Resistance of Ampicillin, Ceftazidime, and Cefotaxime in Poultry’s *Escherichia coli*

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Abstract

Beta-lactam antibiotics are important antibiotics that are widely used in the field of human and animal health. Ampicillin resistance has been widely reported. Another increase in resistance is 3rd generation cephalosporins. The purpose of this study was to compare the ampicillin resistance profiles in 2019 and 2021 in the same *E. coli* isolates and to determine the resistance profiles of ampicillin, ceftazidime, and cefotaxime in live chicken *E. coli*. The research stages were the preparation of isolates; culture on differential selective media and checking the uniformity of bacterial cell morphology; biochemical test; bacterial DNA extraction; uspA gene amplification; visualization of amplification results; manufacture of bacterial suspensions; Kirby-Bauer disk diffusion resistance test; measurement of inhibition zones and determination of isolate status; and compared the ampicillin resistance test data. All isolates were confirmed positive for *E. coli*. The uspA gene (884 bp) was detected in all isolates. Ampicillin resistance in 2019 and 2021 in the same *E. coli* isolates when compared, there was no difference. Resistance test showed *E. coli* was resistant to ampicillin (100%), ceftazidime (15.4%), and cefotaxime (64.5%). The conclusion of the study was that there was no difference between the ampicillin resistance in 2019 and 2021 in *E. coli* isolates. *Escherichia coli* in this study had the highest resistance profile to ampicillin, followed by cefotaxime, and the lowest was ceftazidime.

Key words: *Escherichia coli*, resistance, ampicillin, ceftazidime, cefotaxime

Introduction

During the late 1950s and early 1960s, antibiotic resistance was detected for the first time, in enteric bacteria including *Salmonella*, *Shigella*, and *Escherichia coli*. These resistant strains cause great clinical, economic and mortality losses, especially in developing countries. However, in developed countries, the incidence of antibiotic resistance is considered a minor health problem because it is limited to enteric microbes. Understanding changed in the 1970s when *Neisseria gonorrhoeae* and *Haemophilus influenzae* were found to be resistant to ampicillin, whereas in the case of *Haemophilus* it was further reported to be resistant to tetracycline and chloramphenicol. The increasing use of antibiotics causes the incidence of resistance to accelerate, especially in developing countries because antibiotics are freely accessible without any prescription (Rossolini et al., 2014).
Beta-lactam antibiotics are important antibiotics that are widely used in the field of human and animal health (World Health Organization, 2021). Ampicillin belongs to the penicillin class of beta-lactam antibiotics. According to Roth et al. (2019) The penicillin class is used to treat diseases caused by E. coli in chickens in several countries. The World Health Organization has identified several classes of beta-lactams, namely 3rd, 4th, and 5th generation cephalosporins, and carbapenems for use in humans (WHO, 2022). Several beta-lactam antibiotics are exclusively used in the field of veterinary medicine, including ceftiofur and cefquinome, which consist of 3rd and 4th generation cephalosporins (Cameron-Veas et al., 2015). The alarming increase in 3rd generation cephalosporin-resistant bacteria reinforces suspicions of possible “unauthorized” use in chicks (Dutil et al., 2010). In the United States, the use of cephalosporins in poultry and other species is prohibited by the Food and Drug Administration (FDA) (Food and Drug Administration, 2012).

Ceftazidime and cefotaxime belong to the 3rd generation cephalosporins. Both antibiotics will be used in this study, along with ampicillin. Chicken farms do not use ceftazidime and cefotaxime but there are many reports abroad (Vinueza-Burgos, 2019) and few domestically (Witaningrum et al., 2020) regarding antibiotic resistance in chickens. Chen et al. (2014) also stated that ceftazidime resistance increased from 1993–2003 (18–27.2%). Cadena et al. (2007) said that bacteria with environmental conditions without exposure to antibiotics increased their sensitivity to certain antibiotics. Resistance to ampicillin, ceftazidime, and cefotaxime in this study was detected in E. coli. This study aimed to compare the resistance profile of ampicillin in 2019 and 2021 in the same E. coli isolates and determine the resistance profile of ampicillin, ceftazidime, and cefotaxime in E. coli from chicken.

Materials and Methods

Isolate

The identified E. coli isolates were archive isolates from the Medical Microbiology Laboratory, School of Veterinary Medicine and Biomedical Science, IPB University, Bogor, Indonesia. The number of E. coli isolates was 52 isolates (25 isolates from Sukabumi, 17 isolates from Bogor, and 10 isolates from Cianjur) derived from chicken cloacal swab samples. All from chicken cloacal swab samples. All isolates were stored at -20 ºC in tryptic soy broth (TSB) + 15% glycerol.

Microbiological Analysis

All E. coli isolates from TSB + 15% glycerol were grown on tryptic soy MacConkey agar (MCA) and eosin methylene blue agar (EMBA) media. Incubate for 18–24 hours at 37 ºC. The uniformity of bacterial cell morphology was seen by Gram staining (Markey et al., 2013).

Biochemical tests were carried out on triple sugar iron agar (TSIA), urea, and indol-methyl red-Voges Proskauer-citrate (IMViC) media. Bacterial incubation for the TSIA test, urease production, indole test and citrate test were carried out for 18–24 hours at 37 ºC. Bacterial incubation for methyl red and Voges-Proskauer assays was 24–48 hours at 37 ºC (Markey et al., 2013). Isolates identified as pure E. coli were phenotypically grown in TSA and stored at -4 C for further testing.
Isolate Confirmation

Bacterial DNA extraction using Presto™ Mini gDNA Bacteria Kit (Geneaid) according to manufacturing procedures. Polymerase chain reaction using forward 5'-CCG ATA CGC TGC CAA TCA GT-3' and reverse 5'-ACG CAG ACC GTA GGC CAG AT-3' (Mishra et al., 2017) with a PCR product of 884 bp. The PCR and visualization of PCR result using a previously described method (Hardiati et al., 2020).

Antibiotics Resistance Test

A total of 52 isolates of E. coli stored in TSA at 4 ºC were rejuvenated by re-culturing them in new TSA. The bacteria were incubated at 37 ºC for 18–24 hours. The rejuvenated bacterial colonies were suspended in sterile physiological NaCl until they reached the standard of 0.5 McFarland (1.5 × 10⁸ CFU/ml).

Resistance testing using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (MHA) media refers to the Clinical Laboratory and Standards Institute (CLSI) year 2020. The antibiotics used in the test were ampicillin (AMP) 10 g, ceftazidime (CAZ) 30 g, and cefotaxime (CTX) 30 g. The bacterial suspension that has been made is cultured evenly on MHA media using a sterile cotton bud. For 10–15 minutes, the cultured MHA was left until the surface of the medium dries. The three discs containing antibiotics were placed on the surface of the MHA using sterile tweezers. Bacteria were incubated at 35 ºC for 16–18 hours. This test was carried out in triples.

The zone of inhibition is a clear area around the antibiotic disc. The zone of inhibition was measured in millimeters (mm). The results of the inhibition zone measurements were averaged and matched with the standard antibiotic inhibition zone in the CLSI 2020 guidelines.

Results and Discussion

All of the archived E. coli isolates (100%) showed conventional test results according to the literature (Markey et al., 2013). The resistance of E. coli isolates when stored in TSB + 15% glycerol at -20 C in this study showed a 100% match with the initial observations before the bacteria were stored. The statement of Setiaji et al. (2015) support the results of this study. Setiaji et al. (2015) storage of Aeromonas hydrophilla in TSB + 15% glycerol media at -20 C provides growth resistance and does not change the characteristics of bacteria. TSB + 15% glycerol is the medium recommended by Handbooks Clinical Microbiology Procedures for maintaining bacterial cultures (Isenberg, 2004).

Universal stress proteins are proteins that are significantly expressed under unfavorable environmental stresses, such as nutrient starvation (lack of carbon, nitrogen, phosphate, sulfate, and amino acids), heat/cold stress, oxidative stress, heavy metal toxicity, transport chain release, electrons, exposure to polymyxin, cycloserine, ethanol and antibiotics and others (Kvint et al., 2003). Escherichia coli has six different USPs namely USPA, USPC, USPD, USPE, USPF and USPG. Each protein is encoded by a different gene. The uspA, uspC, uspD, uspE, uspF and uspG genes are genes encoding universal stress proteins (USP) A, C, D, E, F and G, respectively. Each USP in E. coli has its own specific function under certain environmental stresses (Nachin et al., 2005).
A total of 52 DNA samples were amplified against the uspA gene. The uspA gene was successfully amplified with an amplification product of 884 bp (Figure 1). According to Chen and Griffiths (1998) performing PCR amplification using the flank region primer of the uspA gene is a fast and effective method for screening non-pathogenic and pathogenic E. coli. Detection of E. coli by Mirzarazi et al. (2015) using primers from the uspA gene showed that all UPEC (Uropathogenic E. coli) isolates were positive. According to Godambe et al. (2017) and Bhowmik et al. (2022) the uspA gene is a genetic marker for the identification of E. coli by PCR method.

Figure 1. The results of uspA gene amplification with a product of 884 bp. M: 100 bp marker, 1–12: sample number 1–12

Ampicillin, ceftazidime, and cefotaxime used in this study are beta-lactam antibiotics. The condition of antibiotic resistance in E. coli from the three locations (Sukabumi, Bogor, and Cianjur) was dominated by ampicillin resistance. This is in line with research conducted by Khoirani et al. (2019) in chickens in West Java that 100% of E. coli isolates were resistant to ampicillin. Apart from chickens, E. coli resistance in various samples, animals and regions in Indonesia is considered quite high. Data on ampicillin resistance in E. coli isolated from cat samples at the Depok City veterinary clinic was 66% (Yaddi et al., 2020). Escherichia coli originating from Bali cattle was recorded as 80% ampicillin resistant (Mustika et al., 2015). Escherichia coli is considered as a reservoir bacterium and disseminator of antibiotic resistance (Tawfick et al., 2022). Ampicillin is a broad-spectrum antibiotic that has long been used in both humans and animals so it is not surprising that the level of bacterial resistance to ampicillin is high.

Figure 2. Antibiotics resistance tests on E. coli

Figure 3 presents ampicillin resistance profiles in E. coli isolates from three locations at different
testing times. The first test was carried out immediately after *E. coli* was isolated and identified from chicken cloacal swab samples. The second test was conducted 2 years later. The results of the first test showed that all isolates of *E. coli* were resistant to ampicillin. Resistance properties of *E. coli* still showed resistance after 2 years stored in TSB + 15% glycerol media at -20 °C. Dunai *et al.* (2019) cultured bacteria on media without exposure to antibiotics for 60 days by transferring to new media every day. Antibiotic resistance in bacteria can be reduced in potency within 480 generations during exposure to an antibiotic-free environment. Therefore, the rotation of the use of antibiotics is a very good policy and needs to be adhered to. Restricting the use of certain antibiotics will reduce the exposure of bacteria to certain antibiotics.

Figure 3. Comparison of ampicillin resistance profiles in *E. coli* tested in 2019 and 2021

Ceftazidime is a 3rd generation cephalosporin with activity against many Gram-negative bacteria that are resistant to other antibiotics. Ceftazidime is not an antibiotic that is approved for use in food-breeding animals by the FDA but is often used in zoos, exotics and pets. Ceftazidime has been used to treat infections from enteric Gram-negative bacteria in dogs and cats. It is also used to treat skin infections, tissue wounds, and before surgery (Papich, 2016).

Resistance of *E. coli* to ceftazidime (Figure 4). Ceftazidime resistance in this study was 15.4%, still quite low. This is in line with research conducted by Davis *et al.* (2018) that ceftazidime resistance from *E. coli* from chicken meat did not reach 20%. However, one study related to cephalosporin resistance in *E. coli* in laying hens in Blitar showed the prevalence of ceftazidime resistance was 94% (Witaningrum *et al.*, 2020). Nguyen *et al.* (2015) found the prevalence of ceftazidime resistance in *E. coli* was higher at 44.2%. *Escherichia coli* was isolated from chicken feces on farms. In livestock, differences in bacterial resistance to antibiotics are caused by many factors including treatment procedures, management systems, breeding environments and so on (Manyi-Loh *et al.*, 2018).

The use of ceftazidime in chickens has not been found. However, cephalosporins are one of the most commonly used classes of antibiotics in human medicine (Nguyen *et al.*, 2013). Therefore, there may be transmission of resistance traits from humans or other species (eg pigs) to chickens. A finding related to possible transmission of antibiotic resistance is the presence of third-generation cephalosporin resistance in ESBL-producing *E. coli* from fish ponds in integrated farms. This relationship is related to the contact of chickens with fish pond water. The relevance of human activity can also be correlated with antibiotic resistance in poultry (Van Minh *et al.*, 2013). Early attention to ceftazidime antibiotic resistance is needed because ceftazidime has the ability to fight bacteria that are resistant to other antibiotics (Papich, 2016).

Cefotaxime is a broad-spectrum antibiotic and belongs to the 3rd generation of cephalosporin
antibiotics. Cefotaxime is most commonly used to treat birds with bacterial infections of the brain but is also useful for other serious infections. Cefotaxime is not adequately absorbed after oral administration and must be administered intramuscularly or intravenously to be effective (Flammer, 2006). Although cefotaxime was not used in chickens, data on cefotaxime resistance in E. coli from the three sites (Sukabumi, Bogor, and Cianjur) ranked second after ampicillin. The prevalence of cefotaxime resistance in this study was 63.4% (Figure 4). Cefotaxime resistance in E. coli studied by Hering et al. (2016) from various samples (chicken feces, shoe swabs of coop officers, and cage dust) also showed a fairly high number, namely 77.6%. Even Vinueza-Burgos et al. (2019) found very high cefotaxime resistance results (98.3%) in chickens in Ecuador. However, Januari et al. (2019) stated that cefotaxime resistance to E. coli from chicken meat was still low (12%). Nevertheless, these conditions cannot be ignored and must remain a concern. According to Van Minh et al. (2013) there is a relevance between human activities and antibiotic resistance in poultry. Significant genetic similarities between strains of resistant E. coli from poultry and those found in humans were found in the study of Kluymans et al. (2013).

Figure 4. Resistance profile of ampicillin (AMP), ceftazidime (CAZ), and cefotaxime (CTX) in E. coli

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<th>Sukabumi</th>
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<td>CTX</td>
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**Figure 4. Resistance profile of ampicillin (AMP), ceftazidime (CAZ), and cefotaxime (CTX) in E. coli**
Conclusion

The properties of ampicillin resistance in 2019 and 2021 in the same *E. coli* isolates showed no difference. *Escherichia coli* in this study had the highest resistance profile to ampicillin, followed by cefotaxime, and the lowest was ceftazidime.

References


