Antimicrobial resistance in aerobic bacterial isolates from broiler lungs

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ABSTRACT

Antibacterial resistant bacteria cause a big concern to poultry and to public health in general because some bacterial poultry pathogens can infect humans or transfer their resistance ability to human pathogens. Nontherapeutic use of antibacterial in poultry, especially as growth promoters to increase feed conversion efficiency is thought to be one of the main causes of resistance. The study included pulmonary swab samples collected during necropsy from 120 poultry farms showing respiratory symptoms with mortality. The disc diffusion method for antibiotic sensitivity testing was performed and antibiotic disks for 21 antibiotics were used. The results showed that three antibacterial were sensitive to more than 50% of the isolates. The first is doxycycline and 69.9% of the isolates were sensitive. The second is Cefalexin with 60.5% sensitive isolates and third is Chloramphenicol with 55.2% sensitive isolates. In the rest of antibacterial, less than 50% were sensitive. Five isolates were found resistant to all antibacterial. Moreover, three samples were found to be negative with no bacterial growth. The present study concluded that 50% of the aerobic bacteria isolated from poultry lungs are resistant to 85% of the 21 antibiotics tested in the study.

Keywords: Antibiotics; Antimicrobial Resistance, Poultry, Iraq

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1. Introduction

An important challenge to the poultry industry is the bacterial resistance to antibiotics [1]. The failure to mitigate this challenge has an effect extending to the rest of bioecosystems and affects our ability to achieve one-health goals. The resistance is reportedly increasing in the past years. This development in resistance is thought to be due to excessive and injudicious application of antibacterials to animals [2]. The trend toward intensive animal production systems is also a contributing factor aggravating the need for a prolonged use of treatment. Poultry meat and eggs are essencial types of food in most parts of the world. Resistant avain pathogens can be transferred to human through consumption or handling of meat or eggs. Once inside the human body, these organisms can carry their resistance ability to the human pathogens since most resistance genes are transferable between bacteria [3]. The phenomenon of resistance is expected to increase simply because the administration of antibacterials to treat illnesses in the presence of resistant bacteria will kill other sensitive bacteria that minimize the numbers of the first by competitive exclusion. Hence, removing the competing bacteria will lead to domination of the resistant population [4].

There are therapeutic and non-therapeutic uses of antibiotics. Non-therapeutic usage especially as growth promoters to increase feed conversion efficiency is thought to be a primary reason for the development of resistance. In this practice, an antibiotic is used in the feed in a sub-lethal dose: one tenth or one hundredth of the therapeutic dose. After many years of use as growth promoters, according to scientists, antibiotics that were used as growth promoters have more resistant bacteria when compared to antibiotics not used for this purpose [5–7]. Another important side effect of antibacterial administration to poultry is the residual traces in meat,



eggs, and manure. Residues in manure can pollute water, soil and plants which will further increase the chance of the spread of resistant pathogens or exposure of sensitive ones to those antibiotics and hence developing resistance [8]. Antibacterial resistance has been documented in a number of poultry pathogens including: Escherichia coli [9,14], Salmonella [15–22], Campylobacter jejune [23–27] and Staphylococcus aureus [28]. Due to the wide spread of resistance and the huge impact of this phenomenon on poultry health and human health, this study was performed for the estimation of the spread of resistance against 21 widely used antibiotics in Iraq in bacterial pathogens isolated from the respiratory system of broiler poultry.

2. Materials and methods

2.1. Sampling

The study included pulmonary swab samples collected during necropsy from 120 poultry farms with respiratory symptoms with mortality. The samples were collected from cases submitted by poultry farmers to the poultry diseases laboratory at Fallujah University during January 2018 to August 2020. Based on the gross lesions, many cases have been diagnosed with a primary viral disease. Viral diseases were accompanied by bacterial pathogens or there was a primary bacterial pathogen that requires special microbiological techniques for isolation such as mycoplasma gallisepticum or avibacterium paragallinarum. Those bacterial pathogens were not included in the study because we only used ordinary culture media incubated aerobically.

2.2. Bacteriology

The disc diffusion method for antibiotic sensitivity testing was performed followed the methods that were published by by Markey et al., [29]. Sample swabs were streaked on solid agar media including MacConkey, Nutrient and Brain heart infusion agar. Single colonies were transferred into Soybean casein digest broth to purify the culture, then spread into Mueller Hinton agar with antibiotic disks for 21 widely used antibiotics.

3. Results and discussion

Table 1 shows the proportions of resistant and sensitive isolate for each antibacterial arranged according to the percentage of sensitivity in a descending order.

Three antibacterials were sensitive to more than 50% of the isolates. The first is doxycycline and 69.9% of the isolates were sensitive. The second is Cefalexin with 60.5% sensitive isolates and third is Chloramphenicol with 55.2% sensitive isolates. In the rest of antibacterials, less than 50% were sensitive to them. Five isolates were found resistant to all antibacterials. More than 90% of the samples were resistant to three antimicroblias these are oxytetracycline, thiamphenicol and spiramycin (Figure 1). Moreover, three samples were found negative with no bacterial growth. The present study concluded that 50% of the aerobic bacteria isolated from poultry lungs are resistant to 85% of the 21 antibiotics tested in the study.

Those kind of isolates should alarm all workers in the medical fields as they are a serious public health concern especially if those bacteria were able to infect humans or transfer their resistance plasmids to human pathogens [2], [30]. Moreover, three samples were found negative with no bacterial growth. Those could be caused by a viral pathogen and have not yet been complicated by a bacterial agent at the time of sampling [31–33].

The main way to minimize the spread on resistant bacteria is through the practice of antimicrobial susceptibility testing prior to administration. The success of this practice depends upon many factors among which: (1) The timing of the test which will be more relevant and can be more correlated to clinical outcome if the sampling was done at an early stage of the course of the disease, [34] (2) The accuracy of sampling which should be from the site of infection without contamination by bacteria from the hands of the operator or other tissues. Otherwise, the susceptibility results will not reflect the traits of the causative pathogen and (3) The ability of the veterinarian to interpret the results correctly and choose an antibacterial that can reach the site of infection in high enough concentrations. For example, in vitro results are not reliable for a non-absorbable antibacterial to be used in respiratory diseases [35].

Research addressing the issue of antibacterial resistant bacteria in Iraq includes many works in the recent years. Most researchers focused on bacterial species such as: Salmonella species [36–42], Campylobacter species [43–45], Staphylococcus aureus [46, 47] and Escherichia coli [48] and [49].

Our study emphasizes the significance of the problem of antibacterial resistance in poultry pathogens because more than 50% of the samples were resistant to 18 of the 21 tested drugs. More research studying antibacterial

resistance is needed especially in Iraq and should involve more animal species and wider geographical area. Additionally, efforts should be made to tackle this challenge by spreading knowledge about the problem and how to minimize it. More regulatory restrictions are needed to prevent non veterinary practitioners from misusing antibiotics. Poultry veterinarians need to be informed about pharmacodynamics and pharmacokinetics of antibiotics, especially absorption and whether or not the antibiotic will reach the site of infection.

The present study concluded that 50% of the aerobic bacteria isolated from poultry lungs are resistant to 85% of the 21 antimicrobials tested.

| Antibiotic | Resistant isolates | Sensitive isolates |
|--------------------|---------------------------|--------------------|
| Doxycycline | 30.1% | 69.9% |
| Cefalexin | 39.5% | 60.5% |
| Chloramphenicol | 44.8% | 55.2% |
| Cefixime | 51.3% | 48.7% |
| Furaltadone | 51.7% | 48.3% |
| Levofloxacin | 56.5% | 43.5% |
| Ciprofloxacin | 62.6% | 37.4% |
| Azithromycin | 63.5% | 36.5% |
| Enrofloxacin | 70.8% | 29.2% |
| Fosfomycin | 73.0% | 27.0% |
| Trimethoprim/sulfa | 78.5% | 21.5% |
| Lincomycin | 78.6% | 21.4% |
| Amoxicillin | 80.4% | 19.6% |
| Erythromycin | 83.7% | 16.3% |
| Tilmicosin | 85.9% | 14.1% |
| Tylosin | 86.0% | 14.0% |
| Florfenicol | 88.5% | 11.5% |
| Chlortetracycline | 89.7% | 10.3% |
| Oxytetracycline | 91.5% | 8.5% |
| Thiamphenicol | 94.4% | 5.6% |
| Spiramycin | 95.4% | 4.6% |

Table 1. The percentage of resistant and sensitive isolates for each antibacterial





Figure 1. A colored bar-chart representing the percentage of resistant and sensitive isolate for each antibacterial. Red bars represent the percentage of resistant isolates, while green is the percentage of sensitive isolates

4. Conclusions

Some bacterial poultry infections can infect humans or transfer their resistance ability to human pathogens, making antibacterial-resistant bacteria a major worry for poultry and public health. One of the main sources of resistance is the non-therapeutic use of antibacterial, notably as growth boosters in poultry to improve feed conversion efficiency. Necropsied pulmonary swab samples from 120 poultry farms with respiratory symptoms and mortality were included in the study. Antibiotic disks representing 21 different antibiotics were utilized in

a disc diffusion assay to determine how well each antibiotic worked. Over half of the isolates were responsive to at least three antibiotics. First, doxycycline, which was effective against 69.9% of the isolates. The next most effective antibiotic is cefalexin, with 60.5% of isolates responding favorably to it. Less than half were sensitive to the other antibacterial. All antibiotics were ineffective against five different isolates. And no germs were detected in any of the three negative samples. Half of the aerobic bacteria isolated from chicken lungs were found to be resistant to at least 15 of the 21 antibiotics examined.

Declaration of competing interest

The authors declare that they have no known financial or non-financial competing interests in any material discussed in this paper.

Funding information

No funding was received from any financial organization to conduct this research.

References

- [1] C. Agyare, V. E. Boamah, and C. N. Z. and F. B. Osei, "Antibiotic Use in Poultry Production and Its Effects on Bacterial Resistance," in *Antimicrobial Resistance, A global threat*, IntechOpen, 2018, pp. 33–51. doi: 10.5772/intechopen.79371.
- [2] B. M. Marshall and S. B. Levy, "Food animals and antimicrobials: impacts on human health," *Clin. Microbiol. Rev.*, vol. 24, no. 4, pp. 718–733, 2011.
- [3] C. L. Hofacre, R. S. Singer, and Timothy J. Johnson, "Antimicrobial therapy (including resistance)," in *Diseases of Poultry*, 13th ed., John Wiley & Sons, Inc., 2013, pp. 52–57.
- [4] S. R. Collett and J. A. Smith, "Principles of Disease Prevention, Diagnosis, and Control," in *Diseases of Poultry*, John Wiley & Sons, Ltd, 2020, pp. 1–87.
- [5] H. W. Smith and W. E. Crabb, "The effect of continuous administration of diets containing low levels of tetracyclines on the incidence of drug-resistant Bacterium coli in the faeces of pigs and chickens: the sensitivity of the Bact. coli to other chemotherapeutic agents.," *Vet. Rec.*, vol. 69, pp. 24–30, 1957.
- [6] C. H. L. Howells and D. H. M. Joynson, "Possible role of animal feeding-stuffs in spread of antibioticresistant intestinal coliforms.," *Lancet*, pp. 156–7, 1975.
- [7] B. L. Ngangom, S. S. A. Tamunjoh, and F. F. Boyom, "Antibiotic residues in food animals: Public health concern," *Acta Ecol. Sin.*, vol. 39, no. 5, pp. 411–415, 2019.
- [8] M. Tian *et al.*, "Pollution by antibiotics and antimicrobial resistance in livestock and poultry manure in China, and countermeasures," *Antibiotics*, vol. 10, no. 5, p. 539, 2021.
- [9] S. Bhave *et al.*, "Phylogrouping and antimicrobial resistance analysis of extraintestinal pathogenic Escherichia coli isolated from poultry species," *Turk. J. Vet. Anim. Sci.*, vol. 43, no. 1, pp. 117–126, 2019.
- [10] J. E. Dominguez *et al.*, "Simultaneous carriage of mcr-1 and other antimicrobial resistance determinants in Escherichia coli from poultry," *Front. Microbiol.*, vol. 9, p. 1679, 2018.
- [11] V. Furtula, C. R. Jackson, E. G. Farrell, J. B. Barrett, L. M. Hiott, and P. A. Chambers, "Antimicrobial resistance in Enterococcus spp. isolated from environmental samples in an area of intensive poultry production," *Int. J. Environ. Res. Public. Health*, vol. 10, no. 3, pp. 1020–1036, 2013.
- [12] M. M. Hassan, K. B. Amin, M. Ahaduzzaman, M. Alam, M. S. Faruk, and I. Uddin, "Antimicrobial resistance pattern against E. coli and Salmonella in layer poultry," *Res J Vet Pr.*, vol. 2, no. 2, pp. 30–35, 2014.
- [13] K. M. Osman *et al.*, "Poultry hatcheries as potential reservoirs for antimicrobial-resistant Escherichia coli: A risk to public health and food safety," *Sci. Rep.*, vol. 8, no. 1, pp. 1–14, 2018.
- [14] A. K. Yassin *et al.*, "Antimicrobial resistance in clinical Escherichia coli isolates from poultry and livestock, China," *PloS One*, vol. 12, no. 9, p. e0185326, 2017.
- [15] R. D. Abdi *et al.*, "Determination of the sources and antimicrobial resistance patterns of Salmonella isolated from the poultry industry in Southern Ethiopia," *BMC Infect. Dis.*, vol. 17, no. 1, pp. 1–12, 2017.

- [16] L. A. Andoh, A. Dalsgaard, K. Obiri-Danso, M. J. Newman, L. Barco, and J. E. Olsen, "Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry in Ghana," *Epidemiol. Infect.*, vol. 144, no. 15, pp. 3288–3299, 2016.
- [17] K. A. Borges, T. Q. Furian, S. N. de Souza, C. T. P. Salle, H. L. de S. Moraes, and V. P. do Nascimento, "Antimicrobial resistance and molecular characterization of Salmonella enterica serotypes isolated from poultry sources in Brazil," *Braz. J. Poult. Sci.*, vol. 21, 2019.
- [18] K. A. Liljebjelke, C. L. Hofacre, D. G. White, S. Ayers, M. D. Lee, and J. J. Maurer, "Diversity of antimicrobial resistance phenotypes in Salmonella isolated from commercial poultry farms," *Front. Vet. Sci.*, vol. 4, p. 96, 2017.
- [19] D. H. Shah, N. C. Paul, W. C. Sischo, R. Crespo, and J. Guard, "Population dynamics and antimicrobial resistance of the most prevalent poultry-associated Salmonella serotypes," *Poult. Sci.*, vol. 96, no. 3, pp. 687–702, 2017.
- [20] K. Shang, B. Wei, H.-K. Jang, and M. Kang, "Phenotypic characteristics and genotypic correlation of antimicrobial resistant (AMR) Salmonella isolates from a poultry slaughterhouse and its downstream retail markets," *Food Control*, vol. 100, pp. 35–45, 2019.
- [21] R. Singh, A. S. Yadav, V. Tripathi, and R. P. Singh, "Antimicrobial resistance profile of Salmonella present in poultry and poultry environment in north India," *Food Control*, vol. 33, no. 2, pp. 545–548, 2013.
- [22] C. G. Velasquez *et al.*, "Prevalence and antimicrobial resistance patterns of Salmonella isolated from poultry farms in southeastern United States," *Poult. Sci.*, vol. 97, no. 6, pp. 2144–2152, 2018.
- [23] L. García-Sánchez, B. Melero, I. Jaime, M. Rossi, I. Ortega, and J. Rovira, "Biofilm formation, virulence and antimicrobial resistance of different Campylobacter jejuni isolates from a poultry slaughterhouse," *Food Microbiol.*, vol. 83, pp. 193–199, 2019.
- [24] D. M. Hull, E. Harrell, A. H. van Vliet, M. Correa, and S. Thakur, "Antimicrobial resistance and interspecies gene transfer in Campylobacter coli and Campylobacter jejuni isolated from food animals, poultry processing, and retail meat in North Carolina, 2018–2019," *PLoS One*, vol. 16, no. 2, p. e0246571, 2021.
- [25] F. Marotta *et al.*, "Antimicrobial resistance genotypes and phenotypes of Campylobacter jejuni isolated in Italy from humans, birds from wild and urban habitats, and poultry," *PloS One*, vol. 14, no. 10, p. e0223804, 2019.
- [26] C. Varga, M. T. Guerin, M. L. Brash, D. Slavic, P. Boerlin, and L. Susta, "Antimicrobial resistance in Campylobacter jejuni and Campylobacter coli isolated from small poultry flocks in Ontario, Canada: A two-year surveillance study," *PLoS One*, vol. 14, no. 8, p. e0221429, 2019.
- [27] K. Wieczorek, T. Wo\lkowicz, and J. Osek, "Antimicrobial resistance and virulence-associated traits of Campylobacter jejuni isolated from poultry food chain and humans with diarrhea," *Front. Microbiol.*, vol. 9, p. 1508, 2018.
- [28] I. Benrabia, T. M. Hamdi, A. A. Shehata, H. Neubauer, and G. Wareth, "Methicillin-resistant Staphylococcus aureus (MRSA) in poultry species in Algeria: Long-term study on prevalence and antimicrobial resistance," *Vet. Sci.*, vol. 7, no. 2, p. 54, 2020.
- [29] B. Markey, F. Leonard, M. Archambault, A. Cullinane, and D. Maguire, *Clinical veterinary microbiology*. Elsevier Health Sciences, 2013.
- [30] H. D. Hedman, K. A. Vasco, and L. Zhang, "A review of antimicrobial resistance in poultry farming within low-resource settings," *Animals*, vol. 10, no. 8, p. 1264, 2020.
- [31] D. F. Apata, "Antibiotic resistance in poultry," Int. J. Poult. Sci., vol. 8, no. 4, pp. 404–408, 2009.
- [32] C. L. Gyles, "Antimicrobial resistance in selected bacteria from poultry," *Anim. Health Res. Rev.*, vol. 9, no. 2, pp. 149–158, 2008.
- [33] N. T. Nhung, N. Chansiripornchai, and J. J. Carrique-Mas, "Antimicrobial resistance in bacterial poultry pathogens: a review," *Front. Vet. Sci.*, vol. 4, p. 126, 2017.
- [34] G. V. Doern and S. M. Brecher, "The clinical predictive value (or lack thereof) of the results of in vitro antimicrobial susceptibility tests," *J. Clin. Microbiol.*, vol. 49, no. 9 Supplement, pp. S11–S14, 2011.
- [35] J. L. Watts, M. T. Sweeney, and B. V. Lubbers, "Antimicrobial susceptibility testing of bacteria of veterinary origin," *Microbiol. Spectr.*, vol. 6, no. 2, pp. 6–2, 2018.

- [36] D. A. Almashhadany, "Occurrence and antimicrobial susceptibility of Salmonella isolates from grilled chicken meat sold at retail outlets in Erbil City, Kurdistan region, Iraq," *Ital. J. Food Saf.*, vol. 8, no. 2, 2019.
- [37] Z. H. Al-Safi, "Molecular detection of Quinolone Resistant-Salmonella isolates from poultry farms in Alkut, IRAQ.," in *IOP Conference Series: Earth and Environmental Science*, 2021, vol. 790, no. 1, p. 012045.
- [38] A. Harb *et al.*, "Occurrence, antimicrobial resistance and whole-genome sequencing analysis of Salmonella isolates from chicken carcasses imported into Iraq from four different countries," *Int. J. Food Microbiol.*, vol. 284, pp. 84–90, 2018.
- [39] T. O. Hasan and I. J. Lafta, "Identification and Antimicrobial Susceptibility Profiles of Salmonella spp. Isolated from Chicken Flocks and their Feed and Water in Karbala, Iraq," *Indian J. Ecol.*, vol. 48, no. 5, pp. 1542–1550, 2021.
- [40] E. R. Hassan and A. O. Alhatami, "Antimicrobial susceptibility profile of Salmonella enterica isolated from poultry farms using Vitek-2," *Kufa J. Vet. Med. Sci.*, vol. 10, no. 1, 2019.
- [41] A. A. Jassim and N. M. Al-Gburi, "Virulence genes and antimicrobial resistance of Salmonella isolated from milk in Wasit Province, Iraq," *Plant Arch.*, vol. 20, no. 1, pp. 2033–2039, 2020.
- [42] Z. M. Taha, M. S. Ahmed, and J. M. Abdo, "Occurrence and antimicrobial resistance of Salmonella serotypes isolated from chicken carcasses in Duhok, Kurdistan Region/Iraq," JZS, vol. 17, no. A, pp. 119– 128, 2015.
- [43] M. H. G. Kanaan, A. J. O. Al-Isawi, and F. A. Mohamme, "Antimicrobial Resistance and Antibiogram of Thermotolerant Campylobacter Recovered from Poultry Meat in Baghdad Markets, Iraq," Arch. Razi Inst., vol. 77, no. 1, pp. 231–237, 2022.
- [44] M. H. G. Kanaan and F. A. Mohammed, "Antimicrobial resistance of Campylobacter jejuni from poultry meat in local markets of Iraq," *Plant Arch*, vol. 20, no. Suppl 1, pp. 410–415, 2020.
- [45] Z. M. Shakir, A. O. Alhatami, Y. Ismail Khudhair, and H. Muhsen Abdulwahab, "Antibiotic Resistance Profile and Multiple Antibiotic Resistance Index of Campylobacter Species Isolated from Poultry," Arch. Razi Inst., vol. 76, no. 6, pp. 1677–1686, 2021.
- [46] A. E. Almousawi, A. O. Alhatami, N. A. Neama, and A. M. Baqir, "Characterization and molecular evaluation of Staphylococcus aureus isolated from poultry and dairy cattle milk in Iraq," in *AIP Conference Proceedings*, 2022, vol. 2386, no. 1, p. 020014.
- [47] M. S. Assafi, H. A. Hado, and I. S. Abdulrahman, "Detection of methicillin-resistant Staphylococcus aureus in broiler and broilers farm workers in Duhok, Iraq by using conventional and PCR techniques," *Iraqi J. Vet. Sci.*, vol. 34, no. 1, pp. 15–22, 2020.
- [48] A. O. Alhatami, H. Muhsen, F. Al-Araji, I. Raheem, and H. Ayad, "Escherichia coli strains as Major secondary bacterial pathogen isolated from an outbreak of swollen head syndrome in layers, in Al-Diwaniyah, Iraq," *Al-Qadisiyah J. Vet. Med. Sci.*, vol. 17, no. 1, pp. 81–88, 2018.
- [49] N. M. Saeed, "Detection of extended spectrum beta-lactamase gene production by E. coli isolated from human and broiler in Sulemania province/Iraq," *J. Zankoy Sulaimani-Part A*, vol. 16, p. 2, 2014.