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Biological Hazard on Multidrug Resistance (MDR) of Escherichia Coli Collected From Cloacal Swab of Broiler Chicken on Wet Markets Surabaya

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Abstract

Objective: The purpose of this study was to determine the multidrug resistance (MDR) profile of Escherichia coli from broiler chicken from Wonokromo Market and Tambahrejo Market Surabaya to some antibiotic groups, namely streptomycin, levofloxacin, cefotaxime, trimethoprim and chloramphenicol.

Materials and Methods: Seventy samples were taken from Wonokromo market and Tambahrejo market, each of which had thirty-five samples. Sampling was used cloaca swab technique. Then inoculated on Eosin Methylene Blue Agar (EMBA) media, purification of bacteria on EMBA media, and tested biochemically with Sulfide Indol Motility (SIM) and Triple Sugar Iron Agar (TSIA) media.

Antibiotic sensitivity test was used the Kirby-Bauer method. Muller-Hinton Agar (MHA) media incubation. Then the inhibition zone was measured according to the Clinical and Labortary Standard Institute (CLSI) standards. Detect positive multidrug resistance (MDR) bacteria characterized by resistance to ≥3 types of antibiotics.

Results: Detection results of Escherichia coli multidrug resistance (MDR) at Wonokromo market was 85.7%, and at Tambahrejo market was 51.4%. Presumptive Extended Spectrum Beta Lactamase (ESBL) producing E. coli at Wonokromo market was 14.3% and at Tambahrejo market was 2.9%.

Conclusion: The high level of MDR of Escherichia coli from cloacal swabs of broiler chicken was a threat to public health and the environment, and is an important concern to reduce the rate of its spread.

Keywords: *Broiler chicken, Multidrug resistance, Escherichia coli, Cloacal swab, Public health*

Introduction

Chicken is one of the sources of animal protein needed by Indonesian people[1]. Some of the products produced by chickens and used by the community include meat and eggs. Increased population growth affects the food needs of the community as seen from the data consumption of chicken meat each year[2]. Chicken production industry is inseparable from the use of antibiotics. Antibiotics are used as treatments and growth triggers[3]. The reason for the use of antibiotics is because chickens are very susceptible to pathogenic diseases due to the high density of cages which causes the chickens to become stressed. Uncontrolled use of antibiotics will leave residues and cause pathogenic microbes to become resistant to antibiotics.

Escherichia coli can also act as a reservoir for the spread of antibiotic resistance because it can easily move

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resistance genes to other bacteria. Multidrug resistance (MDR) Escherichia coli has been isolated from food from animals, hospital environments, plants, and feces. Some studies also report the high prevalence of MDR Escherichia coli in food-consuming animals, food products, and the environment. Escherichia coli is a contaminant bacterium commonly found in meat[4].

Based on this background, a study was conducted on the detection of multidrug-resistant (MDR) Escherichia coli in broilers isolated from cloacal swabs of broiler chicken in Wonokromo Market and Tambahrejo Market Surabaya.

Materials and Methods

Sampling

Sampling by cloacal swab was 70 broilers taken from Wonokromo Market and Tambahrejo Market. Samples were taken using a sterile cotton swab with a cloaca swab and then put in a tube filled with 0.9% Sodium Chloride solution. Sampling must be aseptic and the samples were taken to the Bacteriology and Mycology Laboratory of the Faculty of Veterinary Medicine, Airlangga University using a cool box for further research.

Bacteria Isolation and Identification

Samples inoculated on EMBA media were incubated by the streak method at 37^0C for 24 hours and their growth was observed. On EMBA selective media, E. coli bacteria which grow their colonies are metallic green[5]. After that proceed with the purification process. Purification is done by scraping using heated ose that has been preheated until it is red and left to stand for a while then used to extract bacterial colonies from inoculation EMBA media, streaking the EMBA media for purification[6].

Planting in Triple Sugar Iron Agar (TSIA) media is carried out by stabbing to the bottom of the tube and streaking on the oblique portion of the media using an ose needle. Positive sample E. coli on TSIA is marked by the change in color at the base of the media from orange to yellow on the slant. Planting in Sulfide Indol Motility (SIM) media, bacterial inoculation was carried out by means of a single prick in the center of the media using an ose needle. Positive samples of E. coli bacteria will form indole and positive motility[7].

Bacteria Sensitivity Test

Bacterial sensitivity testing was used by the Kirby-Bauer diffusion method using Mueller-Hinton Agar (MHA) media. The E. coli suspension was taken using a sterile cotton swab and rubbed onto the surface of the Mueller Hinton Agar (MHA) medium and allowed to stick on. Then put a 10µg streptomycin antibiotic disc, levofloxacin 5µg, cefotaxime 30µg, trimethoprim 5µg and chloramphenicol 30µg placed on Muller Hinton Agar (MHA) media using tweezers and incubated at 37^0C for 24 hours. The clear area formed around the antibiotic disc after incubation, measured in diameter and referred to as the diameter of the antibiotic inhibitory zone against the growth of test bacteria[8,9].

Results and Discussion

The results of isolation and identification with biochemical tests of 70 samples tested showed that all positive samples were identified as E. coli bacteria which can be seen in Table 1. The diameter of the inhibition zone results of the bacterial sensitivity test on 70 samples taken from Wonokromo Market and Tambahrejo market are shown in Table. 1 grouped into three categories namely Resistant, Intermediate, and Sensitive according to the standards of the Clinical and Laboratory Standards Institute[8].

Table. 1. Antibiotics sensitivity test on 70 samples of *E. coli*

No	Antibiotic	R (%)	I (%)	S (%)
1.	Streptomycin (10µg)	52 (74%)	7 (10%)	11 (16%)
2.	Levofloxacin (5µg)	42 (60%)	9 (13%)	19 (27%)
3.	Cefotaxime (30µg)	6 (9%)	3 (4%)	61 (87%)
4.	Trimethoprim (5µg)	55 (79%)	1 (1%)	14 (20%)
5.	Chloramphenicol (30µg)	30 (43%)	3 (4%)	37 (53%)

Legend : R= Resistant, I= Intermediate, S= Sensitive

Table 1. showed that E.coli bacteria obtained from Wonokromo market and Tambahrejo market have the highest resistance levels in Trimethoprim antibiotics in 55 samples (79%), Streptomycin in 52 samples (74%), Levofloxacin in 42 samples (60%), Chloramphenicol in 30 samples (43%) and Cefotaxime in 6 samples (9%).

Table. 2. Multidrug Resistnace (MDR) and Presumtive ESBL on Wet Markets Surabaya

Wet market	Positive MDR	Presumtive ESBL	Non MDR
	n (%)	n (%)	n (%)
Wonokromo	30 (85.7%)	5 (14.3%)	5 (14,3%)
Tambahrejo	18 (51.4%)	1 (2.9%)	17 (48.6%)
TOTAL	48 (68.6%)	5 (9%)	22 (31%)

Determination of multidrug resistance (MDR) if there is resistance to ≥3 classes of antibiotics[10], as shown on Fig. 2. Bacteria that are not resistant> 3 antibiotic groups are categorized as MDR negative bacteria. Determination of MDR presumptive Extended Spectrum Beta Lactamase (ESBL) if the bacteria are resistant to ≥3 classes of antibiotics and third-generation sephalosporin (Cefotaxime). Bacteria that are resistant to ≥3 classes of antibiotics but are not resistant to cefotaxime, bacteria including non presumptive MDR ESBL. Bacterial sensitivity test results can be obtained by E. coli MDR positive bacteria, MDR negative bacteria, and also presumptive ESBL E. coli bacteria, as shown on Fig.1.

Table 2. shows the highest levels of E. coli-positive MDR bacteria found in the Wonokromo Market of 30 out of 35 samples or 85.7%. Whereas in the Tambahrejo Market it was found that E. coli had positive MDR of 18 out of 35 samples or 48.6%. The results of detection of E. coli MDR, presumptive ESBL, and MDR negative are made in the diagram in Figure 1. to facilitate the reading of research results.

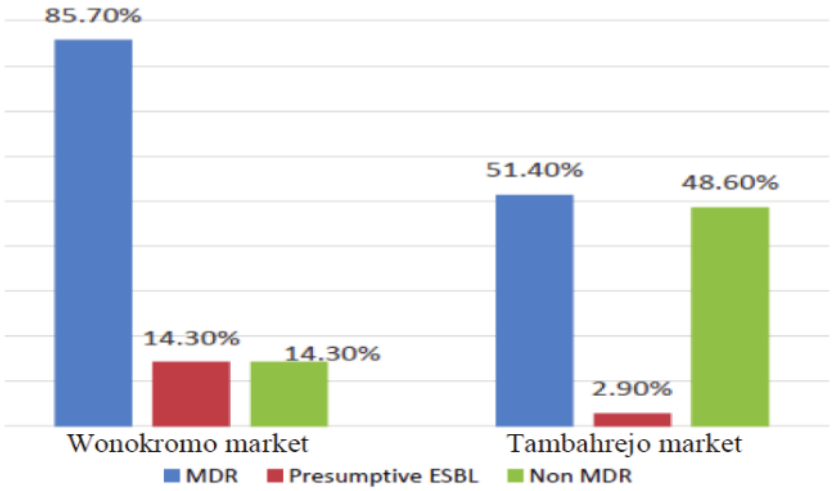

Figure 1. Diagram of percentage of MDR, presumptive ESBL, and Non MDR on two markets.

Bacteria that are resistant indicate that bacteria can still grow even though they are exposed to these antibiotics. Bacteria that are still sensitive to antibiotics indicate that antibiotics can still inhibit bacterial growth and the possibility of therapeutic success is still high[11].

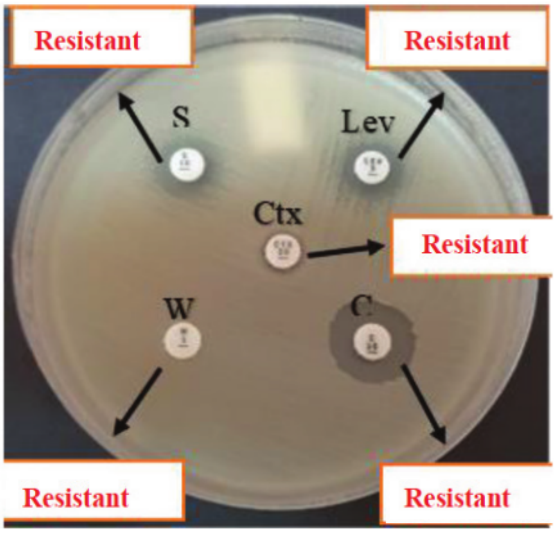

Figure 2. Result of MDR E. coli from wet markets, Surabaya

Legend : S = Streptomycin; Lev= Levofloxacin; Ctx= Cefotaxime; W= Trimethoprim; C= Chloramphenicol

The level of sensitivity of bacteria that experienced the greatest resistance was trimethoprim as many as 55 of 70 samples or 79%, intermediates as much as 1 of 70 samples or by 1% and sensitive as many as 14 of 70 samples or by 20%. Trimethoprim is an antibiotic that works by inhibiting dihydrofolic acid to become tetrahydrofolic acid so that there is an inhibition of bacterial growth[12]. The cause of resistance to trimethoprim modification in the target enzyme dihydrofolate reductase[13].

The antibiotics that experienced the second most resistance were streptomycin found in 52 of 70 samples or 74%, intermediates in 7 out of 70 samples or 10% and sensitive in 11 out of 70 samples or 16%. Streptomycin is an aminoglycidid class of antibiotics which means it works by modifying or inhibiting protein synthesis. The cause of resistance to streptomycin is due to mutations in the S12 protein ribosome gene[14].

Antibiotics that experienced the third largest resistance were Levofloxacin in 42 out of 70 samples or 60%, intermediates in 9 out of 70 samples or 13% and sensitive in 19 out of 70 samples or 27%. Levofloxacin is a quinolone class of antibiotics that works to influence the gyrase enzyme and IV topoisomeration enzymes which become toxic enzymes on bacterial chromosomes. The cause of resistance to levofloxacin is the mutation of these two enzymes so that the interaction between quinolones and enzymes is reduced[15].

E. coli bacteria isolated from Wonokromo Market and Pasar Rejo are still sensitive to cefotaxime antibiotics with data obtained 61 of 70 samples or 87% are still sensitive, intermediates are 3 out of 70 samples or 4% and are resistant as many as 6 out of 70 samples or 9%. Cefotaxime is a third generation sephalosporin that is widely used for Gram negative bacterial infections. Resistance to third generation sephalosporin is often associated with extended spectrum β-lactamase[16, 17, 18].

The isolated E. coli bacteria were still sensitive to the chloramphenicol antibiotic found 37 out of 70 samples or 53% were still sensitive, intermediates were 3 out of 70 samples or 4% and resistant were 30 out of 70 samples or 43%. Chloramphenicol is an antibiotic that inhibits or modifies bacterial protein synthesis. Chloramphenicol inhibits protein synthesis by binding to the 50S ribosomal subunit and inhibiting the extension of the peptide chain. Resistance to chloramphenicol is caused by bacteria that produce chloramphenicol acetyltransferase that damage drug activity[19].

The results of the detection of Escherichia coli multidrug resistance were most commonly found in the Wonokromo Market, 30 of 35 samples were taken or 85.7%. At Tambahrejo market found 18 out of 35 samples taken or 51.4%. In total, 48 out of 70 samples were E. coli multidrug resistance, or 68.6%. E. coli presumptive ESBL in Wonokromo Market was 5 out of 35 samples or 14.3% while in Tambahrejo market there were 1 in 35 samples or 2.9%. Overall in the two markets it was found that E. coli presumptive ESBL bacteria were as many as 6 out of 35 samples or 9%.

The high level of antibiotic resistance or multidrug resistance is caused by the use of antibiotics that are not the right dose, not the right diagnostic, and not the right bacteria causing[18]. Antibiotics also have a minimum dose to achieve the therapeutic effect, the use of antibiotics above the dose gives a stronger selection pressure against bacteria so that they can mutate and eventually become resistant[20].

The discovery of multidrug resistance bacteria isolated from the cloaca swab at Wonokromo Market and Tambahrejo market indicates that bacteria originated

from individual chickens and is thought to originate from farms that still use antibiotics. On farms, antibiotics are given as feed additives, to spur growth (growth promoters) and increase feed efficiency[21, 22, 23]. The use of antibiotics as feed additives and growth promoters kills some bacteria, but there are also those that survive and experience mutations and resistance. This situation is a serious problem and can endanger humans. Bacteria that are already immune to several antibiotics will continue to replicate, spread and contaminate water, food, soil and the environment. Humans are exposed to resistant bacteria through food consumed (foodborne) and contaminated environment.

Conclusion

The conclusion from the results of this study is that as many as 70 out of 70 samples can be isolated Escherichia coli or as much as 100% through broilers cloacal swabs in Wonokromo market and Tambahrejo market, Surabaya. Multidrug resistance (MDR) E. coli can be found in Wonokromo market was 85.7% and in Tambahrejo market was 51.4%. It can be concluded that the isolates were biological hazard for spreading MDR to human health

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Designation :

Nenny Harijani as a researcher on Universitas Airlangga,

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Mustofa Helmi Effendi as an associate professor on Universitas Airlangga,

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

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Conflict of Interest: Nil

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