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Multiple Resistance of Pathogenic Bacteria in Poultry from Malang District, Indonesia

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Abstract : Infectious diseases caused by bacteria can cause enormous economic losses due to an acute, fatal and sporadic. Now there is still no research on a survey of infectious diseases caused by bacteria that infect chickens in Tumpang district, Malang, East Java. So it needs to investigate the spread of bacteria and antibiotics is still used in the poutry farm. The purpose of research is to know the kind of bacteria that infects and antibiotics that can be used against the bacteria in three poultry farm in Tumpang district, Malang. The method used is descriptive exploratory with observed clinical symptoms and conduct a necropsy to take pathology of organs then performed the isolation and identification of bacteria include bacterial culture, Gram staining and biochemical tests as well as carried out also the sensitivity test of bacteria using the Kirby Bauer. The results showed that the chicken has undergone co infection caused by the bacteria in the third poultry farm in the district Tumpang, caused by bacteria *Escherichia coli (E.coli), Staphylococcus aureus (S.aureus), Klebsiella oxytoca* (*K. oxytoca*) and *Proteus vulgaris* (*P.vulgaris*). Test of the sensitivity of bacteria to antibiotics showed that S. aureus bacteria resistant to antiotika such as penicillin, ampicillin, ampicillin-sulbactam, and Amoxicillin/clavulanic, while K. oxytoca resistant to the antibiotic tetracycline and erythromycin. All test antibiotics-resistant E. coli and P. vulgaris. The conclusion was that in the third poultry farm have occurred coinfection to chicken and most of the bacteria are resistant to antibiotics.
Keywords: polymicrobial infection, poultry, Malang, antibiotic resistance.

Introduction and Experimental

Based on statistical data on chicken farms in the district of Malang showed an increase mainly laying hens and broilers in 2011-2012 compared to previous years. The population of laying hens in 2013 reached 2,920,857 tails compared to the previous year of 2,733,458 tails. This amount is greater than domestic chicken[1]. Total population of chickens that causes many Tumpang districts, one of the center of laying hens in Malang can meet market demand for meat and eggs[2].

The high of chicken population at risk transmission of infectious disease. Infectious diseases caused by bacteria top five in broilers while on infectious diseases in laying hens caused by viruses was ranked first in 2010[3]. Until now In the district of Malang yet scientifically reports on infectious diseases caused by bacteria invade the chicken along with antibiotics is still sensitive against these bacteria so that the results of this study are expected as a preliminary step surveilace activities to detect bacteria that often circulate in chicken farms and cause illness. In the modern poultry industry, antibiotics are used for the prevention and treatment of infectious diseases[4]. The existence of bacteria strain that resistant to antibiotics in foods such as poultry can lead to the emergence of new problems in society healthy.

Experimental

This research is the descriptive exploratory laboratory to obtain qualitative data by identify the bacteria on the poultry farm. Samples were taken from swab and organs chicken that show pathologic changes. This study was conducted in August to October in 2015. The location of the research conducted at Ranch Chicken Laying, in Tumpang District,Malang with the target of 2 chicken farm population are considered representative.

Preparation of sample, Isolation and Identification of bacteria

Organ samples from dead chickens (heart, liver, intestine) taken and added with a physiological saline solution (0.85%), then mashed with a mortal and put in a conical sterile. sample is taken and inoculated streak into TSA medium[5](OXOID) + 5% sheep blood, MC Conkey Agar (OXOID), Agar Blood Base (OXOID, Triple Sugar Iron Agar (OXOID), MIO (motile, indole and Ornithine) (OXOID) then bacteria growth was observed after incubation for 24-48 hours at 37 ° C. It is hereby the examination Mycrobact System. Colonies growth on the surface then is identified by gram staining, morphological and biochemical characteristics according to the standard testing methods of bacteriology.

Sensitivity antibiotic test :

TSA (Trypticase soy agar) medium containing bacterial suspension with a concentration 10^6-10^8 CFU/ml streak on all surfaces of MHA Medium (Muller Hinton Agar) by cotton bud and then put disc antibiotic (Gentamicin, Ampicillin, Ampicillin-sulbactam, penicillin, tetracycline, Amoxicillin/clavulanic acid, Erythromycin, Ciprofloxacin, Sulfamethoxazole and Trimethoprim, then incubated at 37C for 24 hours and measured the diameter of inhibition zone by clear zone[6].

Statistical Analysis :

Analysis Data in the descriptive

Results and Discussion

The first farm with a population of as many as 50,000 laying hens. Chickens suspected to be sick there were only 10. Clinical symptoms generally appeared in the tenth chicken was snoring and discharge nasal. In the fourth chicken (P4) excreted the green stools and ascites in the abdomen. Microbiological examination results showed in Table 1, that respiratory disease in poultry caused by a polymicrobial bacterial infections, be founded in systemic such as Proteus vulgaris (P. vulgaris), Escherichia coli (E. coli), Klebsiella oxytoca (K. oxytoca), and Staphylococcus aureus (S. aureus). Test of Gram-negative bacteria such as Klebsiella and Proteus also continued through biochemical tests using Microbact system to know species. Bacteria E. coli is basically a normal flora in the intestine but can become pathogenic if to enter through inhalation and exist in the respiratory tract causes chicken became sick[7]. These bacteria are pathogenic in chickens caused colibacillosis. It is a major cause of respiratory and gastrointestinal diseases with mortality and less than 5% and morbidity of more than 50%. In the layer chickens can decrease the production of eggs and cause fertility and cause airsacculitis, pericarditis, septicemia, and can spread to the skin, joints, eyes, head, heart, yolk sac, and peritoneum[8]. In the field, E. coli can cause Coli septicemia with an incubation period of 5-7 days after infection. E. coli bacteria inhabit in the small intestine with a concentration of 10^6/g are can inhibit the growth of bacteria Salmonella. In the healthy chicken, 10-15% of coliform bacteria in the small intestine is pathogenic serotypes. Coliform bacteria are more common in newly hatched chicken than eggs. The source of the infection can come from infection eggs, feed, litter and fecal contamination through penetration into the egg and shell membranes. Concentrations of dust containing E. coli more in outdoors than indoors[9]. Identification of E. coli used the medium MC Conkey Agar indicated the lactose fermentation, red color colony and in medium EMBA (Eosin Methylene Blue Agar) looked dark colonies with a metallic sheen. Bacteria P. vulgaris is a normal flora of the gastrointestinal tract of animals and humans but if immune system decreases can cause disease so that it can be found in the stool. Proteus vulgaris bacteria commonly found the digestive tract of humans and animals, but it also on the water, soil, and feces. Bacteria K. oxytoca is an opportunistic bacterial pathogen associated with nosocomial infections such as septicemia, pneumonia, and urinary tract infections. The bacteria can be transmitted through the chain of origin of poultry feed and fecal contamination may occur during slaughtering, transportation of products and storage phase[10].

Tabel 1 : The result of bacteria identification

The sample code	Pathology of organ	TSA medium + sheep bload	Gram staining	Mc Conkay Agar medium	Blood Agar medium	MIO Test	TSIA medium	Bacteria
P1 cloaca swab (P1K)	Normal	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+),and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
P1 nasal swab (P1N)	discharge	White colonies, cloudy, moderate, mucoid	Rods, Gram negative	Red colonies	Non hemolisa	Motil (-), indol (-) and ornithine (-)	Acid butt/acid slant, H2S (-) and gas (-)	*K. oxytoca*
P1 trachea swab (P1F)	-	White colonies, cloudy, moderate, mucoid	Rods, Gram negative	Red colonies	Non hemolisa	Motil (-), indol (-)and ornithine (-)	Acid butt/acid slant, H2S (-) and gas (-)	*K. oxytoca*
P2 nasal swab (P2N)	Discharge	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
P2 trachea swab (P2F)	normal	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
P3 cloaca swab (P3K)	normal	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*

P3 nasal swab (P3N)	Discharge	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
P4 cloaca (P4K)	Diarrhea	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*
P4 ovarium	edema	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*
P4 spleen (P4)	Odem	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*
P5 swab nasal (P5N)	Disharge	White colonies, cloudy	Coccus, positive gram	Not growth	Non hemolisa	-	-	*S. aureus*
P5 swab trachea (P5F)	normal	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
P6 nasal swab (P6N)	disharge	White colonies, cloudy	Coccus, positive gram	Not growth	Non hemolisa	-	-	*S. aureus*
P6 trachea swab (P6F)	normal	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*

P7 swab trachea (P7F)	disharge	1. White colonies, cloudy, moderate, mucoid 2. White colonies, cloudy 3. Whit e colonies, cloudy	1.Rods, Gram negative 2. Rod, short, coccobacil, negative gram 3. Coccus, positive gram	1.Red colonies 2. Red colour 3. Not growth	1.Non hemolisa 2. Non hemolisa 3. Non hemolisa	1.Motil (-), indol (-) and ornithine (-) 2. Motil (+), indol (+) and ornithine (+) 3. -	1.Acid butt/acid slant, H2S (-) and gas (-) 2. Acid butt/Acd slant, H2S (+) and gas (-) 3. -	1.*K. oxytoca* 2. *E. coli* 3. *S. aureus*
P7 cloaca swab (P7K)	normal		Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
P8 swab trachea (P8F)	-	1.White colonies, cloudy, swarming 2.White colonies, cloudy 3.White colonies, cloudy	1.Rods, Gram negative 2. Rod, short, coccobacil, negative gram 3. Coccus, positive gram	1.colourless 2. Red colour 3. Not growth	1.Non hemolisa 2. Non hemolisa 3. Non hemolisa	1. Motil, indol (+), and ornithine (+) 2. Motil (+), indol (+) and ornithine (+) 3. -	1.Acid butt/acid slant, H2S (-) and gas (+) 2. Acid butt/Acd slant, H2S (+) and gas (-) 3. -	1. *P. vulgaris* 2. *E. coli* 3. *S. aureus*
P9 swab trachea (P9F)	normal	1.White colonies, cloudy, moderate, mucoid 2.White colonies, cloudy	1.Rods, Gram negative 2.Coccus, positive gram	1.Red colonies 2. Not growth	1.Non hemolisa 2. Non hemolisa	1.Motil (-), indol (-) and ornithine (-) 2. -	1.Acid butt/acid slant, H2S (-) and gas (-) 2. -	1.*K. oxytoca* 2. *S. aureus*

P10 swab trachea (P10F)		1.White colonies, cloudy, swarming 2.White colonies, cloudy 3.White colonies, cloudy	1.Rods, Gram negative 2. Rod, short, coccobacil, negative gram m	1.colourless 2. Red colour	1.Non hemolisa 2. Non hemolisa	1. Motil, indol (+), and ornithine (+) 2. Motil (+), indol (+) and ornithine (+)	1.Acid butt/acid slant, H2S (-) and gas (+) 2. Acid butt/Acd slant, H2S (+) and gas (-)	1. *P. vulgaris* 2. *E. coli* 3. *S. aureus*

The second farm has a total of the population as many as 5000 chickens. Chickens suspected of sick only two. Clinical symptoms showed that chicken looked thin, also, the first chicken looked torticollis, but the second chicken looked the swelling in the facial and nasal discharge. Torticollis can also be caused due to viral infections such as avian influenza, Newcastle Disease and Marek's. On the farm also was found polymicrobial infection by bacteria E. coli, S. aureus and P. vulgaris showed in Table 2.

Table 2 : The result of bacteria identification

The sample code	Pathology of organ	TSA medium + sheep bload	Gram staining	Mc Conkay Agar medium	Blood Agar medium	MIO Test	TSIA medium	Bacteria
M1 nasal swab (M1N)	discharge	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*
M1 cloaca swab (M1K)	Normal	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*
M1 ocular swab (M1O)	discharge	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*
M2 trachea swab (M2F)	normal	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
M2 swab nasal (M2N)	discharge	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
M2 cloaca swab (M2K)	normal	White colonies, cloudy, moderate, swarming	Rods, Gram negative	Colourless colonies	Non hemolisa	Motil, indol (+), and ornithine (+)	Acid butt/Alkali slant, H2S (-) and gas (+)	*P. vulgaris*
M2 Heart (M2H)	edema	White colonies, cloudy	Coccus, positive gram	Not growth	Non hemolisa	-	-	*S. aureus*
M2 trachea (M2T)	hemorraghi	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*
M2 Liver (M2L)	Hepatomegaly, streak colour	White colonies, cloudy	Rod, short, coccobacil, negative gram	Red colour	Non hemolisa	Motil (+), indol (+) and ornithine (+)	Acid butt/Acd slant, H2S (+) and gas (-)	*E. coli*

Bacteria K. oxytoca of samples had shown resistance to some antibiotics such as Penicillin, Tetracycline, and Erythromycin showed in Table 3 that had been classified into multiple antibiotic resistance (MAR) which caused resistance towards to three or more antibiotics. The bacterial resistance to tetracycline supported by research[11], which states that 9.8% of the test sample of 102 chickens had resistant isolates. Aminogikosida class antibiotics such as gentamicin are active against gram-negative rod bacteria. Bacteria P. vulgaris also showed resistance from all types of discs used antibiotics test. This was in accordance with[12], which showed that this bacteria also are resistant to many antibiotic such as piperacillin, amoxicillin, ampicillin, cefoperazone, cefuroxime, and cefazolin. Bacteria S. aureus is still sensitive to the antibiotic gentamicin and tetracycline while on the other disc is resistant. Bacteria E. coli had been resistant to all antibiotics test. All the tested bacteria were resistant to erythromycin because it is often used in poultry farms. These antibiotic resistance genes encoded by Erma and ermC thought to be caused by the presence of Ca2 + or Mg 2+[13]. Bacteria S. aureus is a nosocomial infection. The resistance of bacteria to penicillin caused by penicillinase enzyme that breaks down the molecular structure of penicillin beta-lactam[14].

Tabel 3 : The sensitivity antibiotic test toward bacteria test

Kind of bacteria	CN_{10}	AMP_{10}	SAM_{20}	P_{10}	TE_{30}	AMC_{30}	E_{15}	SXT_{25}
Klebsiella oxytoca	**2,5 cm (S)**	**3,3 (S)**	**3,7 (S)**	**4,4 (S)**	1 cm (R)	**3,4 (S)**	0 (R)	**3 cm (S)**
Proteus vulgaris	1,5 cm (R)	0 (R)	0,9 (R)	0 (R)	0,2 cm (R)	0,6 cm (R)	0,3 cm (R)	0,9 (R)
Staphylococcus aureus	**2 cm (S)**	0,8 cm (R)	1,5 cm (R)	1,8 cm (R)	**1,9 cm (S)**	0 (R)	0 (R)	2,5 cm (S)
Escherichia coli	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	1,5 cm (R)	1 cm (R)	0 (R)

Description : CN= Gentamicin, , AMP = Ampicillin, SAM = Ampicillin sulbactam, P= penicillin, TE = tetracyclin, AMC= Amoxicillin/clavulanic acid, E= Erythromicin, CIP = Ciprofloxacin, SXT = Sulphamethaxazole and Trimethoprim[15]

Antibiotic sensitivity test using the Kirby-Bauer disk diffusion assay. Antibiotics are often used on poultry farms such as bacitracin, chlortetracycline, erythromycin and penicillin for the control and treatment of diseases[11]. Antibiotics are also often used for prophylaxis and growth promoters such as Bacitracin, chlortetracycline, tylosin, avoparcin, neomycin, oxytetracycline, and virginiamycin[14] based on antibiogram[15]. The use of antibiotics that are not rational and often used to trigger resistance of strains of pathogenic bacteria. Antibiotics were used in this study were Gentamicin, Streptomycin, Amoxicillin, penicillin, tetracycline, Chloramphenicol, Methicillin, Erythromycin, Ciprofloxacin, and Sulfamethoxazole and Trimethoprim. Research on antibiotic sensitivity was also performed on samples that have been identified as polymicrobial infections. In the sample code P7F (bacteria K. oxytoca and E. coli) and P10K (bacteria S. aureus and E. coli) showed resistance to all antibiotics test (Gentamicin, Streptomycin, Amoxicillin, penicillin, tetracycline, Chloramphenicol, Methicillin, Erythromycin, Ciprofloxacin, Sulfamethoxazole and Trimethoprim) showed in Table 4. Resistance to antibiotics is not only caused by the natural ability of bacteria to change but also due to the transmission capability of progeny through extrachromosomal fragments of DNA called plasmids. Resistance mechanism can occur by the synthesis of enzyme inactivation, the cell wall configuration changes, and system modifications career membrane[14].

Table 4 : The sensitivity antibiotic test toward polymicrobial infection

Sample code	CN_{10}	S_{10}	AML_{25}	P_{10}	TE_{30}	C_{30}	MET_5	E_{15}	CIP_5	SXT_{25}
P7F	1 cm (R)	0,5 cm (R)	0 (R)	0 (R)	0,3 cm (R)	0,9 cm (R)	0 (R)	0 (R)	0 (R)	0 (R)
P10F	**2 cm (S)**	0 (R)	0 (R)	0 (R)	0 (R)	**2,3 cm (S)**	0 (R)	0 (R)	0 (R)	0 (R)

Descriptive : CN= Gentamicin, S=,Streptomycin AML= Amxicillin, P= Penicillin, TE = tetracyclin, C=Chloramphenicol, MET= Methicillin, E= Erythromicin, CIP = Ciprofloxacin, SXT = Sulphamethaxazole and Trimethoprim

Conclusions

The results of these study suggest that there is a polymicrobial infection in both farm in District Tumpang that showed respiratory and gastrointestinal disorder caused by bacteria Escherichia coli, Proteus

vulgaris, Staphylococcus aureus, and Klebsiella oxytoca. Multidrug resistance was found in test samples, especially Proteus vulgaris and Escherichia coli. The antibiotic sensitivity test against polymicrobial infections (K. oxytoca and E. coli) found resistance to all types of antibiotics while polymicrobial infections (S. aureus and E. coli) was found still sensitive to Gentamicin and chloramphenicol.

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