Abstract. *Effendi MH, Tyasningsih W, Yurianti YA, Rahmahani J, Harijani N, Plumeriastuti H. 2020. Presence of multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) of* Escherichia coli *isolated from cloacal swab of broilers in several wet markets in Surabaya, Indonesia. Biodiversitas 22: 304-310.* The purpose of this research was to identify multidrug resistance (MDR) and extended-spectrum beta-lactamase (ESBL) of *Escherichia coli* from cloacal swab of broiler chicken in several wet markets in Surabaya. This study used 60 broiler chicken samples, with cloacal swab method. The samples were isolated and identified to find *Escherichia coli* with several procedures, MacConckey Agar (MCA), Eosin Methylene Blue Agar (EMBA), Gram staining, indole test, Methyl Red-Voges Proskauer (MR-VP), citrate, and Triple Sugar Iron Agar (TSIA). Antibiotic sensitivity test was tested by using Kirby-Bauer (Disc Diffusion Method) with antibiotics: Aztreonam, Gentamicin, Chloramphenicol, Tetracycline, and Ciprofloxacin. From research, it can be illustrated that the isolates resistant to antibiotic Ciprofloxacin were 67% and Tetracycline was 65%. Total 97% isolates were found sensitive for Aztreonam, 73% for Chloramphenicol, and 55% for Gentamicin. Twelve isolates identified for MDR and two were ESBL. It can be explained that broiler chicken from wet market should be considered as a source of transmission for MDR and ESBL of *E. coli* to the public health.

Keywords: *Escherichia coli*, MDR, ESBL, Broiler chicken, Wet markets, Public health

INTRODUCTION

The human diseases from food products of animals origin can be categorized as foodborne diseases The diseases caused by consumption of broiler chicken as food is one of them. Broiler chicken production is relatively high because of the high level of consumption, especially in the part of chicken meat. Meat product of animal origin contains antibiotic residues which make these products bacterial resistant. Meat contaminated resistant *E. coli* bacteria can transfer it from animals to humans via food chain pathway or direct contact. The use of antibiotics in the long term can affect the resistance of bacteria, both pathogens or normal microflora in the body of living things (Effendi et al. 2019, Wibisono et al. 2020).

Poultry can act as an important reservoir of bacterial agents. Infected poultry can be a source of disease transmission. Pathogenic microorganisms can cause infectious disease which is the main cause of death in animals and humans (Suardana et al. 2014). The increase in the incidence of infectious diseases is mainly because of inappropriate use of antibiotics is the most dominant in poultry farms (Wiedosari and Wahyuwardani 2015; Wibisono et al. 2020). The high use of antibiotics can lead to an increase in antibiotic resistance. The combination preparation of amoxicillin and colistin (60.8%) is most widely used in several farms. The perception of farmers that the use of antibiotics has no side effects and is a cheap effort to prevent disease (Niasono et al. 2019; Masruroh et al. 2016). This ultimately leads to an increase in the factors causing the emergence of antibiotic resistance in poultry as food of animal origin.

A research carried out by Suandy (2011) revealed that the resistance level of *E. coli* isolated from broiler chicken meat from the traditional Bogor market was 80.6% against Tetracycline, 14.2% against Gentamicin, and 11.4% against Chloramphenicol. From these data, it was concluded that the level of resistance of *E. coli* to some antibiotics was quite high due to their excessive use. Broiler chicken meat isolated in testing by Akmal et al. (2017) showed high resistance levels in the antibiotics Tetracycline, Ciprofloxacin, and Gentamicin.

The selection of appropriate antibiotics based on bacterial resistance patterns is important for the assessment of bacterial resistance factors and controlling the incidence of resistance from bacteria in animal food to humans (Putra et al. 2019; Vasilakopoulou et al. 2020). Aztreonam, Gentamicin, Chloramphenicol, Tetracycline, and

Ciprofloxacin are broad-spectrum antibiotics from several classes of effective antibiotics and are often used in cases of *E. coli* bacterial infections (Karaman 2015).

This study was conducted to obtain an overview of the profile of antibiotic resistance from broiler chickens related to biosafety based on the high cases of resistance of *E. coli* bacteria in humans that can be transmitted from food products of animal origin. Broiler chickens for research were taken from three wet markets in Surabaya by cloacal swab. The market was chosen because of the high supply of broiler chickens and a large number of purchases by consumers in the market.

MATERIALS AND METHODS

Samples

The sample consisted of 60 broilers cloacal swabs were taken from three Surabaya, Indonesia wet markets, i.e., Pucang Market, Keputran Market, and Wonokromo Market.

Isolation and Identification

Sixty samples taken by the cloacal swab method were put into a vacutainer tube containing Buffered Peptone Water (BPW) and put into a cool box. Samples were cultured on Mac Conkey Agar (MCA) media for 24 hours at 37ºC. *E. coli* bacterial colonies on MCA media were observed for color and texture and processed for identification of *E. coli* (Effendi et al. 2018; Putra et al. 2020).

Colonies suspected of being *Escherichia coli* bacteria on EMBA media were again stained with Gram stain to confirm the morphology and nature of the bacteria. Separate colonies that had been tested for Gram staining were followed by biochemical IMViC tests (Indol, MR-VP, citrate) and TSIA. *E. coli* bacteria showed positive indole results and motility on the SIM media. In the Methyl-Red (MR) test, *E. coli* bacteria showed positive results and Voges-Proskauer (VP) with negative results. In the citrate test, *E. coli* bacteria showed negative results. TSIA test results showed Acid/Acid results, negative H_2S, and positive gas (Putra et al. 2019; Kristianingtyas et al. 2020).

Antibiotic Sensitivity Test

The suspension *E. coli* bacterial isolate was made that was synchronized with McFarland 0.5 standard and then tested for antibiotic sensitivity using the Kirby-Bauer diffusion method against Aztreonam, Gentamicin, Chloramphenicol, Tetracycline, and Ciprofloxacin antibiotics on Mueller Hinton Agar (MHA) media. For this, the suspension was inoculated on MHA media for 16-18 hours at 37ºC. Inhibitory zone diameters were measured using a calipers measuring instrument with an accuracy of 0.02 millimeters (mm) and adjusted Clinical and Laboratory Standards Institute (CLSI 2017; Rahmahani et al. 2020).

ESBL confirmation test by DDST

ESBL producing *E. coli* can be confirmed by using Double Disc Synergy Test (DDST). This confirmation test is to evaluate the presence of a inhibitory zone of ESBL activity with clavulanic acid. This conventional method was carried out by using the Kirby-Bauer disk diffusion method on Mueller - Hinton agar (Merck, Germany). The DDST confirmation test used an antibiotic (Oxoid, England) disc Amoxicillin-clavulanate 30µg, Cefotaxim 30µg, and Ceftazidime 30µg. The culture was incubated at temperatures of 35-37 ºC for 18-24 hours. Evaluation results after incubation showed the presence of inhibitory zones that appeared in the cup were measured according to the Clinical and Laboratory Standards Institute (Effendi et al. 2018; Wibisono et al. 2020).

RESULTS AND DISCUSSION

Antibiotic Sensitivity Test

The results of isolation and identification of *E. coli* that grew with red, convex characteristics and clear boundaries on MCA media, were followed by biochemical IMViC tests. Positive results of IMViC test continued with antibiotic sensitivity testing as shown in Figure 1 and Table 1.

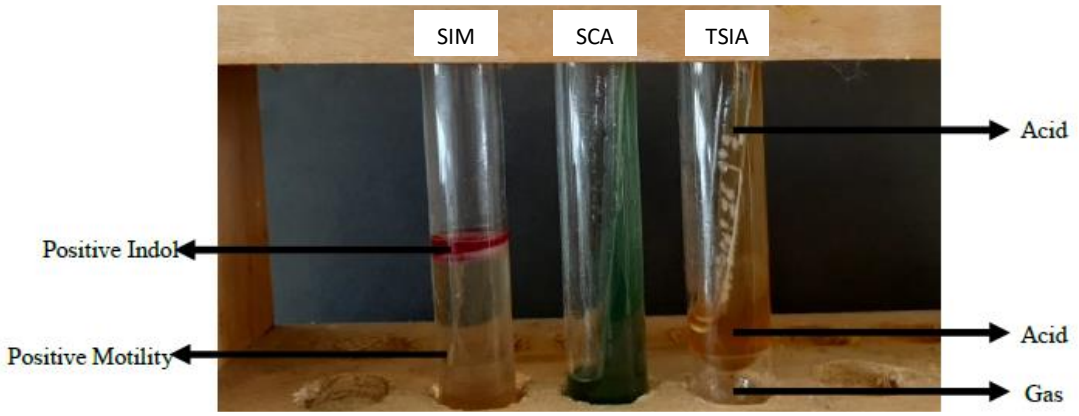

Figure 1. The results of identification of Escherichia coli bacteria on Sulfide Indole Motility (SIM), Simons Citrate Agar (SCA), and Triple Sugar Iron Agar (TSIA) media

Tabel 1. Results of antibiotic sensitivity test for 60 isolates of *E. coli*

Antibiotics	Sensitive samples		Intermediate samples		Resistant samples	
	N	%	N	%	N	%
Aztreonam	58	97	0	0	2	3
Gentamicin	33	55	5	8	22	37
Chloramphenicol	44	73	2	3	14	23
Tetracycline	18	30	3	5	39	65
Ciprofloxacin	12	20	8	13	40	67

Results of Antibiotic Sensitivity Test for 60 Isolates of *E. coli* illustrated that 2 samples (3%) were resistant to Aztreonam 30µm antibiotic with inhibition zone ≤ 17 mm and 58 sensitive samples with inhibition zone ≥ 21 mm. Chloramphenicol 30µm antibiotics contained 14 samples (23%) resistant to inhibition zones ≤ 12 mm and 44 samples (73%) sensitive to inhibition zones ≥ 18 mm. Tetracycline 30µm antibiotics showed that 39 samples (65%) were resistant with 11 mm inhibition zone and 18 samples (30%) were sensitive with 15 mm inhibition zone, as shown in Table 2.

Bacterial resistance is the nature of the disruption of bacterial cell life to antibiotics. Resistance arises due to excessive use of antibiotics that poses long enough impacts on humans (Nahar et al. 2014; Wibisono et al. 2020). Antibiotic sensitivity test using the Kirby-Bauer method. The media was incubated at 37ºC for ± 24 hours and the inhibition zone was measured using a caliper that was used to determine resistance, intermediate, and sensitivity compared to the 2017 CLSI standard.

Antibiotic sensitivity test results of 60 *E. coli* bacterial isolates showed 67% resistance towards Ciprofloxacin and 65% towards Tetracycline resistant. Isolates were found sensitive to antibiotics Aztreonam (97%), Chloramphenicol (73%), and Gentamicin (55%). The results of the sensitivity test of *E. coli* bacteria towards Ciprofloxacin revealed that 67% of the isolates were found resistant. These results are in accordance with the research of Baharutan et al. (2015) which showed that 62.5% of *E. coli* isolates were resistant to Ciprofloxacin. Ciprofloxacin antibiotics belong to the class of fluoroquinolones which work to influence DNA Gyrase acids in bacteria, thus inhibiting DNA synthesis. The sensitivity test of *E. coli* bacteria to ciprofloxacin cannot inhibit the existing DNA Gyrase enzyme, causing antibiotic resistance. Ciprofloxacin resistance is the result of a mutation of the gyrA subunit from Gyrase that reduces the ability of antibiotics to bind to bacteria.

The results of the sensitivity test of *E. coli* bacteria towards Tetracycline showed the resistance of 65% isolates. A study carried out in patients at Fatmawati Hospital revealed that Tetracycline antibiotics have a high level of resistance against *E. coli* (Refdanita et al. 2004). Suandy (2011) showed the pattern of Tetracycline resistance and observed this antibiotic highly resistant *to E. coli* bacteria. Tetracycline antibiotics inhibit bacteria by inhibiting the process of the 30S ribosome of prokaryotic by binding to aminoacyl-tRNA.

Table 2. Antimicrobial Resistance Profile of *Escherichia coli* isolated from Broiler Chicken of several wet markets in Surabaya

Sample code	Antibiotics (mm)				
	Aztreonam 30µm	Gentamicin 10µm	Chloram-phenicol 30µm	Tetracycline 30µm	Ciprofloxacin 5µm
BPS 1	33.43	NCZ	26.00	21.24	7.08
BPS 2	32.64	17.43	25.56	NCZ	8.42
BPS 3	32.46	NCZ	22.60	18.18	6.63
BPS 4	29.52	17.60	21.78	NCZ	NCZ
BPS 5	33.54	NCZ	22.72	21.25	7.24
BPS 6	33.36	9.94	NCZ	9.82	13.74
BPS 7	35.74	NCZ	25.62	20.18	9.68
BPS 8	34.34	8.46	24.84	14.00	24.86
BPS 9	24.31	13.86	28.18	9.88	28.14
BPS 10	28.86	11.96	20.14	5.93	18.36
BPS 11	34.48	NCZ	27.32	7.74	26.88
BPS 12	30.82	16.36	NCZ	6.36	21.98
BPS 13	32.31	16.39	8.06	7.44	9.36
BPS 14	15.72	6.26	28.04	20.28	29.52
BPS 15	15.58	NCZ	31.86	31.98	26.06
BPS 16	31.78	NCZ	29.18	12.68	11.66
BPS 17	30.62	NCZ	23.56	NCZ	17.86
BPS 18	35.58	NCZ	24.88	22.60	9.88
BPS 19	36.36	23.18	26.26	15.80	NCZ
BPS 20	24.64	15.54	19.98	7.12	NCZ
BKS 1	18.02	18.38	24.82	8.82	17.60
BKS 2	34.68	NCZ	NCZ	NCZ	10.18
BKS 3	32.04	20.70	6.46	10.76	12.02
BKS 4	30.46	8.44	24.14	10.38	10.06
BKS 5	29.66	14.46	24.42	22.80	9.50
BKS 6	28.24	17.82	6.94	6.86	8.74
BKS 7	30.00	20.74	23.58	13.48	24.24
BKS 8	31.76	8.82	24.78	19.92	13.84
BKS 9	34.26	19.60	22.52	11.80	17.24
BKS 10	34.18	18.18	23.52	12.44	5.00
BKS 11	26.94	17.76	23.08	6.46	22.98
BKS 12	31.80	17.28	8.00	17.82	22.62
BKS 13	26.84	NCZ	20.40	15.82	8.04
BKS 14	29.68	17.78	18.02	7.74	17.80
BKS 15	28.00	16.88	21.62	6.90	17.36
BKS 16	30.66	NCZ	25.44	8.10	7.20
BKS 17	33.16	NCZ	23.66	21.34	7.80
BKS 18	31.14	20.68	20.10	6.92	7.96
BKS 19	30.52	17.96	24.62	8.14	19.66
BKS 20	30.96	19.86	20.22	NCZ	8.66
BWS 1	32.64	NCZ	24.74	16.06	NCZ
BWS 2	26.94	16.08	16.56	5.82	16.68
BWS 3	30.58	16.34	20.58	10.78	NCZ
BWS 4	33.86	NCZ	25.50	8.84	24.42
BWS 5	30.1	12.38	NCZ	6.60	13.64
BWS 6	36.72	16.94	NCZ	12.30	NCZ
BWS 7	29.98	16.60	19.78	15.22	31.06
BWS 8	30.98	17.66	NCZ	NCZ	8.74
BWS 9	30.52	19.82	21.90	9.32	NCZ
BWS10	33.76	17.30	NCZ	NCZ	NCZ
BWS 11	24.18	15.98	9.96	NCZ	23.46
BWS 12	26.60	13.52	21.88	NCZ	10.44
BWS 13	33.96	9.84	NCZ	NCZ	14.66
BWS 14	29.84	14.44	NCZ	8.48	6.26
BWS 15	28.10	15.34	19.2	NCZ	8.96
BWS 16	30.22	16.22	21.64	7.92	10.04
BWS 17	29.78	16.58	17.34	6.92	NCZ
BWS 18	26.64	16.28	21.20	NCZ	9.88
BWS 19	27.50	15.68	21.44	8.10	NCZ
BWS 20	29.40	15.96	19.80	8.76	NCZ

Note: NCZ: No clear zone, BPS: Samples from wet market of Pucang, BKS: Samples from wet market of Keputran, BWS: Samples from wet market of Wonokromo, ▭: MDR samples (twelve isolates), ▭: ESBL samples (two isolates)

High levels of resistance to Tetracycline antibiotics can be caused by people often consuming animal food that contains lots of antibiotic residues. The occurrence of tetracycline resistance is due to the transfer of plasmids from resistant bacteria to sensitive bacteria and occurs when bacteria that were initially sensitive are exposed to antibiotic exposure. This antibiotic is often used by the community and as an additive to animal feed as a growth trigger. This is one of the causes of antibiotic resistance in Indonesia (Wibisono et al. 2020).

The sensitivity of bacteria to Aztreonam antibiotic is only 3% and 97% sensitive. Research conducted by Anggraini et al. in patients at Arifin Achmad Hospital in Pekanbaru that the sensitivity pattern of E. coli bacteria to aztreonam was 0% which showed that Aztreonam antibiotics were sensitive to E. coli bacteria (Anggraini et al. 2018). This is confirmed by research on isolates from cow feces that E. coli is sensitive to aztreonam antibiotics by 100% (Normaliska et al. 2019). Aztreonam works by inhibiting bacterial cell wall synthesis to overcome severe infections by aerobic Gram-negative bacteria. The aztreonam antibiotic spectrum is similar to aminoglycosides, so Aztreonam is an alternative to aminoglycosides, specifically for Gram-negative bacterial infections.

The sensitivity of *E. coli* bacteria to chloramphenicol antibiotics is 23% while 73% sensitive. A study conducted by Susanto (2014) stated that *E. coli* bacteria isolated from local chickens showed 2.6% resistance against Chloramphenicol. Chloramphenicol is an antibiotic that is prohibited from being used on farms, so resistance occurs due to the illegal use of antibiotics through feed or drinking water (Susanto 2014). Chloramphenicol is a strong inhibitor of protein synthesis in bacteria. Blocking the attachment of amino acids to the newly arisen peptide chain in the 50S unit of the ribosome, by interfering with the action of peptidyl transferase. Chloramphenicol resistance occurs due to destruction of the drug by an enzyme that is controlled by plasmids.

The pattern of antibiotic sensitivity of *E. coli* bacteria was 37% against Gentamicin and 55% sensitive. According to Katarnida et al. (2013) in the culture of pediatric patients that Gentamycin is still said to be sensitive to *E. coli* by 87.5%. Research was also carried out on broiler chicken meat isolates that which stated that *E. coli* bacteria were resistant to antibiotic Gentamycin by 12.5% and 62.5% were said to be sensitive (Barus et al. 2013). Gentamicin is an aminoglycoside class of antibiotics that have two or more amino groups that are bound to the benzene group and are bacteriosides.

The results of *E. coli* bacterial profile to antibiotics were found to be resistant isolates such as Ciprofloxacin and Tetracycline. *E. coli* bacteria resistant to antibiotics can transfer genetic factors to humans through the food chain or direct contact. The use of antibiotics in animals contributes to the occurrence of foodborne bacterial resistance in humans and animals (Pitout and Laupland 2008; Widodo et al. 2020).

Multidrug-resistant (MDR) commonly denotes the resistance of bacteria/ microbes to three or more antimicrobial classes (Magiorakos et al. 2012), as shown in Fig. 2. One method that is often used by various researchers to characterize organisms as MDR is based on *in vitro* antimicrobial susceptibility test results, when researchers tested resistance to multiple antimicrobial agents, classes or subclasses of antimicrobial agents (Okonko et al. 2009). An overview of this variability of definitions is given in a comprehensive review of MDR by Falagas et al. (2006), which is used as a reference by some researchers, as shown in table 2. The most common definitions used to determine the MDR of Gram-positive and Gram-negative bacteria that are resistant to three or more classes of antimicrobials (Exner et al. 2017).

In this study, ESBL-producing *E. coli* were obtained from cloacal swab samples, as shown in table 2 and figure 3, which showed that transmission of *E. coli* produced in the environment in poultry farms (Kwoji et al. 2019). In the production of food-producing animals, high concentrations of microorganisms in the air often occur in the environment in cages (Gao et al. 2015; Shoaib et al. 2016). The concentration of microorganisms is closely related to environmental quality. A bad environment can benefit the spread of ESBL-producing *E. coli*. These microbes in such an environment can survive in the form of aerosols for a long time in the air and transmit with airflow (Brower et al. 2017).

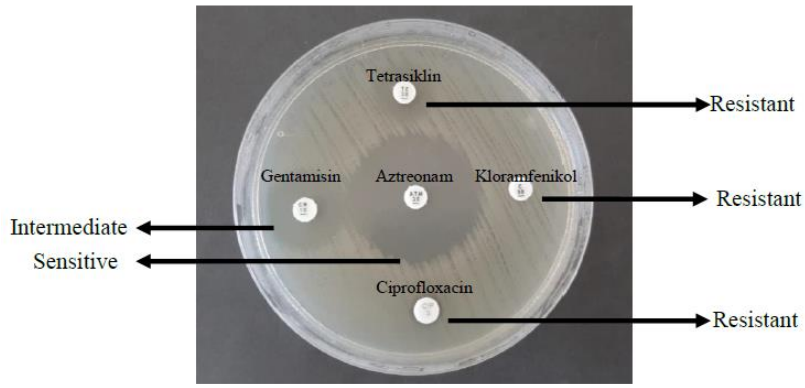

Figure2. MDR of *E. coli* from Antibiotic Sensitivity test

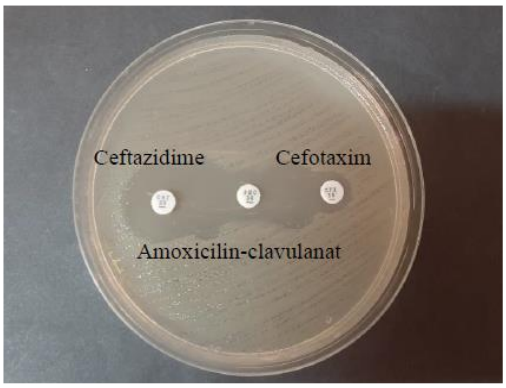

Figure 3. Extended Spectrum Beta-Lactamase (ESBL) producing *E. coli* by DDST

Previous studies have shown the spread of ESBL-producing *E. coli* from the surrounding area (Niasono et al. 2018). The human population can be exposed to antimicrobial resistant bacteria through encounter interactions with poultry sold in wet markets, which are the source of the presence of MDR and ESBL bacteria. This requires humans to be careful of poultry that can spread these isolates. However, more research is needed to understand how persistence and spread can be minimized (McEwen and FedorkaCray 2002).

In conclusion, this study confirmed that *E. coli* were found 100% from broiler chicken swabs. The isolates were resistant to Ciprofloxacin antibiotics by (67%), Tetracycline (65%), Gentamicin (37%), Chloramphenicol (23%), and Aztreonam (3%). The study identified twelve isolates for MDR and ESBL were two isolates. It can be concluded that broiler chicken from wet market should be considered as a source of transmission for MDR and ESBL of *E. coli* to the public health.

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