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Effect of Probiotic, Prebiotic and/or Fosfomycin in Control of Drug-Sensitive and Drug-Resistant *E. coli* O78 Infection in Broiler Chicken and Antibody to ND Vaccines

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Abstract

THE STUDY was goaled to compare the usage of probiotic and/or Fosfomycin in control of *E. coli* O78 in broiler chicken and the possible impact of these treatments on birds' immune reaction to ND vaccines. Two hundred and twenty birds were allocated to 11 with 20 birds per group and each group include two replicates with 10 birds each, the groups were as follows; G1 control negative birds; from G3 to G6 birds were challenged with strain 1(antibiotic resistant) *E. coli* O78 and G3, G4, G5 and G6 treated with Fosfomycin, Fosfomycin + probiotic, prebiotic and probiotic, respectively while G2 kept as control positive untreated birds. Birds from G7 to G11 were infected with strain 2 (antibiotic sensitive) *E. coli* O78 and G8, G9, G10 and G11 treated with Fosfomycin, Fosfomycin + probiotic, prebiotic and probiotic, respectively while G7 kept as control positive untreated birds. The results concluded that Fosfomycin may be a valuable tool in the management of antibiotic-resistant *E. coli* infections in broiler production in addition, the incorporation of probiotic supplementation may enhance the efficacy of Fosfomycin-based treatment by supporting intestinal health and the host's natural defense mechanisms.

Keywords: Boiler chickens, Drug resistance, Prebiotics, Probiotics, Fosfomycin, ND antibody.

Introduction

Escherichia coli (*E. coli*) is a common bacterial pathogen that can cause significant health and economic problems in poultry production [1]. One particularly problematic strain is *E. coli* O78, which is known to be a primary cause of colibacillosis in broiler chickens [2]. Colibacillosis is an invasive bacterial infection that can lead to high mortality and reduced growth performance in affected flocks [3, 4].

There are two main strategies for controlling *E. coli* O78 in broiler chickens are the use of antibiotics and the use of probiotics [5]. The administration of antibiotics has long been a common method for controlling *E. coli* infections in poultry [2,3]. Certain antibiotics, such as enrofloxacin and trimethoprim-sulfamethoxazole, have demonstrated effectiveness against *E. coli* O78 in broiler chickens [6.7]. Antibiotics work by directly killing or inhibiting the growth of the bacterial pathogen, thereby reducing the severity of the infection and minimizing its impact on bird health and productivity [8]. However, the widespread and indiscriminate use of antibiotics in poultry production has led to concerns about the development of antibiotic-resistant strains of *E. coli* and other bacteria [2]. This poses a significant threat to both animal and human health, as resistant bacteria

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can be transferred to humans through the food chain or other means of exposure [9].

An alternative approach to controlling *E. coli* O78 in broiler chickens is the use of probiotics [10]. Probiotics are live microorganisms, typically lactic acid bacteria or Bacillus species, that are administered to animals to improve gut health and enhance the immune system [11]. Probiotics inhibit the growth of pathogenic *E. coli* strains, including *E. coli* O78, through various mechanisms such as competitive exclusion, production of antimicrobial compounds, and modulation of the immune response [3, 12]. The advantage of using probiotics is that they do not contribute to the development of antibiotic resistance and can provide long-term benefits for the overall health and performance of the birds [13]. However, the efficacy of probiotic-based interventions can be variable and may depend on factors such as the specific probiotic strain, dosage, and method of administration [3, 14].

To maximize the effectiveness of *E. coli* O78 control in broiler chickens, a combination of antibiotic treatment and probiotic supplementation may be the most promising approach and that could be adopted by using antibiotics judiciously to quickly control acute outbreaks of colibacillosis, and then incorporating probiotics into the birds' feed or water to support long-term gut health and immune function, producers can potentially achieve better disease management while mitigating the risk of antibiotic resistance [2,11].

The problem is further compounded by the emergence of antibiotic-resistant strains of *E. coli* O78, rendering traditional treatment approaches less effective [15]. In recent years, the antibiotic including Fosfomycin is a bactericidal, low-molecular weight, broad-spectrum antibiotic, with putative activity against several bacteria, including multidrug-resistant Gram-negative bacteria, by irreversibly inhibiting an early stage in cell wall synthesis [16, 17].

It has shown promise in the treatment of *E. coli* infections, including those caused by the pathogenic serotype O78, which is commonly found in broiler chickens [18,19]. The use of Fosfomycin in the control of *E. coli* O78 in chickens has been explored, *in vitro* susceptibility of *E. coli* O78 isolates from broiler chickens to Fosfomycin, where most isolates were susceptible with minimum inhibitory concentrations (MICs) ranging from 0.5 to 8 μg/mL [20. 21]. The Fosfomycin treated infected chickens showed that Fosfomycin treatment significantly reduced the *E. coli* O78 counts in the intestines and livers of the infected chickens compared to the untreated control group [21]. The use of Fosfomycin in the control of *E. coli* O78 in chickens may have several advantages [21]. Firstly, Fosfomycin has a different mechanism of action compared to commonly used antibiotics in poultry production, which could help mitigate the development and spread of antibiotic resistance [20]. Secondly, Fosfomycin has been shown to be effective against both antibiotic-susceptible and antibiotic-resistant strains of *E. coli* O78 [21].

Multidrug resistance was detected to 42%-83.3% of tested 12 antibiotics. Only 15% of tested isolates showed a relationship between phenotype and genotype, most strains are sensitive and show resistant genes (P-G+) presented in three isolates for beta-lactam, one for Macrolide (ERI), as well as 5 isolates for trimethoprim (pyrimidine inhibitor) [22]. *E. coli* isolates had resistance and lacked gene (P+G-) reported meanly in 2 isolates for tetracycline, 4 isolates for ERI, 7 isolates for trimethoprim, and 9 isolates for aminoglycoside) [22]. Ahmed et al [23] stated that the most predominant isolated serotypes were O91, O128, O78, O124, O2 and O44. These strains were related to EHEC, EPEC, ETEC, and EIEC and these *E. coli* isolates are MDR to extensively drug-resistant (XDR). Addressing avian colibacillosis and the associated problem of multidrug resistance requires a comprehensive approach, including improved management practices, prudent use of antibiotics, and ongoing research into alternative treatments and preventative measures [24]. The rise in MDR not only impacts poultry health and productivity but also poses a risk of spreading resistance genes to other bacterial populations, with potential implications for both animal and human health [25]. Abd Elatiff et al [26] found that probiotics was of great value in protection against the *E. coli* infection and improve the performance parameters of chicks, including feed consumption, weekly body weight gain and FCR. Also, prebiotics (Lysozyme and Betaine) which could improve antibody titers of inactivated ND and AI vaccine [27]. An increase in the humoral immunity against ND was noticed after ND vaccination. The HI geometric mean was 5.9 and 4.2 for probiotic and prebiotic, respectively [5]. Therefore, this study was carried out to compare the usage of probiotic and/or Fosfomycin in control of *E. coli* O78 drug sensitive and drug resistance strains in broiler chicken and the possible effect of these treatments on birds' immune response to ND vaccines.

Material and Methods

Ethical approval

The institutional animal care and use committee of the Faculty of Veterinary Medicine, University of Cairo, Egypt, ensured that the handling of chickens and all experimental procedures were followed all applicable measures (Vet CU 18042024933).

Chicks:

Two hundred and twenty (220) commercial broiler (Arbor Acres plus) chicks were bought as hatched from Cairo poultry Co hatchery. The chicks were caged hygienically in experimental cages of Department of Poultry Diseases; Faculty of Veterinary Medicine; Cairo university under the requirement of the breed manual, on wood saving deep litter and given feeds and water *ad-libitum* under strict sanitary and biosecurity standards.

Ration:

All chickens were fed on the same commercial broiler pelleted ration kindly supplied by Cairo poultry Co. poultry company based on the NRC [28] *ad-libitum*. The starter ration which holds 23% Crud protein was given to the chickens for the first two weeks, followed by the grower ration contain 21% crud protein for the next two weeks, and finally the finisher (contain 19% Crud protein) ration for the last week.

E. coli strains:

Avian Enteropathogenic *E. coli* O78 (AEPEC O78) isolated from infected chicken flocks [23]. Strain 1 was antibiotic resistant, and strain 2 was sensitive.

ND Vaccines and vaccination:

The birds were vaccinated with Groups 1-6 were vaccinated against ND La Sota virus at 17th day of age via eye drop. The vaccine was produced by Boehrimger Ingelhiem "Volvac" Lot No 2207062C1A.

Antibiotic:

Calcium Fosfomycin (Adwiafos) was obtained from ADWIA company, each 100 gm of Adwiafos contain 25 gm of Ca Fosfomycin. It administrated orally (according to company's recommendation) at the dose rate 40 mg/kg body weight for 5 successive days in drinking water**.**

Supplements:

The following two different commercial products including probiotics bacteria, probiotics yeast, organic acid, one symbiotic were used in drinking water for five days before infection (day 9: day 13). Doses were used in drinking according to the manufacture guide. The products were as follow:

P1: Protexin®: It is Commercial probiotic manufactured by ADM Protoxien LTD, UK (Batch no. 124496) holds per kg: *Enterococcus faecium* (NCIMB 11181) 4b 1708. <1.0% Total Viable Count $2x10^{12}$ CFU/kg. Ingredients: Dextrose up to 1kg. Crude Protein < 1.0%. Crude Fiber < 1.0%. Crude Oil < 1.0%. Crude Ash < 1.0%. Trace. It was used according to a manufacture guide in drinking water. Dose 1 gm/2 Liter water/day.

P4: Amino-Zyme®: It is commercial product manufactured by 2M group, Egypt (Batch no. 2389). It is composed of Beta glucan 48.6 gm, Fructo oligosaccharide 8.3 gm, DL-methionine 0.5 gm, L-carnitine 15.3 gm, L-lycine HCL 4.47 gm, Mono propylene glycol 45.25 gm, Purified water up to 1 liter. Also hold: Spirulina, L-valine, Taurine, Thereonine, L- arginine, Leucine, Isoleucine, *Lactobacillus acidophilus, Lactobacillus subtillis, Bifidobacterium*, Phytase, Protease, Amylase, Xylase. It was used in drinking water according to the manufacturing manual. Dose 1ml / Liter water/day.

Experimental infection:

The *AEPEC* O78 sensitive (strain 1 and resistant strain 2) were used for experimentation. At 6 and 7th day of life, each chicken in the infected groups was orally inoculated with 1 ml of saline containing 10^8 colony forming unit (CFU) *E. coli*/ ml [29].

Experimental design:

Two hundred and twenty (220) commercial broiler chicks were divided into 11 groups with 20 birds each with duplicate including 10 birds with replicate. The used chicks were randomly divided into 11 groups (1-11); 20 chicks each. Each group was reared in separate disinfected room on deep liter. At the 6th and 7th day of life chicks of group 1 was kept a non-infected non-treated group, while chicken of groups 2- 6 and 7-11 were orally infected each chick with 1 ml broth culture containing 10^8 CFU /ml of *E. coli* O78 full drug sensitive (strain 1) and extreme drug resistance (strain 2) [23]. The infected groups were daily observed till the appearance of first clinical signs including diarrhea, decrease feed intake at the 5th dpi. Birds of groups 2 and 7 were kept as strain1 and strain 2 infected non treated control. Birds of groups 3 and 8, 4 and 9, 5 and 10 as well as 6 and 11 were given Fosfomycin, Fosfomycin & Probiotic, Prebiotic as well as Probiotic, respectively. The treatment was done in drinking water at the recommended dose for 5 days (Table 1). All groups were subjected to daily observation for signs of mortality.

Clinicopathological Examination

Chickens in all groups were checked daily for clinical signs and mortality. Clinical signs observed, mortality and the pathological postmortem (PM) findings in dead birds were recorded. The cumulative mortality rate was calculated as the total number of deaths in chickens per group divided by the total population in the same group [30,31].

Organ body weight ratio and bursal index

Organ body weight ratio (OBW ratio) = organ weight/ Body weight× 100 [32]. Bursal weight index (BW index) = BW ratio of infected group/ BW ratio

of control group [32]. The bursa considered atrophied when BW index less than 0.7 [34,35].

Re-isolation of E. coli:

Samples from dead infected chickens including heart, liver, lungs, intestine and spleen) were collected after postmortem examination for *E. coli* re-isolation by bacteriological examination.

Detection of NDVHI Antibody:

a. Blood samples for serum:

Blood was collected from the jugular vein at 1st day and day 14th to detect MDABs while from wing vein 21 days post vaccination to HI determine antibody titer, and serum was obtained after centrifuging at 3000 rpm for 10 min and stored at −20 $^{\circ C}$ for further analysis.

b. Haemagglutination inhibition (HI) assay:

Sera were obtained from all groups at 35 days of age (21 days post vaccination) were tested by HI assay. The HI assay was carried out using (La Sota strain) according to standard procedures with 4 Hemagglutinating units' virus/ antigen in 0.050 ml and HI titer ≤ 2 Log- $_2$ considered negative [36].

Broiler growth Performance Parameters

At the end of the experiment (35 d), Chicks were individually weighed. The live body weight gain (BWG) was calculated by subtracting the initial weight (1 day weight) from the current weight and expressed as (g/bird). The total consumed feed was divided by the number of birds in each group to get the average feed consumption, so it was expressed as (g/bird). To determine the feed conversion ratio (FCR), It has been determined on a weekly basis by dividing the average amount of feed consumed by each bird by the average amount of gain in weight.

Histological investigations

For histopathological evaluation: bursae, thymus, spleen, cecal tonsils and the middle region of the cecum were fixed in 10% formalin, embedded in paraffin blocks that sectioned using a microtome into slices of 4–6 µm thickness then stained with Hematoxylin and Eosin (H&E) stains [37]. The percentage of scoring system for estimated tissues was determined and compared between the experimental groups across a range of 0 to 4 according to the severity as the following 0 means (normal), 1 means (1–25%), 2 means (26–50%), 3 means (51–75 %), 4 means (76–100%) of estimated lesions included lymphoid necrosis and/or lymphocytic depletion, edema and infiltration of plasma cells as well as heterophils. Total mucosal thickness, including the mucosal epithelium and lamina propria of the cecum was determined by morphometric analysis. The caecum mucosa was measured at 5 representative points in each cecum using ImageJ software. The mean of mucosal thickness was calculated for three birds per group.

Statistical analysis:

One-way analysis of variance (ANOVA) -Duncan test was used to compare the mean values of the various groups at a significance level of $P \leq 0.05$. Statistical analysis was performed using the method cited in Petrie and Watson [38].

Identification of Flagellar (H) antigen "Tube agglutination test":

Determination of Flagellar (H) antigens was carried out by using Polyvalent H antiserum for both phase 1 and phase 2 to determine the complete antigenic formula of the isolates. A loopful of H antiserum was added to one drop of the bacterial suspension in the small agglutinating tube and mixed gently by a sterile loop. The agglutination tube was gently agitated for one minute and observed for agglutination under normal lighting conditions.

Statistical analysis

The results of bacterial counts were expressed as mean ± SD (log_{10} CFU/g). The significance difference ($P<0.05$) between the means is calculated using a student t-test according to [25].

Results and Discussion

E. coli is a common bacterial pathogen causing economic problems in poultry production and the disease manifests in various forms, including septicemia, air sacculitis, and peritonitis, disrupting normal production processes and increasing mortality rates [39]. The impact on avian production is multifaceted, infected flocks often experience reduced growth rates, poor feed conversion, and increased mortality, all of which contribute to economic losses additionally, the presence of colibacillosis can compromise overall flock health and welfare, further exacerbating production challenges [39].

E. coli O78, which is known to be a primary cause of colibacillosis in broiler chickens [2]. Colibacillosis is a bacterial infection that can lead to high mortality and reduced growth performance in affected chicken flocks [4,40].

Infected chicks with *E. coli* O78 showed loose dropping and low feed intake at the 2nd day post 2nd dose [41, 42].

By the 3rd day chicks from group 2 infected with strain 1 (sensitive) and 2 from group 6 infected with strain 2 (MDR resistant) died in rate of 5 and 10 %, respectively. The higher mortality rate in the group infected with the MDR strain is likely due to the increased virulence and reduced susceptibility to antimicrobial treatment [42,43]. On the 2nd day from administration of Fosfomycin, probiotic and

prebiotic treated birds started to be active with improvement in feed intake and drooping [19,42]. This suggests that the antimicrobial and gut-health-supporting interventions were effective in mitigating the negative impacts of the *E. coli* infection [12, 44]. Non treated groups 2 and 7 showed more 2 and 3 dead positive chicks with total mortality 3 (15%) and 5 (25%).

E. coli was re-isolated from liver, heart blood and spleen of dead infected chicks, *E. coli* was re-isolated from the liver, heart blood, and spleen of the dead infected chicks, confirming the role of the bacteria in the observed mortality [19,42]. Lesions were moderate to severe enteritis especially mid part of intestine, air-sacculitis, pericarditis, enlarged, congested and hemorrhage in liver, spleen enlarged and congestion, kidney congested with accumulation of ureates in ureters with loss of weight. These are all typical pathological findings in E. coli infections in poultry [15,45, 46], while no marked signs were seen in control negative and treated groups [19, 42].

Feed conversion rate (FCR) at 35 days (Table 2) proved that the noninfected nontreated group 1 showed the best (1.42), and these findings in concur with Osman et al. [47]. Fosfomycin treated groups 3 (1.51) and 8 (1. 48) had lower FCR than probiotic and prebiotic treated (1.43-1.46) also, as reported by Kola et al [48], while groups 4 and 9 received Fosfomycin + probiotic showed better FCR than those given Fosfomycin alone and lover than control group 1 and these findings parallel to those reported by Mountzouris et al. [49]. Probiotics can help maintain a balanced gut microbiome, which can improve nutrient absorption and utilization, leading to better FCR [50]. Probiotics can also directly antagonize the *E. coli* O78 strain, reducing its detrimental impact on the host's digestive function and nutrient utilization [51].

FCR is a crucial metric in poultry production [52], as it reflects the efficiency of feed utilization and ultimately impacts the profitability of the operation. Several studies have investigated the effects of various dietary interventions, including Fosfomycin, probiotics, and prebiotics, on the FCR of broiler chickens infected with the pathogenic *E. coli* O78 strain [19,53,54].

At the two check points (6 and 17 days old), the organ body weight ratio revealed no significant differences among the experimental groups but only liver body weight ratio showed at 17 days old the lowest ratio in G2 (2.60), and the best parameter was reported in G5 (6.29) that administered prebiotic. The intestine body weight ratio at 17 days old showed the best ratio at G9 (12.71) and the lowest ratio at G11 (9.53). The proventriculus body weight ratio at 17 days old revealed the lowest parameters in G6 (0.51) and the highest ratio in G5 and G11 (0.75). The gizzard body weight ratio at 17 days old showed the best ratio in G10 (3.21) and the lowest in G4 (2.24). The administration of both probiotic and/ or prebiotic improves the intestinal health that reflects on nutrients absorbability and feed assembly that reflected on organs weight [13].

Regarding the recorded geometric mean of ND HI antibody titers (Table 3). The geometric mean of ND HI antibody titers is a widely used metric to measure the immune response to Newcastle Disease virus in poultry [55]. In the context provided, the control negative group had the highest geometric mean of 9.2, which is expected as this group was not exposed to any infectious agents or treatments.

The *E. coli* O78 infected groups treated with probiotics or prebiotics had geometric mean titers ranging from 8.2 to 9.2. This suggests that the probiotic or prebiotic treatments were effective in enhancing the immune response in the birds challenged with *E. coli* O78 infection, as the titers were comparable to or higher than the control negative group [3,56,57].

The Fosfomycin treated groups (groups 3 and 8) had higher geometric mean titers of 7.9 and 7.7, respectively, compared to the *E. coli* infected non-treated groups (groups 2 and 7) with titers of 7.4 and 7.2. This indicates that the Fosfomycin treatment was able to improve the immune response in the *E. coli* infected birds, although the titers were lower than the probiotic/prebiotic treated groups [58, 59]. The lower titers observed in the *E. coli* infected non-treated groups (groups 2 and 7) suggest that the *E. coli* infection had a negative impact on the immune response, which was mitigated by the probiotic/prebiotic or Fosfomycin treatments [45,60, 61].

Histopathological examined tissue section revealed that *E. coli* O78 Strain 1 infected birds gr 2 intestine showing sloughing of villi tips and moderate leukocytic cells infiltration lamina propria and submucosa (Fig 1A) and the sloughed villi with severe leukocytic cells infiltration submucosa (Fig 1B). Chickens treated with Fosfomycin (G3) showing epithelial hyperplasia with moderate leukocytic cells infiltration lamina propria and submucosa (Fig 1C), while group given Fosfomycin with probiotic (Gr 4) show epithelial hyperplasia with mild leukocytic cells infiltration lamina propria and submucosa. Intestine of chicken given only probiotic (G5) and those given prebiotic (G6) showing mild histopathological alteration to epithelial hyperplasia with moderate leukocytic cells infiltration lamina propria and submucosa (Fig 1A). Chicken infected with strain 2 (G7) epithelial sloughing with moderate leukocytic cells infiltration in lamina propria and submucosa (Fig 1A) to and severe leukocytes infiltration in lamina propria, submucosa and tunica musculosa (Fig 1E), Fosfomycin G8 show epithelial hyperplasia with

mild (Fig 1D) to moderate leukocytic cells infiltration lamina propria and submucosa (Fig 1A). Probiotic group 9 showing severe epithelial hyperplasia with mild leukocytic cells infiltration lamina propria and submucosa (Fig 1B) while those given prebiotic (G 10) epithelial hyperplasia with mild leukocytic cells infiltration lamina propria and submucosa (Fig 1D). These findings are consistent with the pathogenic effects of *E. coli* infection, which can lead to damage and inflammation of the intestinal epithelium, as reported by Beal et al. [64]. The epithelial hyperplasia observed in this group may suggest a regenerative response to the E. coli infection, as the intestinal epithelium attempts to repair the damage caused by the pathogen. The moderate leukocytic cell infiltration indicates the ongoing immune response to the infection [62]. Chickens treated with Fosfomycin, and a probiotic (Group 4) had Mild leukocytic cell infiltration in the lamina propria and submucosa. The combination of Fosfomycin and the probiotic appears to have resulted in a more favorable intestinal histopathological profile, with reduced leukocytic cell infiltration compared to the Fosfomycin-only treatment group (Group 3). This finding suggests that the probiotic may have had a beneficial effect in modulating the inflammatory response and promoting intestinal healing, as reported [49]. These findings indicate that the probiotic and prebiotic treatments may have had a positive impact on the intestinal histopathology, potentially by supporting the restoration of the intestinal epithelium and moderating the inflammatory response [49,50].

Liver of control negative chicken (G1) showing normal histological structure (Fig 2A), while liver of chicken *E. coli* O78 strain 1 (G2) showing moderate periportal leukocytes infiltration (fig 2 B) as well as focus of leukocytes infiltration (Fig 2 C) [65]. liver of chicken infected with drug resistant strain 2 (G7) showing severe periportal leukocytes infiltration (Fig 2 D). liver of chicken infected with either stain and treated with Fosfomycin + Probiotic (G 4 and 9) showing mild histopathological alteration (Fig 2 E). liver of infected chicken with resistant or sensitive *E. coli* O78 and treated with probiotic and prebiotic (G 5, 6, 10, and 11) showing mild periportal leukocytes infiltration (Fig 2 F). The mild histopathological alterations observed in the treated groups suggest that the dietary supplementation of prebiotics and probiotics, in combination with or without antibiotic therapy, was effective in attenuating the liver damage caused by the E. coli O78 infection, irrespective of the antibiotic resistance status of the infecting strain. In studies examining the effects of *E. coli* O78 infection, researchers have observed various histopathological alterations in the liver, including hepatocellular degeneration, necrosis, inflammatory cell infiltration, and vascular congestion [47]. Supplementation with prebiotics and probiotics has been shown to attenuate the severity of these histopathological changes in the liver during E. coli O78 infection, irrespective of the antibiotic resistance status of the infecting strain. Prebiotics can selectively promote the growth of beneficial gut microbiota, which can help to maintain intestinal homeostasis and reduce the colonization and proliferation of pathogenic *E. coli* strains [51]. Probiotics, on the other hand, can directly antagonize *E. coli* strains through the production of antimicrobial compounds, competitive exclusion, and modulation of the immune system [50]. Antibiotics can help to reduce the bacterial load and the associated inflammatory response, which can contribute to the attenuation of liver damage [48].

The different interventions work together to effectively manage the *E. coli* infection and its associated liver damage, regardless of the antibiotic resistance status of the strain [499]. The study by Shinde et al. [65], examined the histological changes in the livers of broiler chickens infected with different strains of *E coli* O78, including a drug-resistant strain. The authors observed that the *E. coli* O78 infection, particularly the drug-resistant strain, led to significant inflammatory changes in the liver, as evidenced by the moderate to severe periportal leukocyte infiltration. However, the treatment with Fosfomycin and probiotic, as well as the combination of prebiotics and probiotics, showed a mitigating effect on the liver histopathology, reducing the severity of the inflammatory changes in the infected chickens [19,54, 66].

Spleen of control negative and all *E. coli* O78 infected treated groups chicken of 3-6 and 8-11 showing well populated periarteriolar lymphoid sheath and follicles (Fig3 A). This indicates that the prebiotic and probiotic treatments, as well as the combination of treatments, were able to maintain the normal histological structure and lymphoid tissue organization in the spleen of the infected birds [65]. Chicken infected with strain 1 (G2) or strain 2 (Gr 7) showing moderate depletion of periarteriolar lymphoid sheath (Fig 3B). The depletion of the periarteriolar lymphoid sheath suggests a compromised immune response in the spleen, likely due to the pathogenic effects of the *E. coli* O78 infection [65]. Spleen of chicken strain 2 treated with Fosfomycin (G9) showing mild depletion of periarteriolar lymphoid sheath and follicles (Fig 3 C) the milder depletion compared to the non-treated, infected groups (2 and 7) suggests that the antibiotic treatment (Fosfomycin) was able to partially alleviate the immunosuppressive effects of the *E. coli* O78 infection on the spleen [65]. These findings suggest that the antibiotic resistance of the *E. coli* O78 strain did not significantly impact the histological changes observed in the spleen of the infected broiler chickens [67-69].

The use of prebiotics, probiotics, and antibiotics like Fosfomycin has been explored in the

management of E. coli infections in poultry, including both sensitive and resistant strains. The available evidence suggests that these interventions can have varying effects on *E. coli* strains with different resistance profiles. Prebiotics, such as oligosaccharides and fructans, have been found to selectively promote the growth of beneficial gut microbiota, which can outcompete and inhibit the colonization and proliferation of *E. coli*, including resistant strains [70]. The prebiotic-induced changes in the gut microbiome can enhance the host's immune response and improve intestinal barrier function, making the gut environment less favorable for *E. coli* establishment [51]. Probiotics, on the other hand, can directly antagonize *E. coli* strains through the production of antimicrobial compounds, competitive exclusion, and modulation of the immune system [50]. Probiotic strains, such as Lactobacillus and Bifidobacterium species, have been shown to be effective against both sensitive and resistant *E. coli* isolates [11].

Importantly, the efficacy of prebiotics and probiotics may be influenced by factors such as the specific strains used, the dose, and the timing of administration. Combining prebiotics and probiotics (known as synbiotics) can sometimes result in additive or synergistic effects, enhancing their impact on *E. coli*, including resistant strains [49]. Fosfomycin can be effective against both sensitive and resistant E. coli strains, including those harboring extended-spectrum beta-lactamase (ESBL) or AmpC-type beta-lactamase genes [48]. The mechanism of action of Fosfomycin, which involves inhibiting an early stage of bacterial cell wall synthesis, makes it less susceptible to common resistance mechanisms [59]. However, the efficacy of Fosfomycin may be influenced by factors such as the specific resistance mechanisms present in the E. coli strain, the pharmacokinetic properties of the drug, and the dosage regimen used [59]. In some cases, resistant *E. coli* strains may develop resistance to Fosfomycin over time, highlighting the importance of appropriate use and the need for ongoing monitoring of resistance patterns.

Conclusion

Controlling *E. coli* O78 infection in broiler chickens is a critical challenge for the poultry industry. Both antibiotic treatment and probiotic supplementation have their strengths and limitations, but a combination of the two strategies may offer the most effective and sustainable solution. By using antibiotics responsibly and complementing them with probiotic-based interventions, producers can work to protect the health and productivity of their broiler flocks while also addressing the broader issue of antibiotic resistance.

The emergence of antibiotic-resistant *E. coli* O78 strains has posed a significant challenge for the broiler industry. However, the use of Fosfomycin, a novel antibiotic with a unique mechanism of action, has shown promise in controlling these problematic infections. The available evidence indicates that Fosfomycin can effectively reduce bacterial loads, improve bird health, and mitigate the impact of resistant *E. coli* O78 outbreaks in broiler flocks. As the poultry industry continues to grapple with the issue of antibiotic resistance, the utilization of Fosfomycin may provide a valuable alternative approach for the management of *E. coli* O78 and other resistant bacterial pathogens in broiler production. This suggests that Fosfomycin may be a valuable tool in the management of antibiotic-resistant *E. coli* infections in broiler production.

Furthermore, the incorporation of probiotic supplementation may enhance the efficacy of Fosfomycin-based treatment by supporting intestinal health and the host's natural defense mechanisms. As the poultry industry continues to search for effective and sustainable solutions to combat antibiotic-resistant pathogens, the synergistic use of Fosfomycin and probiotics may offer a promising approach for managing *E. coli* O78 infections in broiler chickens.

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Authors' contributions

A.A.A and H.M.S. collected samples, experimental and laboratory investigations. M.M.H. and M.M.A. supervised the work. All team members wrote, revised the original draft, and approved the final manuscript.

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Declarations:

All data included in this paper is an original obtained from our work-by-work team.

Competing interests

The authors declare that they have no competing interests.

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TABLE 1. **Feed conversion rate (FCR) at the 35 day of broiler chicken infected with *E. coli* O78 and treated birds.**

Group No.	Infection *E. coli*	Treatment		FCR
		Fosfomycin	Additive	
1		Control negative		1.42
2	Strain 1	-	-	1.49
3		+	-	1.51
4		+	Probiotic	1.43
5		-	prebiotic	1.43
6		+	probiotic	1.46
7	Strain 2	-	-	1.54
8		+		1.48
9		+	Probiotic	1.46
10		-	probiotic	1.51
11		-	prebiotic	1.44

TABLE 2. Organ body weight ratio of broiler chicken infected with *E. coli* O78 and treated birds.

Group no	Treatment			Age/ days	Organ body weight ratio (Mean ± SD)			
					Liver	Intestine	Proventriculus	Gizzard
	E. coli	Fosfomycin	Additives					
1		Control negative		6	3.24	7.55	0.72	2.16
				17	3.34	13.86	0.54	2.74
2	Strain 1	-	-	6	3.33	7.78	0.74	2.22
				17	2.60	11.38	0.54	2.60
3		+	-	6	3.13	7.29	0.69	2.08
				17	2.99	12.96	0.67	2.29
4		+	Probiotic	6	3.36	7.84	0.75	2.24
				17	3.21	9.83	0.52	2.31
5		-	Prebiotic	6	3.38	7.89	0.75	2.26
				17	6.29	8.12	0.53	2.82
6		-	Probiotic	6	3.38	7.89	0.75	2.26
				17	3.12	11.95	0.51	2.61
7	Strain 2	-	-	6	3.41	7.95	0.76	2.27
				17	3.67	10.92	0.71	3.04
8		+	-	6	3.17	7.39	0.70	2.11
				17	3.38	10.26	0.61	2.40
9		+	Probiotic	6	3.36	7.84	0.75	2.24
				17	2.87	12.71	0.62	2.25
10		-	Prebiotic	6	3.36	7.84	0.75	2.24
				17	3.51	10.21	0.68	3.21
11		-	probiotic	6	3.38	7.89	0.75	2.26
				17	3.18	9.53	0.64	2.82

TABLE 3. **Geometric mean of HI antibody against NDV in vaccinated *E. coli* O78 infected and treated broiler chicken groups (n=15/group).**

Group No	Treatment			HI titer distribution								GMT
	E. coli	Fosfomycin	Additives	0-4	5	6	7	8	9	10	11	
1	Control negative					2		1	3	1	3	9.0
2	Strain 1	-	-			1	6	1	2			7.4
3		+				1	4	1	3	1		7.9
4		+	Probiotic				1	4	2	1	2	8.9
5		-	Prebiotic					3	4	1	2	9.2
6			Probiotic				1	3	4	1	1	8.2
7	Strain 2	-	-			3	3	3	1			7.2
8		+				1	4	3	1	1		7.7
9		+	Probiotic		2		1	1	3	3		8.2
10			Prebiotic			2	1	4	1		2	8.2
11			Probiotic			1		5	2	1	1	8.5

TABLE 4. **Illustrates the intestinal villi measurements (Mean ±SD) in different organs among different groups.**

Group no	Treatment			Length mean +-SD	Width mean +-SD	Depth mean +-SD
	E. coli	Fosfomycin	Additives			
1		Control negative		1336.72 ± 92.4^{a,b}	171.24 ± 22.88	476.69 ± 82.92^{a,b}
2	Strain 1	-	-	1603.88 ± 69.57	139.77±14.17	291.44 ±23.17^{a,b}
3		+	-	644.61 ± 81.1^{a,b}	101.41±20.30^{a,b}	331.86 ± 33.7
4		+	Prebiotics	2002.05±43.2^{a,b}	132.90±11.90	219.79±26.70^{a,b}
5		-	Probiotics	1668.07±43.15	112.34 ±19.47	282.19± 41.87^{a}
6		-	Prebiotics	1829.53±166.5	217.69 ±22.80	358.63±62.87
7	Strain 2	-	-	1729.53±168.5	207.69 ±21.80^{a}	338.63±62.87^{a}
8		+	-	1044.21±55.8	149.10±31.17^{a}	342.03 ± 73.87
9		+	Prebiotics	754.18± 90.03^{a,b}	108.39 ±11.63^{a,b}	263.79 ± 56.23^{a,b}
10		-	Probiotics	1207.68±129.8	170.82±15.87	323.31±47.07
11		-	Prebiotics	1194.64± 97.1	175.33± 21.33^{b}	410.69± 70.00^{a}

Different superscripts (a-d) reveal a significant difference between values within the same column. Statistically significant differences were considered when $p \leq 0.05$.

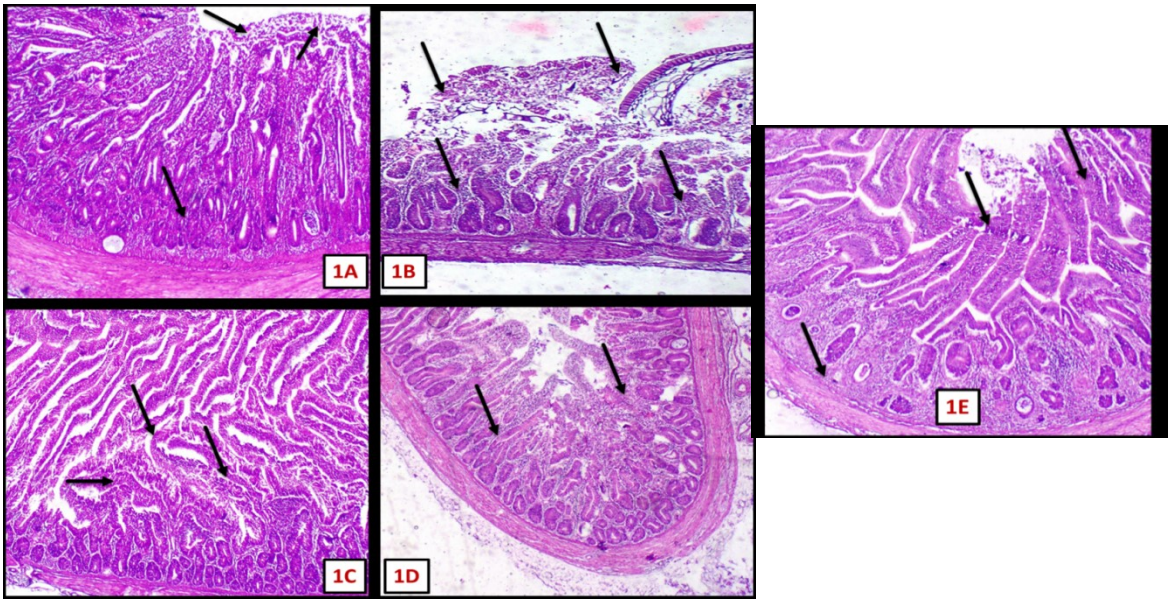

Fig.1. Intestine of chicken infected with *E. coli* strain 1 or 2 followed by treatment with prebiotic, probiotic and/or Fosfomycin (H&E X100) showing: A: Strain 1 (Gr 2): sloughing of villi tips and moderate leukocytic cells infiltration lamina propria and submucosa. B- Strain 1 (G2): sloughing villi and severe leukocytic cells infiltration submucosa. C- Strain 1 Fosfomycin: epithelial hyperplasia with moderate leukocytic cells infiltration lamina propria and submucosa, D- strain 1 Fosfomycin+ probiotic (G4) epithelial hyperplasia with mild leukocytic cells infiltration lamina propria and submucosa. E- Strain 2 (G7): epithelial sloughing with moderate leukocytic cells infiltration in lamina propria and submucosa.

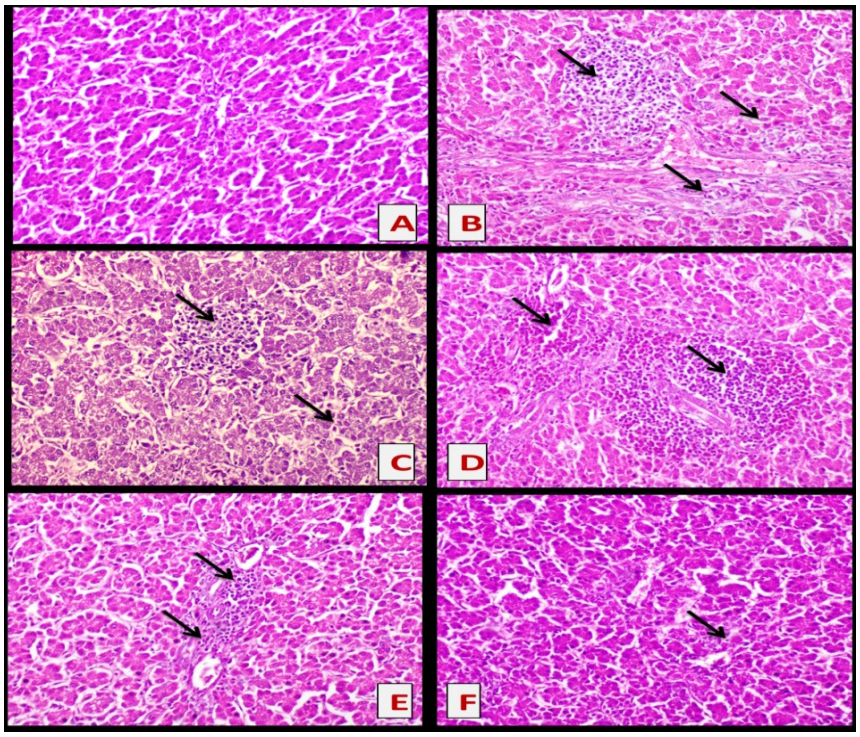

Fig. 2. Liver section of chicken infected with resistant or sensitive *E. coli* O78 followed with treatment with Fosfomycin, Probiotic or prebiotic (H&E X 200) showing A: Control negative chicken (G1) normal histological. B. strain 1 (G2) moderate periportal leukocytes infiltration. C: Strain 1 (G2) focus of leukocytes infiltration D: strain 2 (G7) severe periportal leukocytes infiltration. E: Fosfomycin + Probiotic (G 4 and 9) mild histopathological alteration. F: Probiotic and prebiotic treated (Gs 5, 6, 10,11) mild periportal leukocytes infiltration.

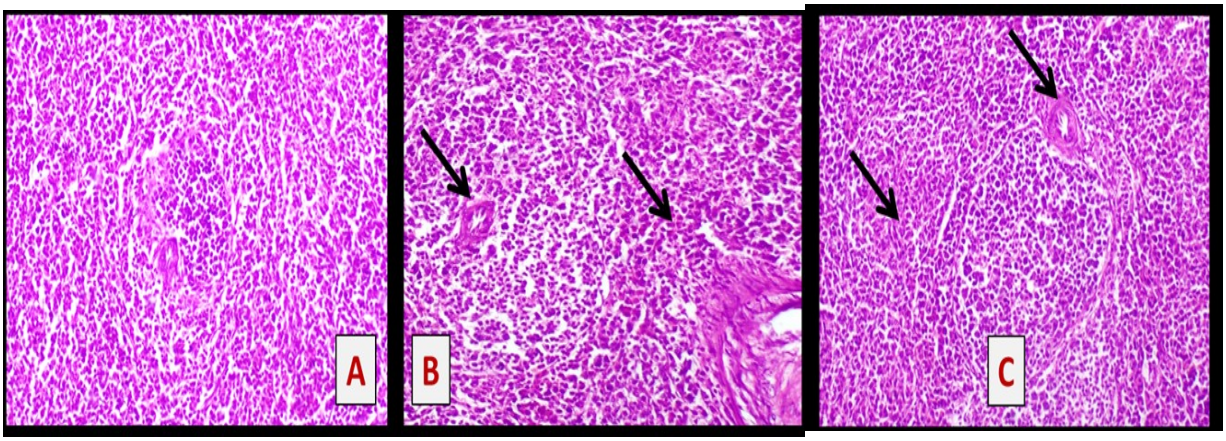

Fig. 3. Spleen sections of chicken infected with E. coli strain 1 or 2 followed by treatment with Fosfomycin, Fosfomycin & Probiotic, prebiotic or probiotic (H&E X200) showing: A: control negative and all infected treated groups 3-6 and 8-11 showing well populated periarteriolar lymphoid sheath and follicles. B. infected with strain 1 (G2) or strain 2 (Gr 7) showing moderate depletion of periarteriolar lymphoid sheath. C. strain 2 treated with Fosfomycin (G9) showing mild depletion of periarteriolar lymphoid sheath and follicles

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مقارنة تأثير البروبيوتيك و البريبايوتك و/أو الفوسفوميسين في السيطرة على الميكروب القولوني عتره O78 ذات الحساسية للأدوية في الدجاج اللاحم و المناعية للقاحات النيوكاسل

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الملخص

داء العصيات القولونية لدى الطيور، والذي يسببه في المقام الأول الإشريكية القولونية (*E. coli*)، هو مرض بكتيري كبير يصيب الدواجن، مما يؤدي إلى خسائر مالية كبيرة في إنتاج الطيور. إحدى القضايا الحاسمة في إدارة داء العصيات القولونية لدى الطيور هي المشكلة المتزايدة المتمثلة في المقاومة للأدوية المتعددة (MDR). لقد أدى الإفراط في استخدام المضادات الحيوية وإساءة استخدامها إلى ظهور سلالات مقاومة من بكتيريا الإشريكية القولونية. وبالتالي، هدفت هذه الدراسة إلى مقارنة استخدام البروبيوتيك و/أو الفوسفوميسين في مكافحة الإشريكية القولونية O78 في الدجاج اللاحم والتأثير المحتمل لهذه المعالجات على رد الفعل المناعي للطيور تجاه لقاحات ND. تم توزيع مائتين وعشرين طيراً على 11 بواقع 20 طيراً لكل مجموعة وكل مجموعة تضم مكررين كل منهما 10 طيور، وكانت المجموعات على النحو التالي: السيطرة على الطيور السلبية G1؛ تم تحدي الطيور من G3 إلى G6 بالسلالة 1 (المقاومة للمضادات الحيوية) *E. coli* O78 وG3 وG4 وG5 وG6 المعالجة بالفوسفوميسين والفوسفوميسين + البروبيوتيك والبريبايوتيك والبروبيوتيك، على التوالي، بينما احتفظت G2 كمجموعة مراقبة إيجابية للطيور غير المعالجة. أصيبت الطيور من المجموعة 7 إلى المجموعة 11 بالسلالة 2 (الحساسة للمضادات الحيوية) الإشريكية القولونية O78 وG8 وG9 وG10 وG11 المعالجة بالفوسفوميسين والفوسفوميسين + البروبيوتيك والبريبايوتيك والبروبيوتيك على التوالي، في حين حافظت الطيور غير المعالجة على المجموعة G7 كمجموعة مراقبة إيجابية. خلصت النتائج إلى أن الفوسفوميسين قد يكون أداة قيمة في إدارة عدوى الإشريكية القولونية المقاومة للمضادات الحيوية في إنتاج دجاج التسمين. بالإضافة إلى ذلك، فإن دمج مكملات البروبيوتيك قد يعزز فعالية العلاج المعتمد على الفوسفوميسين من خلال دعم صحة الأمعاء والدفاع الطبيعي للمضيف.

الكلمات المفتاحية: دجاج تسمين - المضادات الحيوية - مقاومة الأدوية – البريبايوتكس - البروبيوتيك - مناعه النيوكاسل.