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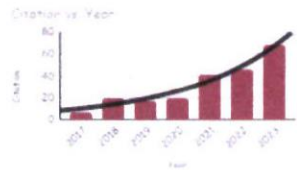
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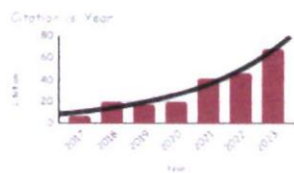


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Resistance Profile of Extended Spectrum Beta Lactamase-Producing *Escherichia coli* Bacteria using Vitek® 2 Compact Method

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ABSTRACT

This study aimed to determine the resistance profile and the nature of multidrug resistance in *Extended Spectrum Beta Lactamase* (ESBL)-producing *Escherichia coli* (*E.coli*) against several classes of antibiotics. Positive isolates of ESBL-producing *E.coli* were tested for antibiotic sensitivity using the VITEK® 2 compact method which then analyzed automatically. The results showed an antibiotic resistance profile against ESBL-producing *E.coli* showed the highest level of antibiotics in beta lactam, amoxicillin, ampicillin, cefazolin, cefotaxime, and ceftriaxone at 100%. Subsequent results found a relatively high level of resistance in the antibiotics aztreonam (86.36%), trimethoprim/sulfamethoxazole (77.27%), gentamicin (72.73%), and ciprofloxacin (68.18%). Antibiotics from carbapenem groups such as ertapenem and meropenem, and antibiotics from the aminoglycosides (amikacin) and tetracycline groups of tetracycline still showed a high sensitivity level of 100%. The most common resistance patterns found in ESBL-producing *E.coli* isolates are AM/AMP/KZ/CTX/CRO/ATM/GM/CIP as much as 22.73%, and AM/AMP/KZ/CTX/CRO/ATM/GM/CIP/SXT patterns of 18.2%. The results of multi-class antibiotic resistance showed that 86.36% had multidrug resistance. The highest multidrug resistance pattern in ESBL-producing *E.coli* occurred with a BL/AG/Q/SP pattern of 50%. Other patterns of multidrug resistance in ESBL-producing *E.coli* that can be found in this study are, the BL/AG/Q/SP pattern is 18.20%, the BL/AG/Q/SP pattern is 13.64%, and the BL/AG/Q pattern is 4.55%. The high profile of resistance and the nature of multidrug resistance in ESBL-producing *E.coli* has the potential to spread these resistant genes, thus risking the use of antibiotics as a public health therapy and animal health, therefore further evaluation and control are needed.

Keywords: ESBL-producing *Escherichia coli*, Multidrug resistance, Vitek® 2 Compact Method

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Introduction

Disease in commercial chicken is one of constraints causing the decline in economic value (Wiedosari and Wahyuwardani, 2015). Pathogenic microorganisms cause infectious diseases, which is one of a leading cause of death in animals or even human. The high prevalence of infectious disease is associated with the high use of antibiotic in health care (Noor and Peoleonga, 2005). A negative economic consequence in commercial chicken production can be caused by *Escherichia coli* (*E.coli*) infection. The existence of Extended Spectrum Beta Lactamase (ESBL)-producing *E.coli* on commercial chicken is highly

associated with *E.coli*, a normal microflora on gastrointestinal tract of commercial chicken. Feces and animal housing environment can facilitate the existence of *E.coli*, although, chicken show no symptoms at all of any diseases caused by the bacteria (Wibisono *et al.*, 2018).

Commercial chicken farm is one of sources of antibiotic resistance. The uncontrolled use of antibiotic causes the emergence of antibiotic resistance. Beta lactam antibiotic is the type of antibiotic commonly used in commercial chicken production. Resistance to beta lactam antibiotic can occur in ESBL-producing *E.coli* (Santos *et al.*, 2013; Hammerum *et al.*, 2014). Extended Spectrum Beta Lactamase is an enzyme that

causes resistance to wider spectrum on third generation of cephalosporins and mobobactams; thus, the antibiotic becomes ineffective. ESBL-producing bacteria can also be resistant to wide array of antibiotic class: aminoglycoside, tetracycline chloramphenicol, and sulfamethoxazole-trimethoprim (Brower *et al.*, 2017; Sudarwanto *et al.*, 2017). Incidence on multidrug resistance to third generation cephalosporins and other antibiotic classes are often found in ESBL-producing bacteria (Masrurh *et al.*, 2016). Multidrug resistance is a resistance to three or more different antibiotic classes (Handayani *et al.*, 2017).

Identification of ESBL bacteria is a problem in confirming a diagnosis at the laboratory, either in the therapeutic approach or in the field to prevent its spread. Clinical and Laboratory Standards Institute (CLSI) states that ESBL examination must be performed routinely. According to CLSI recommendation, ESBL detection consists of two steps. The first is an initial screen test, a filter test to reduce the susceptibility to more than one cephalosporin indicators (cefotaxime, ceftriaxone, ceftazidime, cefpodoxime) and aztreonam. The susceptibility reduction from cephalosporins shows positive results. A positive result from screening test is followed by the second step, ESBL confirmation test. The second step aims to detect the hydrolytic potency of ESBL against antibiotic used in the screening test. The ESBL confirmation test deliver a picture of collaboration action between ceftazidime or cefotaxime, and clavulanate acid (Amelia *et al.*, 2016; CLSI, 2017; Biutifasari, 2018).

Methods of identification, antibiotic sensitivity test, and ESBL-producing bacteria confirmation test are categorized into phenotype and genotype examination methods, with their advantages and limitations. Both phenotypic bacteria identification and antibiotic sensitivity is relatively easier than genotypic examination. Phenotypic examination is performed by using agar testing method or fully automated automatic method as in VITEK[®] 2 compact automated system. The VITEK[®] 2 compact automated system from Biomerieux works on calorimetric principle for identification, through biochemical and antibiotic sensitivity tests. The accuracy of VITEK[®] 2 compact automated system ranges from 97.8% (O'Hara, 2005) to 98.02% (Duggal *et al.*, 2012). This study aimed to determine resistance profile and the nature of multidrug resistance in ESBL-producing *E.coli* against several classes of antibiotic. Antibiotic resistance is a problem occurring across the globe, including Indonesia, both in human and animal health.

Materials and Methods

This study used commercial chicken cloaca swab sample, collected from commercial chicken farms in Blitar Regency. 22 positive ESBL-producing *E.coli* isolate were identified using IMBIC test and confirmed as ESBL-

producing *E.coli* using Double Disc Synergy (DDST) test (CLSI, 2017; Effendi *et al.*, 2018). Identification of ESBL-producing *E.coli* isolate was performed using VITEK[®] 2 GN card, while VITEK[®] 2 AST cards were used to determine the resistance profile. 3 ml of sterile saline solution (0.45 – 0.50%; pH 4.5 – 7.0) was put into plastic tube aseptically to make a bacterial suspension. *E. coli* isolate was isolated on MacConkey Agar (MCA) medium, incubated at 35-37°C for 20-24 hours. *E. coli* isolate was put into saline solution using sterile swab to create bacterial suspension. Bacterial suspensions were homogenized and 0.50 – 0.63 McFarland bacterial turbidity were made using VITEK[®] 2 DensiCHEK. These bacterial suspensions were inoculated to VITEK[®] 2 card no more than after 30 minutes. Bacterial suspension tubes and VITEK[®] 2 cards (GN and AST) were placed into special rack or cassette. Racks containing bacterial suspensions and cards were placed into vacuum chamber station for 30-60 seconds. Bacterial suspension was then transferred into wells. Transfer tubes were cut automatically. Cards were transferred to incubator room after 15 minutes, for approximately 8 hours at 35°C. Cards were then analyzed automatically. Antibiotic sensitivity test on ESBL-producing *E.coli* was carried out using VITEK[®] 2 compact, that consists of 18 antibiotics: amoxicillin, ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, cefotaxime, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, amikacin, gentamicin, ciprofloxacin, tigecycline, nitrofurantoin, dan trimethoprim/sulfamethoxazole. The results were automatically analyzed by system and interpreted as sensitive, intermediate, or resistant (Sugiartha, 2016; Biomerieux, 2017). ESBL-producing *E.coli* multidrug resistance in this study used six different antibiotic classes: beta lactam, aminoglycoside, quinolone, tetracycline, nitrofurantoin, and sulfonamide-potential.

Results and Discussion

The resistance test results of ESBL-producing *E.coli* from commercial chicken cloaca swabs against several classes of antibiotic (Figure 1) shows high level of resistance. The highest percentage of resistance was observed in amoxicillin, ampicillin, cefazolin, cefotaxime, and ceftriaxone, by 100%. This result is according to resistance study on ESBL-producing *E.coli* at RPHR of Bogor City that showed 100% level of resistance on penicillin and amoxicillin (Normaliska *et al.*, 2019). Amoxicillin, ampicillin, cefazolin, cefotaxime, and ceftriaxone are categorized as beta lactam class antibiotic, amoxicillin and ampicillin belong to penicillin, cefazolin belongs to first generation cephalosporins, cefotaxime and ceftriaxone is third generation cephalosporins class. *E.coli* which is often referred as ESBL-producing bacteria has ability to produce beta lactamase enzyme, an enzyme that able to inhibit beta lactam (Paterson and Bonomo, 2005). Beta lactam resistance

commonly occurs in gram negative bacteria, including *E.coli* that can be isolated from animal products. Poultry is known as ESBL-producing *E.coli* reservoir (Schmid *et al.*, 2013; Hammerum *et al.*, 2014). The high resistance level of ESBL-producing *E.coli* from commercial chicken cloaca swab on beta lactam class antibiotic is caused by the presence of ESBL enzyme in *E.coli*, which is not limited on digestive tract, but also in the cage, feces, and surrounding environment (Schroeder *et al.*, 2004). The enzyme is not only able to hydrolyze penicillin, but also third generation cephalosporins and monobactam (Paterson and Bonomo, 2005; Nuangmek *et al.*, 2018).

A relatively high level resistance of ESBL-producing bacteria was observed on aztreonam, trime thoprim/sulfamethoxazole, gentamicin, and ciprofloxacin, by 86.36%, 77.27%, 72.73%, and 68.18% consecutively, as the previous study with relatively high level of resistance of ciprofloxacin (50.0%), gentamicin (60.0%), trimethoprim-sulfamethoxazole (60%, and ciprofloxacin (40.0%) (Cormican *et al.*, 1996; *et al.*Kürekci *et al.*, 2017; Sudarwanto *et al.*, 2017). Other antibiotics such as ertapenem and mempenem from carbapenem class, amikacin (aminoglycoside class), and tigecycline (tetracycline class) still show high level of sensitivity (100%), according to mechanism of action of ESBL which hydrolyze penicillin class antibiotics, first, second, and third generations of cephalosporin, and monobactam antibiotic class, but inactive on carbapenem class (imipenem, meropenem, ertapenem) (Lim *et al.*, 2013; Biutifasari, 2018). Nitrofurantoin has lower resistance level than trimethoprim-sulphamethoxazole and fluoroquinolone (Rank *et al.*, 2018).

The susceptibility result shows a nearly similar pattern for all isolates (Table 1). The most resistance pattern seen on ESBL-producing *E.coli* was AM/AMP/KZ/CTX/CRO/ATM/GM/CIP pattern (22.73% or 5/22), and AM/AMP/KZ/CTX/CRO/ATM/GM/CIP/SXT (18.2% or 4/22). The result of t multiclass antibiotic resistance shows 86.36% (19/22) had multidrug resistance (Table 2). Multidrug resistance is certain bacterial resistance to three or more different antibacterial class (Kurniawati *et al.*, 2015). Multidrug resistance is a problem that hard to resolve in disease treatment. This condition is caused by the practice of the antibiotic use as disease preventive measures in commercial chicken production. Antibiotic has been used to control the level of morbidity, mortality, and infection of *E.coli*. While antibiotics are mostly used as therapeutic, several antibiotic classes are used for sub therapeutic purpose to prevent the emergence of disease in animal production (Niasono *et al.*, 2019). The use of antibiotic at sub therapeutic level can lead to the emergence of multidrug resistance (Wang *et al.*, 2015).

The highest level of multidrug resistance pater of ESBL-producing *E.coli* was observed on BL/AG/Q/SP pattern (50% or 11/22). Other multidrug resistance patterns found in this study were BL/AG/Q/SP (18.20% or 4/22), BL/AG/Q/SP (13.64% or 3/22), and BL/AG/Q (4.55% or 1/22). This study demonstrates that the high incidence of multidrug resistance can indicate the high risk of transmission on other chicken at the farm, to cause a transmission of antibiotic resistance on multi class antibiotics. The condition can result in the high potential failure of antibiotic treatment

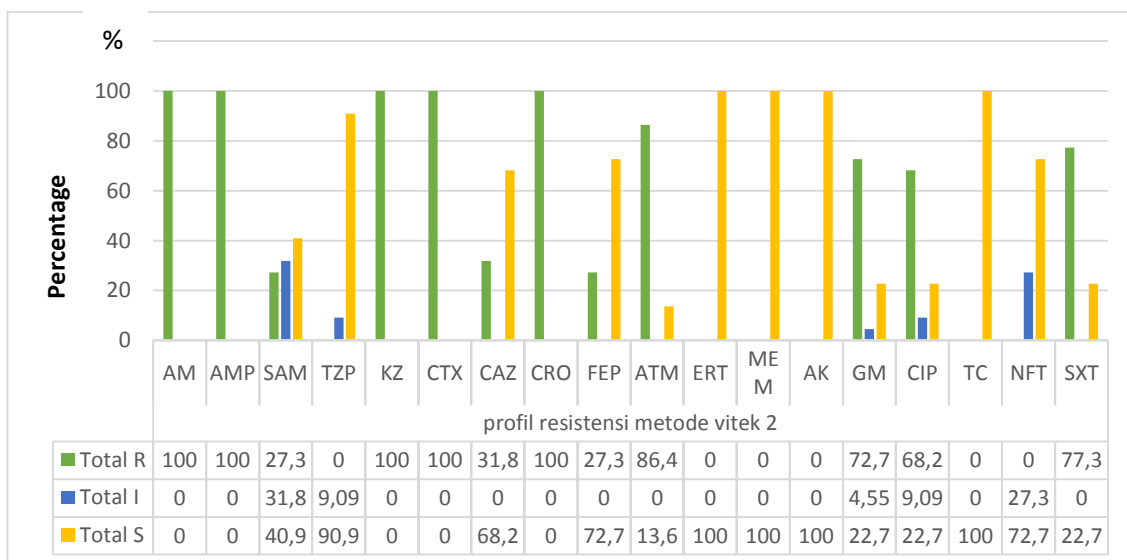


Figure 1. Resistance profiles of ESBL-producing *E.coli* using VITEK[®] 2 method (Amoxicillin = AM; Ampicillin = AMP; Ampicillin/sulbactam = SAM; Piperacillin/Tazobactam = TZP; Cefazolin = KZ; Cefotaxime = CTX; Cefazidime = CAZ; Ceftriaxone = CRO; Cefepime = FEP; Aztreonam = ATM; Ertapenem = ERT; meropenem = MEM; amikacin = AK; gentamicin = GM; ciprofloxacin = CIP; tigecycline = TC; nitrofurantoin = NFT; trimethoprim/sulfamethoxazole = SXT; R = Resistance; I = Intermediate; S = Susceptible)

Table 1. Isolates belonging to individual antibiotic resistance profiles

Code	Antibiotic																Anti-microbial Resistance Profiles		
	A M	A M P	S A M	T Z P	K Z	C T X	C A Z	C R O	F E P	A T M	E R T	M E M	A K	G M	C I P	T C		N F T	S X T
Ec1	R	R	S	S	R	R	S	R	S	R	S	S	R	R	S	S	S	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP	
Ec2	R	R	S	S	R	R	S	R	S	R	S	S	R	R	S	S	R	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP/SXT	
Ec3	R	R	S	S	R	R	S	R	S	R	S	S	R	R	S	S	R	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP/SXT	
Ec4	R	R	I	S	R	R	S	R	S	R	S	S	R	R	S	I	S	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP	
Ec5	R	R	S	S	R	R	S	R	S	R	S	S	R	R	S	S	R	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP/SXT	
Ec6	R	R	I	S	R	R	S	R	S	S	S	S	R	R	S	S	S	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP	
Ec7	R	R	R	S	R	R	R	R	S	R	S	S	S	I	S	S	R	AM/AMP/SAM/KZ/CTX/CAZ/CRO/ATM/SXT	
Ec8	R	R	S	S	R	R	S	R	S	R	S	S	R	S	S	S	R	AM/AMP/KZ/CTX/CRO/ATM/GM/SXT	
Ec9	R	R	I	S	R	R	S	R	S	R	S	S	R	S	S	S	R	AM/AMP/KZ/CTX/CRO/ATM/GM/SXT	
Ec10	R	R	S	S	R	R	S	R	S	S	S	S	R	S	S	I	R	AM/AMP/KZ/CTX/CRO/GM/SXT	
Ec11	R	R	I	S	R	R	S	R	S	S	S	S	R	R	S	S	R	AM/AMP/KZ/CTX/CRO/GM/CIP/SXT	
Ec12	R	R	I	S	R	R	S	R	S	R	S	S	R	R	S	I	S	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP	
Ec13	R	R	S	S	R	R	S	R	S	R	S	S	R	R	S	S	R	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP/SXT	
Ec14	R	R	S	S	R	R	S	R	S	R	S	S	R	R	S	S	S	AM/AMP/KZ/CTX/CRO/ATM/GM/CIP	
Ec15	R	R	S	S	R	R	S	R	S	R	S	S	S	I	S	S	R	AM/AMP/KZ/CTX/CRO/ATM/SXT	
Ec16	R	R	I	S	R	R	R	R	R	R	S	S	R	R	S	S	R	AM/AMP/KZ/CTX/CAZ/CRO/FEP/ATM/GM/CIP/SXT	
Ec17	R	R	R	I	R	R	R	R	R	R	S	S	S	S	R	S	I	AM/AMP/SAM/KZ/CTX/CAZ/CRO/FEP/ATM/CIP/SXT	
Ec18	R	R	R	S	R	R	R	R	R	R	S	S	S	R	R	S	S	AM/AMP/SAM/KZ/CTX/CAZ/CRO/FEP/ATM/GM/CIP/SXT	
Ec19	R	R	R	S	R	R	R	R	R	R	S	S	S	S	R	S	I	AM/AMP/SAM/KZ/CTX/CAZ/CRO/FEP/ATM/CIP/SXT	
Ec20	R	R	R	I	R	R	R	R	R	R	S	S	S	I	S	S	I	AM/AMP/SAM/KZ/CTX/CAZ/CRO/FEP/ATM/SXT	
Ec21	R	R	R	S	R	R	S	R	S	R	S	S	R	S	S	S	R	AM/AMP/SAM/KZ/CTX/CRO/ATM/GM/SXT	
Ec22	R	R	I	S	R	R	R	R	R	R	S	S	S	S	R	S	S	AM/AMP/KZ/CTX/CAZ/CRO/FEP/ATM/CIP/SXT	
Total	R	22	22	6	0	22	22	7	22	6	19	0	0	0	1	1	0	0	1
I	0	0	7	2	0	0	0	0	0	0	0	0	0	0	1	2	0	6	0
S	0	0	9	20	0	0	15	0	16	3	2	2	2	5	5	2	1	5	

(Amoxicillin = AM; Ampicillin = AMP; Ampicillin/sulbactam = SAM; Piperacillin/Tazobactam = TZP; Cefazolin = KZ; Cefotaxime = CTX; Ceftriaxone = CAZ; Cefepime = FEP; Aztreonam = ATM; Ertapenem = ERT; meropenem = MEM; amikacin = AK; gentamicin = GM; ciprofloxacin = CIP; tigecycline = TC; nitrofurantoin = NFT; trimethoprim/sulfamethoxazole = SXT; R = Resistance; I = Intermediate; S = Susceptible)

Table 2. Multidrug resistance pattern of ESBL-producing *E. coli* isolate

Sample code	Beta lactam	Aminoglycoside	Quinolones	Tetracycline	Nitrofurantoin	Sulfonamide potential	MDR	Multidrug Resistance Pattern
Ec1	R	R	R	S	S	S	MDR	BL/AG/Q
Ec2	R	R	R	S	S	R	MDR	BL/AG/Q/SP
Ec3	R	R	R	S	S	R	MDR	BL/AG/Q/SP
Ec4	R	R	R	S	I	S	MDR	BL/AG/Q
Ec5	R	R	R	S	S	R	MDR	BL/AG/Q/SP
Ec6	R	R	R	S	S	S	MDR	BL/AG/Q/NF/SP
Ec7	R	S	I	S	S	R	NEGATIF	BL/SP
Ec8	R	R	S	S	S	R	MDR	BL/AG/SP
Ec9	R	R	S	S	S	R	MDR	BL/AG/SP
Ec10	R	R	S	S	I	R	MDR	BL/AG/SP
Ec11	R	R	R	S	S	R	MDR	BL/AG/Q/SP
Ec12	R	R	R	S	I	S	MDR	BL/AG/Q
Ec13	R	R	R	S	S	R	MDR	BL/AG/Q/SP
Ec14	R	R	R	S	S	S	MDR	BL/AG/Q
Ec15	R	S	I	S	S	R	NEGATIF	BL/SP
Ec16	R	R	R	S	S	R	MDR	BL/AG/Q/SP
Ec17	R	S	R	S	I	R	MDR	BL/AG/Q/SP
Ec18	R	R	R	S	S	R	MDR	BL/AG/Q/SP
Ec19	R	S	R	S	I	R	MDR	BL/AG/Q/SP
Ec20	R	I	S	S	I	R	NEGATIF	BL/SP
Ec21	R	R	S	S	S	R	MDR	BL/AG/SP
Ec22	R	S	R	S	S	R	MDR	BL/AG/Q/SP

(Beta laktam = BL; Aminoglycoside = AG; Quinolones = Q; Tetracycline = TC; Nitrofurantoin = NF; Sulfonamide potential = SP; R = Resistance; I = Intermediate; S = Susceptible).

during the existence of infectious disease at the farm. The different resistance pattern arises due to the varied antibiotic combinations used by farmers, as stated by Bywater *et al.* (2014) in which various type of antibiotics, geographic conditions, and production system are factors causing the different resistance pattern.

Conclusion

The highest antibiotic resistance of ESBL-producing *E.coli* was observed on amoxicillin, ampicillin, cefazolin, cefotaxime, and ceftriaxone (100%). Ertapenem and mempenem from carbapenem antibiotic class, and amikacin (aminoglycoside class), and tigecycline (tetracycline class) still display high sensitivity (100%). The most resistance pattern found in this study was AM/AMP/KZ/CTX/CRO/ATM/GM/CIP (22.73%), and AM/AMP/KZ/CTX/CRO/ATM/GM/CIP/SXT (18.2%). The result of multi class antibiotic resistance exhibits 86.36% are multidrug resistant, with the highest multidrug resistance occurred in BL/AG/Q/SP (50%).

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