Antibiotic resistance profile of *Staphylococcus aureus*, *Streptococcus* spp. and *Klebsiella* spp. isolated from chicken farm in Bogor, Sukabumi, and Cianjur, West Java

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Abstract. West Java province has largest population of chicken poultry, with Bogor, Sukabumi, and Cianjur has highest chicken population. Farmers used antibiotics for prophylaxis and therapy to maintain the production. However, extensive use of antibiotic increased the number of antibiotic resistant bacteria. *Staphylococcus aureus*, *Streptococcus* spp., and *Klebsiella* spp. are the example of flora normal in chicken that affected with abusive use of antibiotic. The aim of this study was to determine the antibiotic resistance profile of *S. aureus*, *Streptococcus* spp., and *Klebsiella* spp. isolated from cloacal swab of chicken poultry in 3 regions. Total of 320 samples were collected and the positive number of *S. aureus*, *Streptococcus* spp., and *Klebsiella* spp. were 61, 8, and 58 isolates respectively. The result of antibiotics susceptibility test showed that *S. aureus* was resistant to ampicillin (98%), erythromycin (95%), nalidixic acid (93%), tetracycline (92%), oxytetracycline (90%), enrofloxacin (69%), and ciprofloxacin (56%). *Streptococcus* spp. was resistant to tetracycline (100%) and doxycycline (87.5%). *Klebsiella* spp. was resistant to ampicillin (100%), ampicillin (94.83%) oxytetracycline (93.10%), tetracycline and nalidixic acid (89.66%), enrofloxacin (86.21%), and ciprofloxacin (81.03%). *S. aureus*, *Streptococcus* spp., and *Klebsiella* spp. has a high level of resistance to antibiotics and most of the isolates were multi-resistant.

1. Introduction

Poultry is widely farmed in several parts of Indonesia. The region that has the largest poultry population in Indonesia is West Java Province. The three regions in West Java that contribute the highest poultry population are Bogor, Sukabumi and Cianjur. This poultry population consists of chicken, duck, broiler, and laying hen. The largest number of poultry populations are broiler and laying hen. The total population of broiler and laying hen in Bogor, Sukabumi and Cianjur areas is 47,562,145 tails [1].
Broilers and laying hens have a high productivity, but their level of immunity against the disease is relatively low. In order to improve the immunity of chickens, farmers usually administer vitamins, vaccinations and antibiotics. Antibiotics are often used in livestock and poultry as a prevention of disease transmission and as a growth promoter [2]. Antibiotics are also used for the treatment of livestock, thereby reducing the risk of death [3].

Since January 2018, the Indonesian government has officially banned antibiotic users as a growth growth promotor by issued Permentan No. 14 year 2017 concerning. Animal Medicine Classification. However, the use of antibiotics in the field is still very high. Collaborative survey conducted by Ministry of Agriculture and FAO in 2018 about the use of antibiotics in 360 broiler chicken farms in West Java, East Java and South Sulawesi. Results the survey revealed that 80% of farmers still use it antibiotics as disease prevention and growth promotor [4].

In spite of its beneficial to human and animal health, as well as increased productivity over the past few years, abusive use of antibiotics has led to resistance to pathogenic and commensal bacteria [5]. Over the past 50–60 years, bacterial resistance has spread and caused death [6]. Based on WHO [7], mortality in humans due to infection resistant bacteria reach 700 thousand per year. The number will continue increased and predicted in 2050 mortality due to resistance antibiotics reach 10 million per year [8]. It is feared that resistant bacteria in chicken can infect humans, thereby reducing the effectiveness of treatment [9]. Further observation of resistant bacteria in chickens is necessary to prevent cases of resistance in animals and humans.

One of the frequent bacteria cause infectious diseases in broiler chickens is *Staphylococcus aureus*, normally found in the skin and mucous membranes animal or human. *S. aureus* infection in broiler chickens can cause diseases such as arthritis, tenosynovitis, gangrenous dermatitis, yolk sac infections, bumble foot, spondylitis and osteomyelitis [10].

*Streptococcus* spp. is a normal microflora in the mucosa digestive tract of broiler chickens. Cases of *Streptococcus* spp. in chicken Broilers have been widely reported, but rarely reported as outbreaks. This matter occurred due to infection with *Streptococcus* spp. generally subclinical and secondary. Some diseases that can be caused by infection. These bacteria include pneumonia, septicemia, cellulitis, osteomyelitis, endocarditis and meningitis with a mortality rate of up to 50% [11].

*Klebsiella* spp. is a Gram negative bacterium which is a normal flora in the oral cavity, skin and intestine, but can be a pathogen in animals and humans in conditions of immunosuppression. This bacterium is a cause of bacterial disease that attacks large livestock and poultry. *Klebsiella* spp. can be transmitted through inhalation, causing pneumonia, bacteremia and nosocomial infections [12]. Chicken meat is a potential reservoir for the transmission of virulent *Klebsiella* spp. that is resistant to antibiotics from animals to humans. Klebsiella is also one of the bacteria producing extended spectrum β-lactamase (ESBL) that produces the CTXM gene, this gene can be transmitted directly or indirectly.

The information about antibiotic resistance from those three bacteria in Indonesia is still limited. The aims of this study is to determine the antibiotic resistance profile of *S. aureus*, *Streptococcus* spp., and *Klebsiella* spp. isolated from cloacal swab of chicken poultry in 3 regions.

2. Method
The samples of this study was collected from chicken farm in Bogor, Sukabumi, and Cianjur. Sample collected from chicken cloacal using cotton swab then stored in sample tube contained phosphate buffered saline (PBS). The samples stored in cool box and the temperature was maintained in 4 °C.

To isolate *S. aureus*, bacterial suspension were cultures in mannitol salt agar medium (MSA) and incubated for 24 hours in 37°C. Positive isolate of *S. aureus* has yellow colony colour and changed the medium colour into yellow. Isolate then cultured in TSA medium and incubated for 24 hours in 37°C. The isolate then tested based on SNI 2332.9:2011, with specified test such as Gram staining, catalase test, glucose fermentation, and coagulase test.

To isolate *Streptococcus* spp., samples from broiler chicken cloaca swabs were isolated and identified. Sample cultured in blood agar (BA), then incubated 37 °C for 18-24 hours. Then the results of a single colony that grows in culture in tryptone soya agar (TSA), incubated 37 °C for 18-24 hours. Next, isolates were carried out Gram staining to determine bacterial morphology. Coloring results with The gram-
positive morphology of cocci was then tested using catalase dripping 3% H$_2$O$_2$ in a few bacterial isolates [14].

The cloaca swab samples taken were then cultured in a selective medium for the Klebsiella spp. in this study using Mac Conkey agar (MCA). The culture was incubated at 37 °C for 18-24 hours. Colonies of bacteria growing on MCA medium were observed. The bacterial colony of the genus Klebsiella is round, convex, smooth, pink and mucoid. A single colony identified as the genus Klebsiella was then recultured on the MCA medium and then a positive colony was observed microscopically by Gram staining. Bacterial morphology was observed under a microscope with a magnification of 10 × 100. The morphology of the Klebsiella bacterium has the shape of a stem and is red in color typical of Gram negative bacteria. Samples that were positive were subcultured on the sloping medium Trypticase Soy Agar (TSA) then incubated at 37 °C for 18-24 hours.

Antibiotic sensitivity test was performed using a Kirby Bauer disc diffusion using Mueller-Hinton agar based on the Clinical and Laboratory Standards Institute Guidelines (CLSI) 2018 [15]. S. aureus culture in an oblique TSA medium that had been incubated for 18-24 hours was suspended on physiological NaCl until it reached physiological NaCl until it reached. McFarland standard 0.5. S. aureus suspension of 100 µL was poured into a cup containing the Mueller-Hinton agar medium and flattened using a sterile spreader Antibiotic discs were placed on top so that Mueller-Hinton used a sterile tweezer. The culture was then incubated at 35ºC for 16-18 hours. Antibiotic inhibition zones formed were measured using caliper. The inhibitory zone measurement results are interpreted according to the CLSI 2018 standard.

3. Result and discussion
A total of 320 samples were isolated from chicken poultry in Bogor, Sukabumi, and Cianjur. 105 samples were isolated from layer chicken farm in Bogor. 105 samples were isolated from broiler chicken farm in Sukabumi. 80 samples were isolated from broiler chicken farm and 30 samples were isolated from layer chicken farm in Cianjur.

S. aureus isolated from Bogor was 29 isolate from layer farm. Fourteen isolate of S. aureus were isolated from layer farm in Sukabumi, and 12 isolate were isolated from layer farm and 6 were isolated from broiler farm in Cianjur. Total positive sample of S. aureus was 61 samples. Total Streptococcus spp. isolated was 8 isolates, with 6 isolates from Bogor, and 2 isolates from Sukabumi. There are no positive isolates found in Cianjur. The positive isolates of Klebsiella spp. was 58 samples, with 16 samples obtained from Bogor, 19 samples obtained from Sukabumi, and 21 isolates obtained from broiler and 2 isolates obtained from layer farm in Cianjur. The description of each positive samples were shown in Table 1.

| Regions | Farm   | Total samples | S. aureus | Streptococcus spp. | Klebsiella spp |
|---------|--------|---------------|-----------|--------------------|---------------|
| Bogor   | Layer  | 105           | 29        | 6                  | 16            |
| Sukabumi| Broiler| 105           | 14        | 2                  | 19            |
| Cianjur | Layer  | 80            | 12        | 0                  | 2             |
|         | Broiler| 30            | 6         | 0                  | 12            |
| Total   |        | 320           | 61        | 8                  | 58            |

Overall, S. aureus from the 3 region were 98% resistant to ampicillin, 95% resistant to erythromycin, 93% were resistant to nalidixic acid, 92% were resistant to tetracycline, 90% were resistant to oxytetracycline, and 56% were resistant to ciprofloxacin. 18% of S. aureus resistant t p gentamycin and chloramphenicol. Streptococcus spp. was resistant to tetracycline (100%) and doxycycline (87.5%). Streptococcus spp. sti ll sensitive against ampicillin and chloramphenicol. There are no CLSI standard for enrofloxacin interpretation for Streptococcus spp.. Klebsiella spp. was resistant to erythromycin (100%), ampicillin (94.83%) oxytetracycline (93.10%), tetracycline and nalidixic acid
(89.66%), enrofloxacin (86.21%), and ciprofloxacin (81.03%). Klebsiella spp. also resistant against gentamycin (55.17%) and still sensitive to chloramphenicol (29.31%). The complete resistance data showed in Table 2.

Table 2. Percentage of bacteria resistance against antibiotics

| Antibiotics        | Bacteria      | Resistant | Intermediate | Sensitive |
|--------------------|---------------|-----------|--------------|-----------|
| Ampicillin         | S. aureus     | 98        | 0            | 2         |
|                    | Streptococcus spp | 100      | 0            | 0         |
|                    | Klebsiella spp.| 94.83     | 3.45         | 1.72      |
| Nalidixic acid     | S. aureus     | 93        | 2            | 5         |
|                    | Streptococcus spp | -       | -            | -         |
|                    | Klebsiella spp.| 89.66     | 6.9          | 3.45      |
| Ciprofloxacin      | S. aureus     | 18        | 10           | 72        |
|                    | Streptococcus spp | -       | -            | -         |
|                    | Klebsiella spp.| 81.03     | 6.9          | 12.07     |
| Enrofloxacin       | S. aureus     | 69        | 16           | 15        |
|                    | Streptococcus spp | -       | -            | -         |
|                    | Klebsiella spp.| 86.21     | 12.07        | 1.72      |
| Tetracycline       | S. aureus     | 92        | 3            | 5         |
|                    | Streptococcus spp | 0       | 0            | 100       |
|                    | Klebsiella spp.| 89.66     | 0            | 10.34     |
| Oxytetracycline    | S. aureus     | 90        | 2            | 8         |
|                    | Streptococcus spp | -       | -            | -         |
|                    | Klebsiella spp.| 93.1      | 1.72         | 5.17      |
| Doxycycline        | S. aureus     | -         | -            | -         |
|                    | Streptococcus spp | 0       | 12.5         | 87.5      |
|                    | Klebsiella spp.| -         | -            | -         |
| Erythromycin       | S. aureus     | 95        | 2            | 3         |
|                    | Streptococcus spp | -       | -            | -         |
|                    | Klebsiella spp.| 100       | 0            | 0         |
| Gentamycin         | S. aureus     | 18        | 7            | 66        |
|                    | Streptococcus spp | -       | -            | -         |
|                    | Klebsiella spp.| 55.17     | 5.17         | 39.66     |
| Chloramphenicol    | S. aureus     | 18        | 10           | 72        |
|                    | Streptococcus spp | 87.5     | 0            | 12.5      |
|                    | Klebsiella spp.| 29.13     | 17.24        | 53.45     |

Antibiotics used in this study are most common used antibiotics by chicken farmer. A research in Malang showed that erythromycin, tetracycline, oxytetracycline, quinolone, ciprofloxacin and ampicillin were often used in chicken farm, either for therapy or prophylaxis [16]. S. aureus and Klebsiella spp. in this study were shown a high level of resistance to those antibiotics. Most of S. aureus
and *Klebsiella* spp. were resistant to three or more than three kind of antibiotics, so it categorized as multidrug resistance bacteria [17]. Similar result found that *S. aureus* isolated from chicken cloaca in Bangladesh experienced resistance to tetracycline (80.76%), erythromycin (73.08%) and gentamicin (34.62%) [18]. *S. aureus* from broiler isolates in Yogyakarta had experienced resistance to gentamicin (26.1%), tetracycline (21.7%), and erythromycin (17.4%) [19]. Similar research in China mentioned *Klebsiella* spp. isolated from broiler chickens in China's Shandong province were resistant to the antibiotic ampicillin 98.9%, ciprofloxacin 80.0%, tetracycline 78.9% and chloramphenicol 92.2% [20].

In contrary, *Streptococcus* spp. showed the low level of antibiotics resistance. *Streptococcus* spp. has a high level resistance to tetracycline and doxycycline. Level of resistance of *Streptococcus galiolyticus* in broiler chicken against tetracycline and doxycycline reaching more than 50%. Resistance level of tetracycline and doxycycline respectively were 56.6% and 68.2% [21]. Some of antibiotics tested do not have an official breakpoint for sensitivity test. EUCAST [22] explains that if there is no breakpoint for an antibiotic, the sensitivity test of that antibiotic is not recommended for species tested. This means antibiotic activity which is bad for the therapy of these species, so the results obtained become resistant without the need for previous tests.

The 3 bacteria showed the low level of resistance against chloramphenicol, possibly because the use of chloramphenicol in poultry farms has been banned since 1994 by the Indonesian government. Decree of the Minister of Agriculture No. 806 / Kpts / TN.260 / 12/94 concerning the classification of veterinary medicines, stated that chloramphenicol is included in the list of hard drugs which are not permitted for animal use at all. Furthermore, through Permenkes Number: 1168 / Menkes / PER / X / 1999 concerning food additives, the government explained chloramphenicol is one of the nine types of food additives that are prohibited in Indonesia. Although it was banned 25 years ago, this study showed the isolates still resistant to chloramphenicol, even in a low level. It is thought that the coding gene for chloramphenicol antibiotic resistance still exists today.

The incidence of resistance in bacteria against antibiotics can occur due to various factors, including the use of chloramphenicol in a long time, excessive use and not according to the dose of use. The excessive use of antibiotics causing a global crisis of antibiotic resistance. Further research needed to prevent and control the antibiotic resistance case globally.

### 4. Conclusion

*S. aureus*, *Streptococcus* spp., and *Klebsiella* spp, found in chicken cloaca swab from layer and poultry farm in Bogor, Sukabumi, and Cianjur. *S. aureus*, *Streptococcus* spp., and *Klebsiella* spp. has a high level of resistance to antibiotics and most of the isolates were multi-resistant.

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