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Pharmacological and biochemical aspects of co-administration of doxycycline and Colistin sulphate against experimentally induced *Salmonella typhimurium* infection in chicken

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ABSTRACT

Globally, Salmonella is considered one of the most infectious zoonotic pathogens. New approaches to reduce the transmission of Salmonella by food and other routes must be enforced to limit its infectious spread. The current study aimed to assess biochemical and pharmacological effects of administration of either doxycycline, colistin sulphate, or their combination on chicken experimentally infected with *Salmonella typhimurium*. A total of 25 healthy chickens were divided equally into 5 groups. Group (1) as control. Group (2): Orally infected with *S. typhimurium*. Group (3): Orally infected with *S. typhimurium* before being administered doxycycline. Group (4): Orally infected with *S. typhimurium* before being administered with colistin sulphate. Group (5): Treated with doxycycline and colistin sulphate after *S. typhimurium* infection. The result illustrated a significant increase in kidney function, as reflected in creatinine, blood urea nitrogen, and liver enzymes, alanine aminotransferase, and aspartate aminotransferase, after *S. typhimurium* infection. At the same level, the results showed an elevation in interleukin-6 in Group 2 when compared with control; this increase was improved after either doxycycline, colistin sulphate or their combination treatment. In conclusion, this study found that either doxycycline treatment or colistin sulphate and their combination are considered a promising treatment for *S. typhimurium* infection.

Keywords: Antibiotic resistance; Chicken; Colistin sulphate; Doxycycline; *Salmonella typhimurium*

1. Introduction

As a Gram-negative bacterium, Salmonella is a major contributor to illness, death, and financial loss in the industry of broilers, and a major source of foodborne illnesses in both people and animals worldwide (Pulido-Landinez, 2019). Most frequently, *Salmonella typhimurium* is linked to cattle illnesses (Thung et al., 2016; Lamas et al., 2016). According to Wyrsch et al. (2019), Salmonella contamination in broilers mostly results in intestinal health issues that significantly impair growth and promote the transmission of drug-resistant genes. Disease prevention for broilers increases chicken output (Hassan et al., 2010). Salmonella causes several illnesses in chickens, including typhoid, paratyphoid, and pullorum (Tatiane et al., 2013). Salmonella causes enteritis and significant mortality (Kee et al., 2015). In chickens, pullorum illness is a septicemia that mostly affects young chicks (Ahmed et al., 2021).

According to Cinquina et al. (2003), tetracyclines show a broad spectrum of antibacterial action against both aerobic and anaerobic Gram-positive and Gram-negative bacteria, which makes them essential for therapeutic application. Doxycycline is a broad-spectrum second-

generation tetracycline that primarily inhibits the growth of aerobic and anaerobic bacteria that are Gram-positive and Gram-negative, as well as Mycoplasma (Bettenay et al., 2003).

Doxycycline is typically recommended to treat Lyme disease, *Helicobacter pylori* infection, acute bronchitis, community-acquired pneumonia, and some Sexually transmitted diseases (STDs) (Singh et al., 2021). It also exerts anti-inflammatory and immunomodulatory properties in addition to its bacteriostatic effectiveness against a range of bacteria (Patel et al., 2020). In general, doxycycline doesn't cause severe side effects. The most frequent adverse effects include diarrhea brought on by antibiotics, nausea, and epigastric discomfort. Numerous investigations confirm that the host microbiota's resistance is unaffected by the long-term treatment of sub-antimicrobial dosages of doxycycline (Walker et al., 2005). Studies have also shown that doxycycline is less likely to cause hepatotoxicity than tetracycline (Heaton et al., 2007).

According to Koyama et al. (1950), *Bacillus polymyxa* subsp. colistinus produces the antibiotic colistin. The primary target of colistin is Gram-negative bacteria. It develops its primary activity in the gut against various strains of Salmonella, Enterobacter, and Coli because it is not absorbed from the gastrointestinal system. The inactivation of bacterial endotoxins (*E. coli*) is another benefit of colistin (Brander et al., 1993).

2. Materials and Methods

Ethical Statement

The experiment protocol was accepted by the Research Committee of the Agriculture Research Center and Animal Health Research Institute (ARC-IACUC), Ministry of Agriculture, Giza, Egypt (approval number ARC-AHRI/153/24).

Bacterial strain

Salmonella typhimurium was purchased from the serology department, Animal Health Research Institute (AHRI), Dokki. One ml *S. typhimurium* suspension, containing $1x10^9$ CFU/ml. *S. typhimurium* was administered to chickens in groups 2, 3, 4 and 5 orally once using a sterile gavage.

Doxycycline

Doxycycline was acquired from Pharma Swede, Egypt, as a powder. Each one gm contains 200 mg doxycycline HCL. Doxycycline was dissolved in water and given orally to each chicken in groups 3 and 5 using a stomach tube.

Colistin sulphate

Colistin sulphate® is manufactured by Pharma Swede, Egypt. It is a water-soluble powder. Each one gm contains colistin sulphate 5,000,000 IU. Colistin sulphate was dissolved in water and given orally to each chicken in groups 4 and 5 using a stomach tube.

Biochemical analysis

Creatinine, and blood urea nitrogen (BUN) were quantitatively estimated according to Wybenga (1971) and Tietz (1986), using kits manufactured by Spectrum Diagnostics.

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were estimated using the technique described by Reitman and Frankel (1957) and Young (1997) using kits manufactured by Spectrum Diagnostics.

Quantitative determination of serum interleukin-6 (IL-6) was carried out using an ELISA kit that was manufactured by GENORISE SCIENTIFIC, INC., catalog No. #106294-2.

Experimental animals and design

The determination of the effectiveness of both doxycycline and colistin sulphate alone and the combination by using MIC, MBC, and FIC.

The present study was carried out at the AHRI, El-Dokki, Giza, Egypt. It was conducted on 25 broiler chickens obtained from a private commercial hatchery fed on a drug-free ration and supplied with water ad libitum before and during the experiments. Antibiotics (doxycycline and colistin sulphate) were administered orally to chicken groups after the appearance of symptoms (clinical signs). Chicken was sectioned into 5 groups. Each group contained 5 chickens:

Group 1: Control negative (salmonella or antibiotics were not given).

Group 2: Control positive (infected with *S. typhimurium* orally to induce infection at a dose of 1*109 CFU for each chicken) (He et al., 2019).

Group 3: Given *S. typhimurium* orally to induce infection at a dose of $1x10^9$ CFU for each chicken. Antibiotic doxycycline was

administered orally. Subsequently, with (10 mg/ Kg b.wt) once a day for 5 consecutive days (Mansour et al., 2014).

Group 4: Given *S. typhimurium* orally to induce infection at a dose of $1x10^9$ CFU for each chicken. Antibiotic colistin sulphate was administered orally. Subsequently, with (100,000 IU/ Kg b.wt) once a day for 5 consecutive days (Elbadawy and Aboubakr 2017).

Group 5: Given *S. typhimurium* orally to induce infection at a dose of $1x10^9$ CFU for each chicken. Antibiotic doxycycline was administered orally. Subsequently, with (10 mg/ Kg b.wt) in combination with oral administration of colistin sulphate (100,000 IU/ Kg b.wt). Each drug will be given once a day for 5 consecutive days.

Clinical signs

Chickens showed dullness, dropping of wings, loss of appetite, ruffled feathers and diarrhea after 24 hours in all infected groups. The symptoms alleviated gradually after administration of doxycycline and colistin sulphate, especially in the group given both drugs (group 5).

Sampling

After 24 hours of administration of the last dose of antibiotics, all birds were sacrificed. Blood samples used in biochemical analysis were collected from the chicken's wing veins using a syringe and left to coagulate at room temperature, and then centrifuged (5 min, 3000 rpm). The resulting serum samples were preserved at -20°C until it was time to use them. The results were statistically analyzed and discussed with providing a conclusion on whether doxycycline or colistin sulphate, alone or the combination, is more effective.

Antimicrobial activity of antibiotics

Determination of Antimicrobial test (AT), minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC) of doxycyclin and/or colistin sulphate against *S. typhimurium*.

The MIC for doxycycline and colistin sulphate was determined using the broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2019) against the *S. typhimurium* strain. Double-fold serial dilutions of Colistin sulphate were prepared in a range of 0.250 to 8 µg/mL and 2 to 64µg/mL for doxycycline (EUCAST, 2024). These dilutions of antibiotics were used in broth with $1*10^6$ CFU/ml of *S. typhimurium*. Two growth control tubes were tested, including a control positive tube containing microbial suspension without antibiotic and a control negative tube containing broth with antibiotic. The tubes were incubated at 37°C for 24h. MIC was the lowest concentration of the antibacterial agent that did not allow any bacterial growth after 24 h of incubation based on turbidity. While MBC was detected through the subculture of all the tubes, not showing any bacterial growth in the MIC test. The plates were incubated at 37˚C for 24 hours. The lowest concentration with no visible growth was defined as the MBC, indicating 99.5% killing of the original inoculum (Amita et al., 2013).

Fractional inhibitory concentration (FIC) index

The combination of doxycycline and colistin sulfate was assessed using the Checkboard technique. Doxycycline concentrations ranging from 1/32 MIC to four times of MIC (4×MIC) were utilized with colistin sulphate at a concentration equivalent to 1/2 MIC, and vice versa (Jarrar et al., 2010). The formula, according to Kamatou et al. (2006), was used to calculate the fractional inhibitory concentration (FIC) value.

The sum of FIC A and FIC B equals the FIC index, where FIC stands for the combination's MIC divided by the drug's MIC. When the drug interaction's FIC index is less than 0.5, it is deemed synergistic, meaning that its overall effect is smaller than the sum of its separate agents' effects. FIC values between 0.5 and 4 are regarded as additive or indifferent, meaning that the sum of the impacts of the various agents equals the overall effect. FIC index values more than 4 indicate an antagonistic impact, meaning that the overall effect is smaller than the sum of the effects of the individual agents.

Statistical analysis

A completely randomized design was selected. Data analysis was carried out using SPSS software (release 20, IBM CO). ANOVA and

Fisher's least significant difference test (LSD) were used to find means, and significance was tested at $p < 0.05$.

3. Results

Minimum inhibitory concentration, minimum bactericidal concentration and fractional inhibitory concentration index

MIC of doxycycline was 8 µg/mL , while MIC of colistin sulphate against *S. typhimurium* was 2µg/mL. MBC of doxycycline was 16 µg/mL, while MBC of colistin sulphate against *S. typhimurium* was 4 µg/mL. However, when used together, they showed an additive effect against *S. typhimurium,* as the recorded FIC index value was 0.531(Table 1)

Table 1. MIC, MBC, and FIC index of doxycycline and colistin sulphate against *S. typhimurium*

	Doxycycline	**Colistin sulphate**
MIC	8 µg/mL	2 µg/mL
MBC	16 µg/mL	4 µg/mL
FIC index	0.03125 µg/mL	0.5 µg/mL
FIC index of both	0.531 µg/mL	

The effects of doxycycline, colistin, and their combination on the kidney, liver functions, and cytokine IL-6 in chickens experimentally infected with *S. typhimurium* are shown in Table 2. Regarding creatinine, BUN, they significantly increased ($p< 0.05$) in the experimentally infected group (group 2) (0.87 ± 0.03 and 7.65 ± 0.26, respectively), in contrast to the negative control (0.50±0.01 and 5.7 ± 0.23, respectively). This increase was significantly decreased after treatment with either doxycycline (0.76 ± 0.02) or colistin (0.67 ± 0.02), and a non-significant decrease in the combination group in creatinine levels.

BUN levels in the doxycycline (7 ± 0.26), colistin-treated (6.47 ± 0.24), and the combination groups (6.97 ± 0.3) showed a non-significant decrease compared to the infected group. At the same level there was a significant elevation in liver function enzymes, AST, ALT and cytokine IL6 in the experimentally-infected non-treated group (53 ± 1.53, 28.67 ± 1.2, and 264.5 ± 11.26, respectively), when compared to the control group. The enzyme activities and IL-6 level were significantly reduced after treatment in all groups.

Biochemical results

Table 2. The effect of Doxycycline, colistin, and their combination on the kidney, liver function, and cytokine IL6 against experimentally induced *S. typhimurium* infection in chicken.

Parameter	Control	*S. typhimurium* Infected	Infected /DOXY	Infected /COL	Infected/DOXY/COL
Creatinine	0.50 ± 0.01^{d}	0.87 ± 0.03^{a}	0.76 ± 0.02^{b}	0.67 ± 0.02^{c}	0.82 ± 0.04^{ab}
BUN	5.7 ± 0.23^{c}	7.65 ± 0.26^{a}	7 ± 0.26^{ab}	6.47 ± 0.24^{bc}	6.97 ± 0.3^{ab}
AST	28.67 ± 0.88^{c}	53 ± 1.53^{a}	39 ± 1.53^{b}	39.67 ± 1.67^{b}	35.67 ± 2.73^{b}
ALT	18 ± 0.58^{d}	28.67 ± 1.2^{a}	20 ± 0.58^{cd}	21.5 ± 1.32^{bc}	24.33 ± 1.2^{b}
IL6	115.7 ± 2.06^{c}	264.5 ± 11.26^{a}	122.9 ± 1.01^{c}	130.37 ± 7.63^{c}	170 ± 5.77^{b}

Mean values are significantly different at $p < 0.05$, DOXY; doxycycline, COL; colistin

4. Discussion

The most common food-borne zoonotic illness worldwide is Salmonella. The main causative agents of salmonellosis in chicken are non-typhoidal Salmonella serotypes. The most prevalent foodborne infection in the world is Salmonella, which infects the gastrointestinal tract and may cause cramps, nausea, and diarrhea in people. According to estimates from the Centers for Disease Control and Prevention (CDC), there are around 420 deaths and 1.35 million infections in the US annually (Majowicz et al., 2010; Cosby et al., 2015; Jung et al., 2022).

Most human instances of salmonellosis are due to non-typhoidal strains of *Salmonella, specifically S. enteritidis* and *S. typhimurium.*

Among animals that produce food, poultry is the main reservoir for a variety of non-typhoidal Salmonella (NTS) serotypes. In the United States, 17.9% of foodborne illnesses between 1998 and 2008 were caused by chicken. 17.4% and 34% of foodborne illnesses linked to chicken were caused by *S. Enteritidis* and *S. typhimurium*, respectively (Painter et al., 2013).

According to estimates, nontyphoidal Salmonella linked to chicken costs society $2.79 billion annually in medical expenses and economic hardship. This dilemma is growing as the need for ready-to-eat foods rises globally. Numerous pre-harvest and post-harvest intervention techniques have been developed to ensure the cleanliness and safety of chicken products. Preharvest treatments include farm-level management practices such as biosecurity measures and feed additives.

Preparing meat and slaughtering carcasses are examples of post-harvest practices. The importance of alternatives in the chicken business is growing due to the appearance of multidrug-resistant bacteria and the ensuing public health issue (Tajkarimi, 2007; Scharff, 2020). To help the fight against pathogens like *Salmonella,* more potent techniques for treatment are needed, such as antibiotic combinations.

Selecting the best course of action may be aided by the least MIC. It describes the degrees of resistance or susceptibility of bacterial strains to a particular antibiotic in vitro (Phillips et al., 1998). Understanding MIC values aids in the development of intelligent, focused treatment, much like deciphering a bacterium's resistance profile. Since January 1, 2019, two susceptible categories and one resistant category have been established in accordance with EUCAST (2019) recommendations. Hence, MIC values help determine how effectively an antibiotic can inhibit bacterial growth. Low MIC values indicate high bacterial susceptibility, meaning the antibiotic is expected to work well at standard doses. Intermediate MIC values reflect borderline susceptibility, suggesting that higher doses or combination therapy may be needed for effective treatment. In contrast, high MIC values indicate bacterial resistance, making the antibiotic ineffective and unsuitable for useThe present study showed that MIC of doxycycline was higher than that of colistin sulphate against *S. typhimurium.* This suggested that colistin sulphate had more efficacy than doxycyclin against the tested *S. typhimurium*. These MIC values point to the necessity of constant monitoring of antimicrobial resistance, particularly in agricultural environments where *S. typhimurium* is common, such as Egyptian chicken farms or duck farms.

These findings agreed with Amer et al. (2020), who isolated *S. enteritidis* from broiler samples. The isolated bacteria showed complete resistance to trimethoprim/sulfamethoxazole, moderate resistance against doxycycline and low resistance to colistin. A study on duck farms in Egypt found that *S. typhimurium* isolates were completely sensitive to both colistin and doxycycline, though resistance to other antibiotics like amoxicillin was high (Khalifa et al., 2021).

The current result revealed that Salmonellosis in broilers revealed a significant increase in kidney function parameters and also liver enzymes ALT, AST. Wells et al. (1986), claimed that high blood creatinine is due to low glomerular filtration rate of the renal kidney, whereas creatinine is the result of muscular creatinine catabolism and is expelled by the trunk kidney (Trumble et al. (2006). Chicks infected with *S. gallinarum* showed the same alterations in their liver and kidney (Kumari et al., 2013).

It is commonly known that ALT and AST are sensitive indicators for evaluating hepatocellular damage (Oh et al., 2017). Salmonella-infected broilers may have elevated AST and ALT because the bacterial toxin may have destroyed their liver cells (Halla et al., 2022). Liver cell destruction is caused by Salmonella toxins, and the release of liver enzymes increases liver enzymes (Doxy, 1983). Additionally, according to Ahmed and Mahmoud (2014), broiler salmonellosis causes a substantial rise in ALT and AST.

According to Monfrecola et al. (2013), monocytes and keratinocytes release the multifunctional cytokine IL-6 in response to

inflammatory stimuli such as exposure to UV light and bacterial endotoxins. IL-6's inflammatory function and its position as a mediator cause the persistence of inflammatory manifestations, especially in resistant cases (Li et al., 2003). Many host and bacterial variables influence the riskiness and impact of Salmonella infection. The host response to intracellular infections was largely coordinated by cytokines (Liles & Van Voorhis., 1995).

Salmonella has distinct bacterial products which induce cytokine expression by immune cells (Wilson et al., 1998; Ciacci- Woolwine et al., 1998; Eman et al., 2018). Because monocytes catalyze antigen by *S. typhi* proteins, elevated IL-6 gene expression in these cells may be the cause of rising IL-6 levels (Kogut, 2005). Furthermore, intestinal epithelial cells understand bacteria use toll-like receptors to begin cellular immune responses, of which elevated IL-6 is one expression (Iankov et al., 2004).

Doxycycline is a bacteriostatic antibiotic based on tetracyclines that prevents the production of bacterial proteins and metalloproteases. Additionally, research has shown that it alters proinflammatory cytokines and has anti-inflammatory qualities (Conforti et al., 2020; MohamadiYarijani and Najafi., 2021). Tetracyclines are excellent antibiotics because of their various qualities, which include their capacity to combat both Gram-positive and Gram-negative bacteria, their shown clinical safety, their tolerability, and the fact that most of the class is available in oral and intravenous (IV) forms (Grossman, 2016).

Serum ALT, AST, and creatinine levels significantly improved when doxycycline was administered to salmonella-challenged chicken. It is possible that this improvement is due to the drug's bacteriostatic activity (Flower et al., 2012), which minimizes the harmful and lethal effects on the liver and kidney (Hosny et al., 2020).

Following doxycycline treatment, there was a noticeable decrese in the level of IL-6 rate. Roberta et al. (2015) found that doxycycline, even at low dosages, effectively modulated the expression of the IL-6 gene, a proinflammatory cytokine generated by lipopolysaccaride. In this regard, doxycycline's ability to control proinflammatory cytokine levels was linked to a notable decrease in circulating levels when taken orally every day. Within seven days of therapy, downregulation was quickly seen (Nishant and Dinesh, 2018).

Paenibacillus polymyxa is one of numerous types of bacteria that produce antibiotic colistin. Colistin sulfate and colistimethate sodium are the two commercial forms of collistin. Colistin can also render bacterial toxins inactive in vitro. To combat gram-negative bacteria like Salmonella spp., the antibiotic polypeptide colistin sulfate is typically administered. Bird enteritis is treated with oral colistin sulfate (Van Hattum et al., 2000; EMEA, 2002).

One of the most remarkable features of colistin sulfate is that it is absorbed orally by the birds' gastrointestinal tract (Collell and Segura, 2013). According to Rhouma et al. (2015) and Rasool and Rasool (2018), colistin sulfate has a particular and selective effect on intestinal bacteria in the intestinal lumen in this scenario. Furthermore, numerous sensitivity studies to colistin sulfate, using various isolates of *Salmonella* sp. in Spain, revealed a very low percentage of resistance to this active ingredient, with most results demonstrating 100% sensitivity. This was consistent with our parameter results in kidney, liver, or cytokine functions, which worsen the effects of Salmonella infection (García, 2011). Colistin increased the secretion of pro-inflammatory cytokines, including TNF-α and IL-6 (Wang et al., 2019).

Conclusion

In conclusion, doxycycline and colistin administration showed a promising treatment against the adverse consequences of *S. typhimurium* infection in chicken through its anti-bacterial, hepato-protective, and anti-inflammatory properties. Whether individually or in combination, the treatments showed different levels of improvement.

5. Reference

Ahmed F, Mahmoud S, 2014. Alterations of Blood Components in Broiler Chicks Experimentally Infected with Salmonella Gallinarum. Global Vet,13 (5):787-793.

Ahmed MFE, Abd El-Wahab A, Kriewitz J-P, Hankel J, Chuppava B, Ratert C, Taube V, Visscher C, Kamphues J, 2021. Mitigating the Spread and Translocation of Salmonella Enteritidis in Experimentally Infected Broilers under the Influence of Different Flooring Housing Systems and Feed Particle Sizes. Microorganisms, 9(4):874. doi.org/10.3390/9040874.

Amer MM, AM Amer, ER Hassan and AM Ghetas, 2020. Salmonella Enteritidis in broiler chickens: isolation, antibiotic resistance phenotyping and efficacy of colistin on control of experimental infection. Int J Vet Sci, 9(2): 267-272.

Amita M , Shashank K, Abhay K, 2013. Scientific validation of the medicinal efficacy of Tinosporacordifolia. Scientific World Journal, P, 1-8.

Bettenay VS, Mueller KD , Friend S, 2003. Prospective study of the treatment of feline plasmacyticpododermatitis with doxycycline. Veterinary Record ,152: 564-566.

Brander GC, Plugh DM, Bywater RJ, 1993 .Veterinary Applied Pharmacology and Therapeutics. Bbailliere Tindall, ova Road, London NWI.

Ciacci-Woolwine F, Blomfied IC, Richardson SH, Mizel, SB, 1998. Salmonella flagellin induces tumour necrosis factor alpha in a human promonocytic cell line. Infect. Immun, 66, 1127-1134.

Cinquina AL, Longo F, Anastasi G, Giannetti L,Cozzani R, 2003. Validation of a high performance liquid chromatography method for the determination of oxytetracycline, tetracycline, chlortetracycline and doxycycline in bovine milk and muscle. Journal of Chromatography A, 987: 223-227.

Clinical and Laboratory Standards Institute (CLSI) ,2019. Performance Standards for Antimicrobial Susceptibility Testing. 29th ed. CLSI supplement M100.Wayne, PA: Clinical and Laboratory Standards Institute.

Collell M, Segura A, 2013. Colistina, unclásico hoy imprescindibleentransición. Avancesentecnologíaporcina. Vol. X (97): 26–28.

Conforti C, Giuffrida R, Zalaudek I, Di Meo N, 2020. Doxycycline, a widely used antibiotic in dermatology with a possible anti-inflammatory action against IL-6 in COVID-19 outbreak. Dermatol. Ther., 33 13437.

Cosby DE, Cox NA, Harrison MA, Wilson JL, Buhr RJ, Fedorka-Cray PJ, 2015. Salmonella and antimicrobial resistance in broilers. A review. J. Appl. Poult. Res, 24:408–426.

Doxy D, l983. Clinical pathology and diagnostic 2nd Ed. Baillier London 56- 60.

Elbadawy M, Aboubakr M, 2017. Efficacy of colimox® (a new combination of amoxicillin and colistin) in the control of experimentally induced necrotic enteritis in broiler chickens. International Journal of Pharmacology and Toxicology, 5 (1): 51-56.

Eman AE, EL-Sayed TI, Attia AA, Rashed ME, Heba M R, 2018. Proinflammatory Cytokines in Plasma of Patients with Typhoid Fever and Resistance to Therapy Egypt. J. Microbiol, 53, pp. 141- 149.

EMEA, 2002. The European Agency for the Evaluation of Medicinal Products. Colistin, Summary Report (1). Committee for Veterinary Medicinal Products. 1-5.

Flower R, Rang HP, Dale MM, Ritter JM, Henderson G, 2012. Rang & Dale's Pharmacology. Edinburgh, Churchill Livingstone press 776 p.

García C, 2011. SalmonelosisporcinaenEspaña: Prevalencia, factores de riesgo y resistenciaantimicrobiana. Tesis Doctoral. León, España, 1-197 p.

Grossman T H, 2016. Tetracycline Antibiotics and Resistance. Cold Spring HarbPerspect Med, 6(4):a025387.

Hassan H., Mohamed M.A., Amani Youssef and Eman Hassan (2010). Effect of Using Organic Acids to Substitute Antibiotic Growth Promoters on Performance and Intestinal Microflora of Broilers. Asian-Australasian Journal of Animal Sciences. 23.

Halla M K, Halla A E , Mona S,Hammada H E, 2022. Evaluation of using some organic acids against salmonella infection in broiler chickens experimentally .Assiut Vet. Med. J. Vol. 68 No. 172 ,18-27.

He Y, Yang Y, Dong Y, Yan C, Zhang B, 2019. The Effects of Flavomycin and Colistin Sulfate Pre-Treatment on Ileal Bacterial Community Composition, the Response to *Salmonella typhimurium* and Host Gene Expression in Broiler Chickens. Microorganisms, 7: 574.

Heaton PC, Fenwick SR ,Brewer DE , 2007. Association between tetracycline or doxycycline and hepatotoxicity: a population based case-control study. J Clin Pharm Ther, 32:483-7. 10.1111/j.1365-2710.2007.00853.x

Hosny A I, Naglaa ZHE, Azza AAG, Walaa TE, El Sayed K a, 2020. Antibacterial Activity of Doxycycline against Aeromonashydrophila in Experimentally Challenged African Catfish (Clariasgariepinus) .Zagazig Veterinary Journal, 44511, 48(1): 46-56, DOI: 10.21608/zvjz.2019.15561.1071.

IankovI, Atanasova G, Maria P, Silvia K, Petrov D, Mitev V,Mitov G I, 2004. Bacterial lipopolysacharide induces proliferation of IL-6 dependent plasma cytom cells by MARK pathway activation. Immunobiology, 208(5): 445-54.

Jarrar N, Adwan KM,Abu-Hijleh A, 2010. Antibacterial activity of Rosmarinus officinalis L. alone and in combination with cefuroxime against methicillin--resistant Staphylococcus aureus. Asian Pacific Journal of Tropical Medicine, 3(2): 121-123.

Jung B, Park S, Kim E, Yoon H, Hahn T, 2022. Salmonella Typhimurium lacking phoBR as a live vaccine candidate against poultry infection. Vet. Microbiol, 266:109342.

Kamatou G.P.P, Viljoen AM, VanVuuren SF, Van-ZylRL, 2006. In vitro evidence of antimicrobial synergy between Salvia chamelaeagnea and Leonotisleonurus. South African Journal of Boteny, 72(4):634-636.

Kee E,Hooi A, Syakima S, Lee L, 2015. Salmonella: review on pathogenesis, epidemiology and antibiotic resistance. Frontier in Life Sci, 8: 284-293.

Khalifa, Z. K. M., Ibrahim, A. A. E., Abd El-Motelib, T. Y., Abd El-Aziz, A. M., 2021. Molecular characterization of antibacterial resistance genes of salmonella in ducks. Assiut Veterinary Medical Journal; 67(171): 52-66. doi: 10.21608/avmj.2021.205171

Kogut,2005. Centers for disease control and prevention. National Centers for Infectious Diseases, Division for Bacterial and Mycotic Diseases.

Koyama Y, Kurosasa A, Tsuchiya A, Takakuta K, 1950. A new antibiotic 'colistin' produced by spore forming soil bacteria. Journal of Antibiotics, 3: 457-458.

Kumari D, Mishra K, Lather D, 2013.Pathomicrobial studies on Sal Gallinarum infection in chickens.Vet. World, 6 (10): 25-29.

Lamas A, Fernandez-No IC, Miranda JM, Vazquez B, Cepeda A, Franco CM,2016.Prevalence, molecular characterization and antimicrobial resistance of Salmonella serovars isolated from northwestern Spanish broiler flocks (2011–2015). Poult. Sci., 95, 2097–2105.

Li Y, Reichenstein K, Ullrich R, Danner T, von Specht BU, Hahn HP, 2003. Effect of in situ expression of human interleukin-6 on antibody responses against Salmonella typhiurium antigens. FEMS Immunol. Med. Microbiol., 37, 135-45.

Liles WC, Van Voorhis WC, 1995. Review: nomenclature and biologic significance of cytokines involved in inflammation and the host immune response . J. Infect. Dis, 172, 1573-1580.

Majowicz SE , Musto J, Scallan E, Angulo FJ, Kirk M, O'brien SJ, Jones TF, Fazil A, Hoekstra RM, 2010. International Collaboration on Enteric Disease "Burden of Illness" Studies. Glob. Burd. Nontyphoidal Salmonella Gastroenteritis Clin. Infect. Dis., 50:882–889.

Mansour E, Allam H, Neseren AS, Reham E, Abdalla SH, 2014. Studies on Effect of *Salmonella pullorum*in Balady Chicks and its Treatment with Doxycycline. Zag. Vet. J., 42(3): 145- 156.

MohamadiYarijani Z, Najafi H, 2021. Kidney injury in COVID-19 patients, drug development and their renal complications: Review study. Biomedicine & Pharmacotherapy, 142: 111966.

Monfrecola G , Gaudiello F, Cirillo T, Fabbrocini G, BalatoA, LemboS, 2013. "Nicotinamide downregulates gene expression of interleukin-6, interleukin-10, monocyte chemoattractant protein-1, and tumour necrosis factor-α gene expression in HaCaT keratinocytes after ultraviolet B irradiation. Clinical & Experimental Dermatology, 38(2) : 185–188.

Nishant, K and Dinesh C K, 2018. A Randomized Controlled Study to Assess the Effect of Doxycycline on IL-6 and TNF Levels among the Patients of Dengue .Journal of Medical Science And clinical Research, 6(2), DOI:10.18535/jmscr/v6i2.168

Oh, RC, Hustead TR, Ali SM, Pantsari MW, 2017. Mildly Elevated Liver Transaminase Levels: Causes and Evaluation. Am Fam Physician, 01;96(11):709-715.

Painter JA, Hoekstra RM, Ayers T, Tauxe RV, Braden CR, Angulo FJ, Griffin PM, 2013. Attribution of foodborne illnesses, hospitalizations, and deaths to food commodities by using outbreak data, United States, 1998–2008. Emerg. Infect. Dis., 19:407.

Patel A, Khande H, Periasamy H, Mokale S ,2020. Immunomodulatory effect of doxycycline ameliorates systemic and pulmonary inflammation in a Murine polymicrobial sepsis model. Inflammation, 43:1035-43. 10.1007/s10753-020-01188-y

Phillips I, Acar J, Bergan T, Degener J, Baquero F, Forsgren A, Schito G, Wiedemann BJ. 1998. Methods for the determination of susceptibility of bacteria to antimicrobial agents. Terminology, EUCAST Definitive Document. Clin. Microbiol. Infect.;4:291-296.

Pulido-Landínez M, 2019. Food safety – Salmonella update in broilers. Anim. Feed Sci. Technol., 250:53–58.

Rasool S, Ali and Rasool T, 2018. Application of Colistin to Combat Bacterial Diseases in Broiler Chickens. Int. J. Nanotechnol. Allied Sci, 2(1): 3-6.

Reitman S, Frankel S, 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol, 28(1): 56-63.

Rhouma M, Beaudry F, Thériault W, Bergeron N, Laurent-Lewandowski S, FairbrotherJM, Letellier A, 2015. Gastric stability and oral bioavailability of colistin sulfate in pigs challenged or not with Escherichia coli O149: F4 (K88). Res. Vet. Sci., 102: 173-81.

Roberta DC, Serena L, Luisa DC, Anna B, Giuseppe M, 2015. Anti-Inflammatory Properties of Low and High Doxycycline Doses: An In Vitro Study. Hindawi Publishing Corporation Mediators of Inflammation Volume, Article ID 329418, 10 Doi.org/10.1155/2015/329418

Scharff RL, 2020. Food Attribution and Economic Cost Estimates for Meat- and Poultry-Related Illnesses. J. Food Prot,83:959–967.

Singh S, Khanna D ,Kalra S, 2021. Minocycline and doxycycline: more than antibiotics. CurrMolPharmacol. 14:1046-65.

Tajkarimi M,2007. Salmonella spp.;Calif. Dep. Food Agric., 1–8.

Tatiane M, Elisabeth G,Cíntia S, 2013. Liver function in broiler infected with Sal Typhi treated with organic acid. Italian J. of Ani. Sci, 12 (3) :15-26.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2024. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST), 2019. Clinical Breakpoints—Bacteria (v 9.0) https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_9.0_Breakpoint_Tables.pdf.

Thung TY, Mahyudin, NA,Basri DF,Radzi CWJW,Nakaguchi Y,Nishibuchi M, Radu S, 2016. Prevalence and antibiotic resistance of Salmonella Enteritidis and Salmonella Typhimurium in raw chicken meat at retail

markets in Malaysia. Poult. Sci., 95, 1888–1893.

Tietz NW,1986. Textbook of clinical chemistry. 1919. Philadelphia: WB Saunders.

Trumble SJ, Castellini MA, Mau TL,Castellini JM, 2006. Dietary and seasonal influences on blood chemistry and hematology in captive harbor seals. Mar Mammal Sci, 22(1): 104-123.

Van Hattum C, Terlow P, De Kleyne J, Grintjes G, 2000. Efficacy of amoxicillin/colistin in gastro-intestinal E. coli infection compared with Streptoccocussuis in weaned piglets. In: Proceedings of the 8th EAVPT Congress, Jerusalem, CD-ROM Abstract N.: K13.

Walker C, Preshaw PM, Novak J, Hefti AF, Bradshaw M , Powala C, 2005. Long-term treatment with sub antimicrobial dose doxycycline has no antibacterial effect on intestinal flora. J ClinPeriodontol, 32:1163-9.

Wang J, Shao W, Niu H, Yang T, Wang Y,Cai Y, 2019. Immunomodulatory Effects of Colistin on Macrophages in Rats by Activating the p38/MAPK Pathway. Front Pharmacol, 26;10:729. doi: 10.3389/fphar.2019.00729.

Wells RM, McIntyre RH, MorganAK, Davie PS, 1986. Physiological stress responses in big game fish after capture: observations on plasma chemistry and blood factors. Comp. Biochem. Physiol, 84(3): 565-571

Wilson M, Seymour R, Henderson B, 1998. Bacterial perturbation of cytokine networks. Infect. Immun, 66: 2401-2409.

Wybenga DR, Di Giorgio J, Pileggi VJ, 1971. Manual and automated methods for urea nitrogen measurement in whole serum. Clin. Chem, 17(9): 891-895.

Wyrsch ER, Hawkey J, Judd LM, Haites R, Holt KE, Djordjevic SP, Billman-Jacobe H Z, 2019. Hybrid Virulence Plasmids Carrying Antimicrobial Resistance genes in S. Typhimurium from Australian Food Animal Production. Microorganisms, 7, 299.

Young DS, 1997. Effects of drugs on clinical laboratory tests. Ann. Clin. Biochem , 34 (6): 579-581