ISSN-0976-0245 (Print) • ISSN-0976-5506 (Electronic)

Volume 11 / Number 03 / March 2020

Indian Journal of Public Health Research & Development

An International Journal

Website:
www.ijphrd.com

Indian Journal of Public Health Research & Development

Print-ISSN: 0976-0245-Electronic-ISSN: 0976-5506, Frequency: Quarterly (Four issues per volume)

Indian Journal of Public Health Research & Development is a double blind peer reviewed international journal. It deals with all aspects of Public Health including Community Medicine, Public Health, Epidemiology, Occupational Health, Environmental Hazards, Clinical Research, and Public Health Laws and covers all medical specialties concerned with research and development for the masses. The journal strongly encourages reports of research carried out within Indian continent and South East Asia.

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Editor

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Printed, published and owned by

Dr. R.K. Sharma
Institute of Medico-legal Publications
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Published at

Institute of Medico-legal Publications
Logix Office Tower, Unit No. 1704, Logix City Centre Mall, Sector- 32, Noida - 201 301 (Uttar Pradesh)

Indian Journal of Public Health Research & Development

www.ijphrd.com

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Volume 11, March 03 March 2020

Potential Hazards of Antibiotics Resistance On Escherichia Coli Isolated From Cloacal Swab In Several Layer Poultry Farms, Blitar, Indonesia

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Abstract

Objective: The study was isolated Escherichia coli from cloacalswab of layer poultry farms in Blitar area to investigate their antibiotic sensitivity pattern.

Materials and Method: Fourty swab cloacal samples were collected from 8 farms where located in Blitar for 3 months. In order for MacConkey agar to be a medium for inoculation and biochemical tests to identify isolates such as IMViC and TSIA test were performed. The method of antibiotic sensitivity pattern of Escherichia coli was tested by disk diffusion.

Results: The result revealed40 samples, 34 samples were exposed to contaminationbyE.coli. The pattern of antibiotic sensitivity showed high resistance against ampicillin (62%), ciprofloxacin (56%), tetracycline and trimethrophim sulfamethoxazole (53%). Sensitive antibiotics were also observed for amoxicillin clavulanic acid, cefepime, ampicillin sulbactam, amikacin and meropenem. The presence of MDR and ESBL-producing *Escherichia coli* isolated from cloacal swabs of layer poultry farms in Blitar were 47.1% (16/34) and 5.9% (2/34), respectively.

Conclusion: This research to find out exposed the layer poultry farms to consider critical antibiotic resistance of E. coli and regarded potential public health hazards.

Keywords: *Layer poultry farms, Escherichia coli, public health hazards, MDR, ESBL.*

Introduction

Antibiotic resistance is major global health in worldwide especially in poultry, such as treatment failure economic losses and source of resistant organism that may represent a risk to human health. Antimicrobial usage in animal production raised with intensive conditions using large amount of antimicrobial to prevent and treat disease[(1)]. Enterobacteria especially Escherichia coli is the pathogenic bacteria who receive antibiotic treatment in the gastrointestinal tract of animals and humans into the environment which not only devolvement of the bacterial but antimicrobial affect more than one antibiotic and become multi drug resistance[(2)]. Antimicrobials are commonly used in animals which human consume the animalsproduct and antimicrobial resistance can controlled by reduce antibiotics use for animals, overuse of antibiotic and residues can lead to more drug resistance among microbe[(3)].

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Escherichia coli is a Gram-negative bacterium commonly found in animal and human intestinal tract. Exposure of antibiotic use in animals to inhibit microbial can cause resistance to antibiotics, antibiotics resistance appearance of Escherichia coli in poultry is main purpose to reduce transmission of resistance in the region. Observation ofantibiotics resistance especially investigate the resistance appearance of microba commonly found in poultry. The bacteria commonly detected in the environment so that it can enter the digestive tract of animals is Escherichia coli[4].

Antibiotics resistance is caused by a factor already arise on the bacteria. E. coli bacteria which genes that preserve to imunity from the influence of antibiotics derived from the plasmid. E. coli was detected possess plasmids to some drug resistance genes [5]. Plasmids can carrying the resistance genes in bacteria sensitive to antibiotics [6]. This study usedsome antibiotics that were often associated with data about the problem of resistance in E. coli and then the antibiotic sensitivity test to determine the profile antibiotics resistance of E. coli. The purpose of this study were also to exhibit occurence of multidrug resistant (MDR) of E. coli and extended spectrum beta-lactamase (ESBL) producing E. coli from layer poultry farms.

Materials and Method

Isolation and Identification of E. coli: The study collected 40 samples and taken from 8 layer poultry farms in Blitar, East Java, shown on table 1. Purposive sampling of layer farm was based on some specification such as low sanitation, lack of cleanliness and proper hygiene management by the farm and low maintenance [7]. Samples obtained from cloacal swab brought to the laboratory in Amies medium transport wrapped sterile conditions and were taken using a cool-box[8]. Samples were inoculated streaked onto MacConkey media agar and incubated at 37 ° C for 18 ± 24 hours [9]. Colonies that showed lactose-fermenting was purified and continued positive presumptive test of E. coli. Identification of bacteria were performed using morphological and biochemistry. Biochemical tests include tests Indole, Methyl Red, Vagos-Pasteur, Simon Citrate (IMViC) and TSIA to determine the level of genus and continued until the sugar fermentation test to determine the species of E. coli[10].

Antibiotic Sensitivity Test: Antibiotic sensitivity testing was done using Kirby-Bauer disc diffusion assay on medium Mueller-Hilton agar[11,12]. Antibiotics and concentration used was ampicillin (10 μg), chloramphenicol (30 μg), gentamicin (10 μg), ciprofloxacin (10 μg), trimethoprim-sulfamethoxazole (25 μg), ceftazidime (30 μg), amoxicillin clavulanic acid (30 μg), cefepime (30 μg), ampicillin sulbactam (20 μg), cephazolin (30 μg), amikacin (30), tetracycline (30 μg), levofloxacin (5 μg) and meropenem (10 μg). Interpretation of the antibiotic resistance use the recommendation of the Clinical and Laboratory Standards Institute was through measurement of inhibitory zone diameter formed in study [13].

Confirmation ESBL using Double Disc Synergy Test (DDST): Test for ESBL in E. coli by used disk antibiotic CAZ/Ceftazidime 30μg, AMC/Amoxicillin Clavulanic Acid 30μg, CTX/Cefotaxime 30μg, ATM/ Aztreonam 30μg and inoculation on Muller-Hinton agar plate, shown on figure 2. The result showed measuring inhibition of the diameter inhibitory zone formed on Clinical and Laboratory Standards Institutions [13].

Results and Discussion

In this study, a total of 40 cloacal swab samples were collected from chickens in layer poultry farms in Blitar and screened for the presence of multidrug resistant (MDR) and Extended Spectrum Beta-lactamase (ESBL)-producing E. coli, shown on table 2. Total prevalence of 47.1% of E. coli was obtained with the MDR casesand ESBL cases was 5.9%inlayer chicken, shown on table 3. This agrees with the findings of Kwoji et al. where a similar occurrence of E. coli from chickens was also reported[14].

This study used fourteen antibiotics against Escherichia coli, the results of antibiotic sensitivity test of Escherichia coli showed that the antibiotic ampicillin occurrence of resistance higher at (62%), ciprofloxacin (56%), tetracycline and trimethrophim sulfamethoxazole (53%), levofloxacin (35%), gentamycin (18%), ceftazidime and cefepime (6%) cephazolin and chloramphenicol (3%) but in this study Escherichia coli sensitive to the antibiotic amoxicillin clavulanic acid, ampicillin sulbactam, amikacin and meropenem, shown on table 2 and figure 1. Sensitivity of microbes to antibiotic can resistant depend on commonly used of antibiotics [15]. These results are ampicillin which the highest of antibiotic resistance against Escherichia coli[16].

Table 1. Location of samples, sample size, results of isolation of E. coli and results of MDR and ESBL cases.

Location	Number of samples	PositiveE.coli	Positive MDR	Positive ESBL by DDST
Farm 1	5	4	0	0
Farm 2	5	4	2	0
Farm 3	5	5	3	0
Farm 4	5	5	5	0
Farm 5	5	4	1	0
Farm 6	5	4	1	1
Farm 7	5	4	2	1
Farm 8	5	4	2	0
Total	40	34	16	2

Table 2. Antibiotic susceptibility profiles of 34 E. coli from cloacal swabs of layer poultry farms in Blitar.

Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)
Ceftazidime (CAZ)	2 (6%)	0	32 (94%)
Amoxicillin Clavulanic Acid (AMC)	0	0	34 (100%)
Cefepime (FEP)	2(6%)	0	32 (94%)
Ampicillin Sulbactam (SAM)	0	0	34 (100%)
Cephazolin (KZ)	1(3%)	0	33 (97%)
Gentamycin (GN)	6(18%)	0	28 (82%)
Amikacin (AK)	0	0	34 (100%)
Tetracycline (TE)	18(53%)	5(15%)	11 (32%)
Ciprofloxacin (CIP)	19 (56%)	3(9%)	12 (35%)
Levofloxacin (LEV)	12 (35%)	6(18%)	16 (47%)
Meropenem (MEM)	0	0	34 (100%)
Chloramphenicol (C)	1(3%)	0	33 (97%)
Trimethrophim-sulfamethoxazole (SXT)	18(53%)	0	16 (47%)
Ampicillin (AMP)	21 (62%)	0	13 (38%)

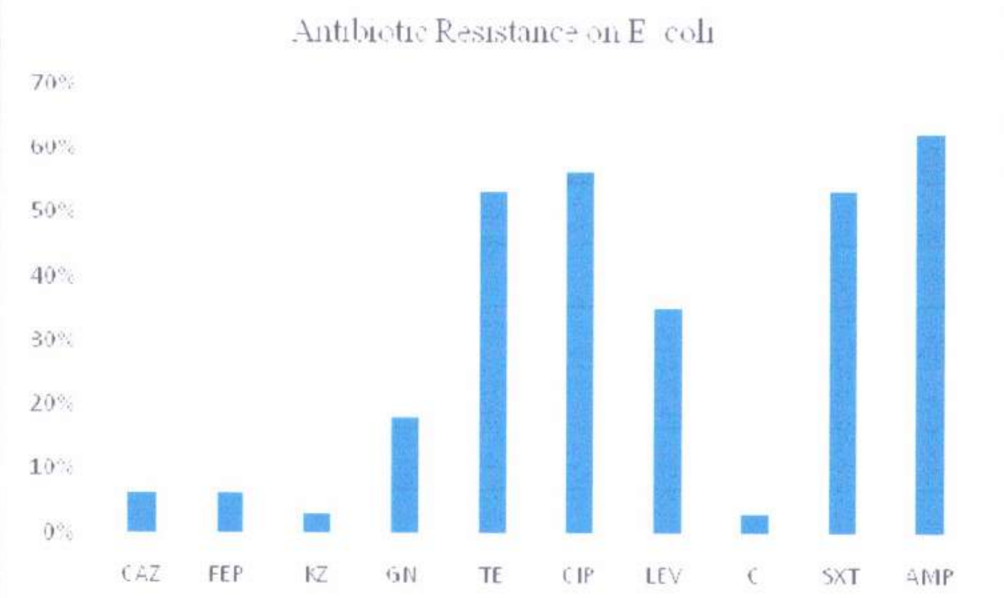

Figure 1. Percentage of antibiotic resistance on E. coli

Multidrug resistant (MDR) as an organism that is resistant to three or more antimicrobial classes [17]. 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 agent[18]. The most commonly used definitions for Gram-positive[19] and Gram-negative bacteria that are resistant to three or moreantimicrobial class [20]. An overview of this variability of definitions is given in a comprehensive review of MDR [21], which is used as a reference by some researchers that a large number of studies do not propose specific definitions for MDR.

There were 16 multidrug resistant (MDR) and 2 extended spectrum beta lactams (ESBL) in this study, shown on table 3.The consume of antibiotics raised antibiotics resistance contain important MDR organisms in poultry. These MDR organisms can transmission to the human through direct contact or consumption and *E. coli* is the most caused high economic losses and food contamination rates which obtained antibiotic-resistant genes with the potential to spread to other populations. The abundant use of antibiotics in poultry farms has been associated with treatment failure and the development of antibiotic resistance itself. A study showed that *E. coli* from poultry in China were resistant to at least 18 different antibiotics [22].

Table 3. Cases of MDR and ESBL of E. coli from cloacall swabs of Layer poultry

Sample Code	Phenotipe Resistance	Type of Resistance
L2A	TE, CIP, SXT	MDR
L2B	CIP, LEV, SXT	MDR
L3A	CIP, LEV, SXT	MDR
L3D	TE, SXT, AMP	MDR
L3E	TE, SXT, AMP	MDR
L4C	TE, CIP, SXT	MDR
L7E	TE, CIP, AMP\	MDR
L8B	TE, SXT, AMP	MDR
L8C	TE, CIP, LEV	MDR
L5C	TE, CIP, SXT, AMP	MDR
L7B	TE, CIP, LEV, AMP	MDR
L4B	GN, CIP, LEV, SXT, AMP	MDR
L4D	GN, CIP, LEV, SXT, AMP	MDR
L4E	GN, CIP, LEV, SXT, AMP	MDR
L6B	TE, CIP, LEV, SXT, AMP	MDR
L4A	GN, CIP, LEV, C, SXT, AMP	MDR
L6A	CAZ, FEP, GN, TE, CIP, LEV, AMP	ESBL
L7D	CAZ, FEP, KZ, GN, TE, CIP, LEV, SXT, AMP	ESBL

In our research findings, 100% of ESBL-producing isolates showed multi-drug resistance to various families of antibiotics. This finding correlates with other studies in other countries such as Switzerland[23], Zambia[24] and in Turkey[25] almost all ESBL-producing E. coli isolates found in animals are multi-resistant.

The nature of multidrug resistance of these isolates may be explained by the fact that ESBL is mediated by plasmids carrying multiresistant genes by plasmids, transposons and integrons and also they are ready to be transferred to other bacteria, not necessarily same species. Bacteria with various resistance to antibiotics are widely distributed in animals and the environment[14]. The facts supported by recent surveys from China[22], Thailand[26] and Indonesia[27], have illustrated an alarming trend related to resistance among ESBL-producing organisms isolated from animals and the environment. Our results support the fact that ESBL producers provide a high level of resistance to not only third generation cephalosporins but also other non beta-lactam antibiotics groups, shown on table 3.

This study also revealed that all ESBL producers and almost isolates that MDR showed resistance to ampicillin. In contrast, better susceptibility was observed to amikacin and no resistance was observed with meropenem. Better susceptibility to amikacin and meopenem were also noted and can be explained by the absence of routine use of amikacin as empirical therapy on poultry farms and the absence of sufficient cross resistance with the beta-lactam antibiotic group.

Figure 2. Confirmation of ESBL by Double Disc Synergy Test (DDST)

Conclusion

The high percentage of drug resistance in E. coli isolates were detected ampicillin, ciprofloxacin, tetracycline and trimethrophim sulfamethoxazole more than 50%. The number of MDR of E. coli isolates was significantly higher in healthy poultry, namely 16 isolates and found also 2 ESBL producing E. coli isolates. This general description of the antimicrobial resistance of these poultry bacteria creates the basis for future investigations and analyzes of resistance development in Blitar, East Java, Indonesia. In view of this, we strongly recommend assessing treatment plans in the poultry industry in Blitar to ensure prudent antimicrobial use and to minimize the potential for the spread of resistant bacteria from poultry to the environment and humans.

Acknowledgement: This study was supported in part with the Penelitian Hibah Mandat Funding from Airlangga University, Indonesia in fiscal year 2019.

Ethical Clearance: Cloacal swabs were used in this study, hence ethical clearance was not necessary. Cloacal swab samples were collected from Blitar area in East Java province, Indonesia.

Conflict of Interest: Nil.

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