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Early Detection of *Escherichia coli* Producing Extended-Spectrum Beta-Lactamase (ESBL) in Cattle Farm Waste from Urban Jakarta

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Abstract – **This study examined the presence of *Escherichia coli* resistant to third-generation cephalosporins in cattle manure originating from an urban farming area in South Jakarta. The study aimed to detect presumptive extended-spectrum beta-lactamase (ESBL)-producing *E. coli* using total plate count analysis, selective culture, Gram staining, and biochemical identification. Urine and fecal waste samples were collected from a cattle farm located in close proximity to residential settlements. Samples were cultured on MacConkey Agar supplemented with cefotaxime, followed by Gram staining and IMViC biochemical tests. All isolates showed lactose-fermenting colonies with pink to red coloration on MacConkey Agar, Gram-negative rod morphology, and biochemical characteristics consistent with E. coli (Indole positive, Methyl Red positive, Voges–Proskauer negative, Citrate negative). Total bacterial counts reached approximately 10^3 colony-forming units per milliliter, with fecal samples exhibiting higher bacterial loads than urine samples. Bacterial growth on cefotaxime-supplemented media indicated phenotypic resistance to third-generation cephalosporins, suggesting the presence of presumptive ESBL-producing strains. These findings demonstrate that urban cattle manure may serve as a reservoir of antibiotic-resistant bacteria and highlight the need for confirmatory ESBL testing, strengthened antimicrobial resistance surveillance, and the implementation of integrated One Health strategies to mitigate environmental and public health risks.**

Keywords *– Antimicrobial Resistance; Cattle Farm Waste; Cephalosporin Resistance; Escherichia Coli; Urban Livestock.*

INTRODUCTION

A serious issue impacting world health for people and animals both, antimicrobial resistance (AMR) could lessens the efficacy of treatments for diseases and increasing the possibility of spreading dangerous bacteria. *Escherichia coli* production of Extended-Spectrum β-Lactamase enzymes, which can degrade medications from the cephalosporin family, is a main cause of AMR. Third generation drugs are those that include cefotaxime and ceftriaxone [1-2]. Unwise use of antibiotics in the livestock industry has been identified as a major contributor to the emergence of ESBL-producing bacteria. Particularly in cattle and poultry, antibiotics of the β-lactam group are frequently given as either preventative or growth boosters, therefore increasing the likelihood of resistant bacterial colonization in animal digestive systems [3]. These resistant bacteria might enter the environment from farm waste, including feces and urine, which, if not properly handled, could contaminate soil and cause problems. Air around it serves as a conduit for human and other animal species [4-5].

According to some research done in Indonesia, animals bear a lot of antibiotic-resistant *E. coli*. For

example, ESBL-producing *E. coli* strains have been found in cows from Badung, Bali [6] and in chickens raised in Surabaya [7]. The prevalence of ESBL-producing *E. coli* in agricultural waste associated with cattle rearing in urban environments, however, has not been thoroughly investigated particularly in Jakarta and the surrounding regions. Traditional cattle farming in densely populated regions such as in South Jakarta, may contribute to local microbiological contamination. Furthermore, Jakarta has seen a rise in ESBL bacterial infections according to certain clinical trials. For instance, a study at Gatot Soebroto Hospital found that over 60% of those having urinary tract infections carried *E. coli* producing ESBL. According to Widianingsih [8] Jakarta is regarded as among the areas most prone to the transmission of resistance genes across various sectors.

Although many clinical studies have been carried out, there is a lack of data concerning the occurrence of *E. coli* producing ESBL in environmental factors such as livestock farming in the Jakarta metropolitan region. Several earlier studies have detected *E. coli* suspected of producing ESBL directly from solid and liquid waste from farms, utilizing straightforward methods based on selective media (MacConkey + cefotaxime) for preliminary identification at laboratory level and examined the possible microbiological contamination from farms in the local community.

The aim of this research is to identify the occurrence of *E. coli* in the urine and feces (stool) of a cattle farm located in Kelurahan Srengseng Sawah, South Jakarta. This will be done by employing growth tests on MacConkey Agar media supplemented with cefotaxime, biochemical identification methods (IMViC), and total plate count (TPC). It is expected that the results of this study will offer initial insights into the possibility of antibiotic resistant bacteria in the city's environment and lay the groundwork for additional research using the One Health methodology.

METHODS

Ethical approval

This research solely focuses on the agricultural waste sample consisting of animal waste (dung and urine) and does not touch on human existence or intrusion. As a result, this research does not necessitate certification in animal ethics. Each phase of the sample gathering and evaluation is performed with attention to biosecurity guidelines and microbiological methodologies, following the standards set by the World Health Organization [2] and the FAO/WOAH One Health AMR criteria [3].

Study period and location

The investigation was conducted on October 2024 – June 2025. Samples were analyzed at University Al Azhar Indonesia's microbiology lab. According to GPS, the site was located at 6°21'56"S, 106°46'17"E.

Experimental design

This research employs descriptive-exploratory designs to identify the presence of *E. coli* by examining the outcomes of Extended-Spectrum β-Lactamase (ESBL) in both urine and feces samples. The design is derived from the Global Tricycle Surveillance Protocol [2] with slight modifications for culturally relevant early detection. The study's findings are as follows: 1. Collection of livestock waste samples, 2. Isolation and identification of suspected *E. coli* bacteria utilizing selective media and biochemical tests, 3. Descriptive analysis of isolation results and Total Plate Count (TPC) values. *E. coli* cultures from the SKHB IPB laboratory serve as positive controls, while sterile aquades are employed as negative controls [9].

Sampling procedure

The Indonesian National Standard (SNI) 6989.59:2008 concerning wastewater sampling techniques was followed for collecting the samples. Urine samples were taken from the cattle pen's drainage channel, and fecal samples were taken straight from the barn floor following defecation. Sterile 50 mL Falcon tubes (Corning Inc., USA) that had been three times cleaned with sterile distilled water were used for the sampling at 7 AM. To maintain microbial viability, all samples were put in bags lined with aluminum foil, kept in a cold box at around 4 °C, and brought to the lab in less than 2 hours [2,10].

Media culture and reagents

The selective medium for ESBL-producing bacteria is MacConkey Agar (MCA; Oxoid, UK) supplemented with cefotaxime (1 mg/L) [11]. Isolate purification using Tryptic Soy Agar (TSA). For IMViC biochemical tests, Simmons Citrate Agar (HiMedia, India), Sulfide Indole Motility (SIM), and MR-VP broth are used in compliance with SNI 2897:2008. When performing serial dilutions, PBS (Phosphate Buffered Saline; Oxoid, UK) is utilized. Biochemical reagents including Kovacs reagent,

methyl red, α-naphthol, and 40% KOH are used in conjunction with Gram staining reagents such crystal violet, iodine, 96% ethanol, and safranin [9]. Incubation was carried out using a digital incubator (Memmert INB200; Memmert GmbH Co. KG, Germany) for 24 hours at 37 °C. Gram staining and microscopic analyses were carried out with a compound microscope (Olympus CX23; Olympus Corporation, Japan).

Sample processing and bacterial isolation

Each sample was homogenized and serially diluted in sterile PBS up to a 10^{-4} dilution factor (in duplicate), following WHO guidelines. A volume of 100 µL from each dilution was inoculated onto MCA containing cefotaxime (1 mg/L) using the four-quadrant streaking technique [12]. After 24 hours of incubation at 37 °C, pink to red colonies with surrounding turbidity were selected as presumptive lactose-fermenting *E. coli*. Pure colonies were transferred to slanted TSA tubes for purification and stored at 4 °C for further testing.

Gram staining and biochemical identification

Gram staining was performed using the classical method [9], involving sequential application of crystal violet, iodine, ethanol, and safranin. Observations were made under 1000× magnification using immersion oil. Biochemical identification was conducted through four IMViC tests—Indole, Methyl Red, Voges-Proskauer, and Citrate—according to SNI 2897:2008. Isolates exhibiting the profile Indole (+), MR (+), VP (–), and Citrate (–) were confirmed as typical *E. coli* strains [13].

Total plate count (TPC)

Colony enumeration was performed using the Total Plate Count (TPC) method in accordance with SNI 2897:2008. Plates containing 25–250 colonies were counted and results were expressed in Colony Forming Units per milliliter (CFU/mL). Final values were reported as the mean of two replicates [14].

Data collection

Field data were obtained through direct sampling using sterile Falcon tubes (Corning Inc., USA) and transported in a temperature-controlled cool box (±4 °C). Laboratory data collection involved the use of a digital incubator (Memmert INB200; Germany) for microbial culture and a compound microscope (Olympus CX23; Japan) for morphological and staining analysis. Quantitative data were derived from colony counts using the TPC method, while qualitative data were obtained from biochemical profiling and Gram staining of bacterial isolates.

Data analysis

All data obtained from culture observations, Gram staining, and biochemical identification were analyzed descriptively, both quantitatively and qualitatively. Total Plate Count (TPC) values were calculated based on colony counts from dilution plates containing 25–250 colonies, and expressed in Colony Forming Units per milliliter (CFU/mL). The final TPC values were reported as the mean of two replicates. By monitoring colony development on MacConkey Agar enhanced with cefotaxime (1 mg/L), ESBL-producing *E. coli* was presumed to be identified. Lactose-fermenting *E. coli* colonies exhibiting distinctive shape, such as pink to red coloring with surrounding turbidity, were thought to be suggestive of possible ESBL activity. Without using statistical software, the data interpretation for this exploratory investigation concentrated on descriptive patterns of microbial presence and resistance features. Presumptive identification of ESBL-producing *E. coli* was determined by growth characteristics on MacConkey Agar supplemented with cefotaxime, following the criteria established by the Clinical and Laboratory Standards Institute [2,15]. No statistical software was applied in this preliminary study, as the analysis focused on descriptive interpretation of microbial presence and resistance indicators.

RESULTS AND DISCUSSION

Figure 1. Sampling location near to the residential area

The study was conducted at cattle farm in South Jakarta, an urban peri-domestic setting with high population density and open drainage. Animal housing was located 5–10 m from residential areas (Figure 1). The liquid waste was discharged directly to surrounding drains and solid manure was stored in open drums for natural composting. These site characteristics increase the likelihood of

environmental dissemination of bacteria and antimicrobial resistance determinants.

All isolates that grew on MacConkey agar supplemented with cefotaxime (1 mg/L) produced pink to red lactose-fermenting colonies with surrounding turbidity consistent with *E. coli* (Figure 2). The recovery of lactose-fermenting colonies on cefotaxime-containing medium indicates the presence of cephalosporin-resistant *E. coli* in both solid and liquid waste streams at this farm.

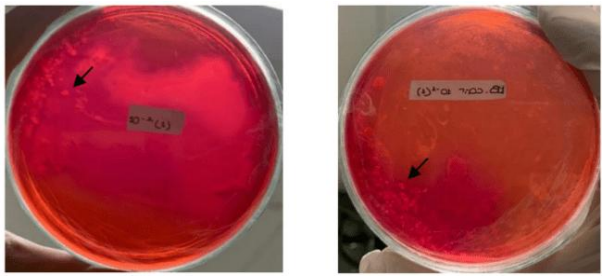

Figure 2. *E. coli* colonies (arrow) growth on MCA supplemented with cefotaxime

The IMViC profile strongly supports presumptive identification of the isolates as *E. coli* and justifies further phenotypic and genotypic confirmation for ESBL from Deni Banteng farm with 2 kinds of samples: urine and feces (Table 1).

Table 1. IMViC results for representative isolates

Test	Feces	Urine
Indole	+	+
Methyl Red	+	+
Voges Proskauer	-	-
Citrate	-	-

The TPC test showed solid waste (feces) yielded higher bacterial loads than liquid waste (urine) (Table 2).

Table 2. Total plate count (TPC) results from cefotaxime suplemented media

Sample type	Mean colony counts	Dilutions	TPC (CFU/mL)
Feces	65 (10^{-1}) / 38 (10^{-2})	10^{-1}–10^{-2}	2.2×10^3
Urine	46 (10^{-1}) / 28 (10^{-2})	10^{-1}–10^{-2}	1.6×10^3

TPC values were on the order of 10^3 CFU/mL for both sample types. The mean difference between urine and fecal material was $0{,}6 \times 10^3$ CFU/mL. TPC magnitudes ($\approx 10^3$ CFU/mL) show significant microbial loads in both solid and liquid agricultural waste and beyond conventional environmental safety thresholds cited in public health guidelines.

The combined results suggest that the observed pattern is supported by two complementary ecological factors. First, resistant *E. coli* populations in animal gut flora can be enriched by selection pressure caused by antibiotic exposure in livestock production which are then expelled in urine and feces [16-17]. Second, the farm's waste management methods which include open drain discharge, near to residents, and open manure storage. These support resistant bacteria and mobile genetic elements (MGEs) like plasmids carrying *bla*CTX-M or *bla*TEM spread and persist in the environment [18-19]. Environmental conditions in drains and composting drums can foster horizontal gene transfer through conjugation among Enterobacterales in microaerophilic or biofilm niches [20-21]. Detection of presumptive cephalosporin-resistant *E. coli* in both liquid and solid waste is consistent with these ecological clues. Our results align with global surveillance observations that animal production systems are important reservoirs of ESBL-producing Enterobacterales [22]. Studies across Southeast Asia have repeatedly reported *bla*CTX-M genes as dominant ESBL determinants in livestock and the environment [23-25]. The presence of presumptive ESBL *E. coli* in urban livestock mirrors findings from environmental surveillance studies reporting ESBL bacteria in surface waters, irrigation systems, and peri-urban farms [26-27]. In Indonesia specifically, most national data derive from clinical surveillance and food sampling [7,12]. However, environmental reservoirs such as urban cattle farms remain under reported. This study fills a local evidence gap and supports inclusion of urban farm waste streams in national anti microbial resistence monitoring.

Total plate count (TPC) values observed ($\approx 10^3$ CFU/mL) exceed several environmental guidance thresholds and indicate potential for human exposure via direct contact, contaminated household water, or agricultural reuse. In highly populated urban environments, even minor environmental contamination can contribute to persistent human colonization risk owing to frequent contact pathways and suboptimal sanitation infrastructure [28-29]. Colonization of humans with ESBL-producing *E. coli* often precedes clinical infections and contributes to community transmission [30]. The proximity of the cattle pen to residences (5–10 m) and the practice of open

drainage establish plausible exposure pathways for both direct and indirect transmission. Consequently, urban livestock operations can act as micro ecological interfaces that bridge animal and human reservoirs of clinically relevant resistance determinants [31].

Although laboratory and sampling techniques were appropriate for preliminary environmental monitoring, they still have limitations. Because selective growth on cefotaxime supplemented MacConkey agar only indicates third generation cephalosporin resistance and cannot distinguish ESBL from chromosomal mutations or AmpC hyperproduction, confirmation of *bla* genes requires targeted PCR or whole genome sequencing together with phenotypic tests such as double disk synergy or combined disk assays to determine plasmid contexts and gene variations [32-33]. The study presents findings from a single urban cattle farm using a small number of plated replicates and isolates due to its small sample size and single site approach. Therefore, there are limitations to external generalizability to other urban farms or regional epidemiology. Another constrain are the usage of selective media at a fixed cefotaxime concentration will preferentially recover resistant strains but may miss low-level resistant subpopulations or non-Enterobacterales carriers of ESBL genes. To address these limitations and translate findings into policy-relevant evidence we recommend some ways. Confirmatory phenotypic and genotypic testing for all presumptive isolates through combined disk or double disk synergy test, PCR for *bla*CTX-M/*bla*TEM/*bla*SHV, Sanger sequencing of PCR amplicons, targeted whole genome sequencing for plasmid and clonal context.

Extension of surveillance will assess prevalence and spatial distribution for several urban farms (soil, vegetables, drain water, and household water) using standardized sampling procedures. The collection of concurrent antimicrobial usage and animal health metadata will enable risk factor analysis. The measurement of resistome abundance throughout agricultural environment, combine culture-based and culture-independent techniques (qPCR, metagenomics tools) [34-35]. Beside that intervention trials such as sanitation improvements (waste treatment, covered manure storage, and artificial wetlands), limitation livestock use of antibiotics, and community education will drive the effects on human colonization and environmental resistance over time.

Affordable on-farm environmental controls should be prioritized to reduce microbial loads and the dissemination of mobile resistance elements. These disseminations include standardized composting protocols with controlled temperature and residence time, containment and treatment of liquid waste (settling, filtration or constructed wetlands), and simple drain interception systems. AMR monitoring frameworks should integrate the urban livestock sites with veterinarian supervision of antimicrobial use. Outlawing nontherapeutic antibiotic applications and implementing ongoing farmer education will support biosecurity.

Scientific evidence indicates that *bla*CTX-M types dominate ESBL ecology in animals and humans globally [23,36]. Indonesia's clinical surveillance shows rising ESBL prevalence in human isolates. This environmental finding suggests community and animal reservoirs may feed into the human epidemiologic pool. Including urban farms in national AMR mapping could improve contribution of community carriage and guide targeted interventions. This study shows that focused environmental surveillance at urban livestock facilities is both feasible and beneficial. A believable potent pathway for the spread of ESBL determinants to people and urban environment was highlighted by the recovery of presumed cephalosporin-resistant *E. coli* from both urine and feces. The findings support quick One Health-focused mitigation strategies such as better waste management, livestock antibiotic stewardship, and integrated surveillance that connects animal and human data. However confirmatory genotyping and wider surveillance are still needed.

CONCLUSION

Presumptive ESBL-producing *E. coli* were recovered from both solid and liquid waste at an urban cattle farm in Srengseng Sawah, Jakarta Selatan, and total bacterial loads exceeded common environmental safety thresholds. These findings identify urban farm waste as a potential local reservoir for cephalosporin-resistant E. coli and support urgent One Health actions including confirmatory laboratory testing, improved waste management, and integrated surveillance.

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