Florfenicol 40% efficacy in piglets with respiratory pathologies

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Abstract. Piglets with large-scale respiratory pathologies caused by a bacterial flora susceptible to phenicol antibacterial drugs received two doses of Florfenicol 40%. On day 4 after administration, this resulted in the complete reversal of clinical pattern, in the recovery of morphofunctional blood parameters, and in the reduction in prevalence of *Klebsiella pneumoniae* by 25%, *Streptococcus suis* by 50%, and *Staphylococcus haemolyticus* by 41.7%. The drug is tolerated with no adverse events. The results of this study allow recommending Florfenicol 40% as an antibacterial therapy in the acute infectious inflammatory respiratory pathologies in the store pigs.

1 Introduction

Respiratory diseases are the main causes of production losses in global pig breeding [6, 7, 9]. This study provides the therapeutic efficacy of a novel amphenicol chemotherapy, a florfenicol-based antibacterial veterinary drug for respiratory infectious pathologies in pigs. This drug is active against the chloramphenicol-resistant bacteria producing acetyl transferase. It inhibits the protein production in a bacterial cell at the ribosome level and has a bacteriostatic effect on the susceptible microorganisms [2, 10].

Florfenicol molecule contains a fluorine and a sulphonyl moiety, in contrast to chloramphenicol which is widely used in veterinary and has a similar structure with hydroxyl and nitro group, respectively. The advantages of this novel drug include: low toxicity for hematopoietic system (does not cause aplastic anemia), bacterial cell death due to peptidyl transferase inhibition and ribosomal protein production arrest, and also lack of resistant bacterial strains to date [5]. Florfenicol lacks a nitro group, an important molecular feature causing aplastic anemia. Therefore, it poses no risk for humans when consuming the animal products [1].

Florfenicol is more active than chloramphenicol or thiamphenicol, and may be more bactericidal towards some pathogens than previously thought. Florfenicol has a wide spectrum of antibacterial activity encompassing all chloramphenicol-susceptible organisms, e.g., Gram negative rods, Gram positive cocci and other atypical bacteria, such as micoplasma. Florfenicol is highly lactophylic which ensures sufficiently high concentrations to treat intracellular pathogens and cross some anatomical barriers [4, 5].

A high value of biological media is noted when defining microbiological activity and predicting the dosages for clinical use. Florfenicol has a concentration-dependent antimicrobial effect [1, 3, 8]. It maintains the therapeutically efficient concentrations, particularly in airway tissues which are the target organs [10]. When administered intramuscularly, florfenicol is rapidly and effectively absorbed (94.1%) and enters all organs and tissues. Its peak concentration persists for 48 h. Florfenicol and its metabolites are primarily eliminated with urine and, to a lesser extent, with feces [11].

Considering the above, Agrovetzashchita NVC LLC suggests using Florfenicol 40% drug in bacterial respiratory diseases in pigs.

Aims of the study:

- to investigate therapeutical efficacy of the Florfenicol 40% veterinary drug in bacterial respiratory diseases in pigs based on clinical, hematological and bacteriological findings;
- to investigate the safety and identify possible adverse effects of Florfenicol 40% when administered to pigs.

2 Materials and methods

The clinical study was conducted in post-weaning piglets aged 40-45 days, with the total of 24 animals owned by Myasoagroprom LLC, Krasnoyarsky district, Samara region. At the beginning of the study, test and control groups were formed, each containing 12 matched animals with clinical signs of respiratory pathology. All animals during the experiment were in the same housing and feeding conditions.

1 mL of the test drug, Florfenicol 40%, contains 400 mg of active substance florfenicol, and excipients. The

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drug is a clear colorless or pale-yellow liquid. Florfenicol contained in the drug is a thiamphenicol derivative whose molecule has a fluorine atom substituted for hydroxy moiety. It has a bacteriostatic effect against *Actinobacillus pleuropneumoniae*, *Pasteurella* spp., *Salmonella choleraesuis*, *Bordetella bronchiseptica*, *Haemophilus* spp., *Staphylococcus* spp., *Streptococcus* spp., *Klebciella pneumonia*, *Moraxella bovis*, and mycoplasma species, *M. hyopneumoniae* and *M. hyorhinis*, including chloramphenicol-resistant bacteria producing acetyl transferase. By binding 50S ribosome subunit in bacterial protoplasm, it inhibits peptidyl transferase slowing protein production at the ribosome level in the susceptible microorganisms. Florfenicol 40% is a moderately hazardous substance (GOST 12.2.007 category 3).

Florfenicol 40% was intramuscularly administered in test group animals in the dose of 1 mL per 30 kg of body weight, two times 48 hours apart. In the control group, we used Florox, a similar drug manufactured by NITA-FARM LLC, Russia. This drug was used according to the same regimen, intramuscularly in the dose of 1 mL per 25 kg of body weight, two times 48 hours apart.

The clinical study was conducted using conventional methods and design. The pathology status and Florfenicol 40% efficacy were assessed by the presence or absence of characteristic clinical signs and common ailment. The animal status was controlled throughout the study.

Blood counts considered RBC, WBC, eosinophils, basophils, lymphocytes, monocytes, platelets, hemoglobin level, and the erythrocyte sedimentation rate (ESR). Blood counts were performed on the Mindray BC-5300 hematological analyzer.

Florfenicol 40% antimicrobial activity was determined using the nasal washings from piglets having signs of bronchial pneumonia, by primary plating on 5% blood agar and universal chromogenic media (BioRad). Cultures were incubated for 2 days at 37 °C. All grown microorganisms were identified using MALDI-ToF mass spectrometry on Microflex LT (Bruker®) by direct application.

Efficacy was calculated using variation statistics and Student's t-test.

3 Results and discussion

At baseline, animals with respiratory pathologies had unsatisfactory appearance reflected by the following criteria: constrained posture (static forelimb astride posture or sitting dog posture, with craned neck), periodical cough, weak body type, poor nutritional status, sallow skin, pale dry conjunctiva and mouth mucosa, hollow eyes, catarrhal purulent nasal discharge. Feed refusal rate was 83.3% in Group 1 and 75% in Group 2. The other animal had poor appetite. All animals in both groups had slightly suprathreshold (above 40 °C) body temperature.

Next day after drug administration, both groups had a slight increase in temperature by 0.3-0.5 °C, however, as early as on day 2 mean body temperature was within the physiological range 39.7-39.9 °C. Hereafter, it dynamically decreased until the end of the experiment, and on day 5 after the end of the treatment, both groups had mean body temperature in the range of 39.2-39.4 °C. The body temperature decreased with the improvement of the overall health status. No differences in temperature by groups were noted.

On day 1 after start of treatment, there were no changes in appearance and activity of animals with respiratory pathologies. Cough was registered in 100% of cases. Imminent changes in appetites were noted. Complete feed refusal rate reduced to 50% in Group 1 and 58.3% in Group 2. On day 1 after start of treatment, all animals with the respiratory pathology had cough, however, changes in general health status were noted. The appearance and physical activity improved by 50% in Group 1 and by 58.3% in Group 2. No one piglet had complete feed refusal. 41.7% and 58.3% of animals in Group 1 and Group 2, respectively, had satisfactory appetites. All the animals still had cough next day after final administration. At the same time, there were no cases of poor appetites, 83.3% of piglets in Group 1 and Group 2 were characterized by satisfactory feed and water intake. The other animal had good appetites. Physical activity and general health improved in 75% and 83.3% animals in Group 1 and Group 2, respectively. Specific clinical parameters have changed on day 2 after final administration. There was no cough in 50% of animals in Group 1 and in 41.7% of animals in Group 2. The appetites significantly improved. 91.7% of animals in Group 1 and 83.3% of animals in Group 2 had good appetites, physical activity and appearance. On day 3 after the final administration, 100% of animals in both groups had good appetites, physical activity and appearance. There was no cough in 66.7% of animals in Group 1 and in 75% of animals in Group 2. 100% recovery of all estimated parameters and, therefore, complete recovery of piglets could be noted on day 4 after the final administration. No physiological abnormalities or clinical signs recurrence were revealed in piglets on day 5 after the final administration and hereafter.

Blood counts at the baseline have shown increase in WBC up to 23.30±1.270 $*10^9$/L in Group 1 and to 27.03±1.86$*10^9$/L in Group 2. By the end of study, WBC was normalized in these groups due to decrease by 24.1% ($P \leq 0.05$) and 27.3% ($P \leq 0.05$), respectively. Between-group differences in this parameter were insignificant.

WBC differential assessment allowed one to reveal the following trends. Band neutrophils level was insignificant at the baseline in all groups and slightly increased by the end of the experiment which had no effect on the general trend. Segmented neutrophils levels were slightly above upper limit of normal in both groups (56.75±1.403% in Group 1 and 53.91±0.958% in Group 2) which is a common phenomenon in the acute phase of inflammatory process. By the end of experiment, this parameter was normalized due to reduction by 16.5% ($P \leq 0.001$) and 13.6% ($P \leq 0.001$) in Group 1 and Group 2, respectively. No between-group differences were noted. On the other hand, lymphocytes showed an increase by 2.57% and 5.24%, respectively, which is indicative of positive dynamics towards the recovery. No differences between these parameters were noted within groups. At

the baseline, eosinophil level was decreased being as small as 1% in both groups which was consistent with the general picture of the acute disease. By the end of experiment, this parameter was increased by 2.42% (P≤0.001) and 2.58% (P≤0.001) in Group 1 and Group 2, respectively. These parameters were comparable between groups. Note that the increase in eosinophils is a beneficial sign of recovery. Similar changes were noted for monocytes. Their level was low in all groups at the baseline, but by the end of experiment, this parameter was increased by 2.33% (P≤0.05) and 1.52% (P≤0.001) in Group 1 and Group 2, respectively. Between-group differences were insignificant.

Red blood parameters showed low hemoglobin level at the baseline in the both groups, however, it was above the lower limit of norm (87.45±0.908 g/L in Group 1 and 92.80±1.662 g/L in Group 2). Hemoglobin level increased at the end of the experiment by 20.2% (P≤0.001) in Group 1 and by 9.1% (P≤0.05) in the Group 2, respectively. There was insignificant difference in this parameter between groups, 3.9% in favor of Group 1. Hematocrit dynamics showed the same trends as the hemoglobin. At the end of the experiment, hematocrit increased by 19.5% (P≤0.001) in Group 1 and by 10.5% (P≤0.05) in the Group 2, respectively. These parameters were comparable in Group 1 and Group 2 (Table 1).

Table 1. CBC dynamics

Parameter	Treatment groups	
	1	2
ESR, mm/h		
Day 0	1.67±0.148	1.64±0.203
Day 9	1.42±0.202	1.83±0.216
WBC, 10^9/L		
Day 0	23.30±1.270	27.03±1.86*
Day 9	17.69±2.399*	19.66±1.350
RBC, 10^{12}/L		
Day 0	6.19±0.151	6.57±0.100
Day 9	6.53±0.095	6.38±0.114
Hemoglobin, g/L		
Day 0	87.45±0.908	92.80±1.662
Day 9	105.15±1.136***	101.22±2.572*
Hematocrit, %		
Day 0	33.69±0.450	36.73±0.625
Day 9	40.26±0.711***	40.58±1.18*
Platelets, 10^9/L		
Day 0	438.42±37.622	411.45±27.508
Day 9	338.50±20.939*	356.50±35.302
Bands, %		
Day 0	0.83±0.117	1.00±0.000
Day 9	2.92±0.505	3.25±0.605
Segmented, %		
Day 0	56.75±1.403	53.91±0.958
Day 9	40.26±0.711***	40.36±1.875***
Lymphocytes, %		
Day 0	39.25±1.343	42.09±1.004
Day 9	41.83±2.603	47.33±2.457
Eosinophils, %		
Day 0	1.00±0.000	1.00±0.000
Day 9	3.42±0.857*	3.58±0.395***
Monocytes, %		
Day 0	1.67±0.235	1.73±0.237
Day 9	4.00±0.704*	3.25±0.532*
Basophils, %		
Day 0	0.50±0.157	0.27±0.195
Day 9	0.42±0.202	1.50±0.375

*Note: *: P≤0.05; **: P≤0.01; ***: P≤0.001 vs. control; ˇ: P≤0.05; ˇˇ: P≤0.01; ˇˇˇ: P≤0.001 vs. baseline*

Platelet counts were significantly increased at the baseline (438.42±37.622 *10^9/L in Group 1 and 411.45±27.508 *10^9/L in Group 2, with the normal range of 180-300 *10^9/L). By the end of the experiment, this parameter reduced by 22.7% (P≤0.05) in Group 1 and by 13.4% in Group 2, respectively. These parameters were comparable to each other.

Analysis of susceptibility of bacteria isolated from nasal washings to Florfenicol 40% has shown that *Streptococcus suis, Moraxella canis, Moraxella pluranimalium, Enterococcus hirae* had the highest susceptibility of 0.5 μg/mL. The susceptibility of *Staphylococcus aureus* was of 1 μg/mL. The susceptibility of *E. coli* and *Staphylococcus haemolyticus* to the test drug was borderline and varied between 1-2 μg/mL. The susceptibility of *Klebsiella pneumoniae* varied within 2-8 μg/mL. *Pseudomonas fulva* had the lowest susceptibility among the cultures tested, which was 8 μg/mL.

The bacterial composition of nasal washings from sick piglets suggested that the disease was caused by a complex of opportunistic microorganisms due to decline in animal immune resistance. At the baseline, the most common microorganisms found in piglets in both groups include *E. coli, Klebsiella pneumonia, Rothia nasimurium, Streptococcus suis, Staphylococcus haemolyticus.* Thus, all bacteria commonly isolated from nasal washings were opportunistic microorganisms able to cause infectious inflammatory disorders with adverse outcomes, with *Rothia nasimurium* having the least rate of displaying its pathological properties.

The picture observed on day 1 of the study was as follows. In test Group 1, 41.7% of animals had *E. coli*, 91.7% had *Rothia nasimurium*; 58.3% had *Streptococcus suis*; and 50% had *Klebsiella pneumoniae and Staphylococcus haemolyticus. Staphylococcus chromogenes, Staphylococcus hominis* were identified in 16.7% of cases; and each of *Acinetobacter gerneri, Lactobacillus johnsonii, Staphylococcus warneri, Staphylococcus cohni, Moraxella canis, Bacillus megaterium* was only identified in 1 animal per group (8.3%).

In control Group 2, *Rothia nasimurium* was found in 66.7% of animals; *Klebsiella pneumoniae* in 58.3% of cases; *Staphylococcus haemolyticus and Klebsiella pneumoniae* in 33.3% of cases; *E. coli, Staphylococcus cohni* and *Streptococcus hyovaginalis* in 25% of cases; and *Enterococcus casseliflavus, Staphylococcus aureus* were found in 16.7% of cases. The following bacteria were found in one case per group (8.3%): *Aeromonas caviae, Stapylococcus saprophyticus, Acinetobacter lwoffii, Enterococcus hirae, Moraxella canis, Acinetobacter baumanii, Pseudomonas fulva, Aerococcus viridians, Staphylococcus muscae, Staphylococcus chromogenes, Staphylococcus xylosus, Staphylococcus hominis, Moraxella pluranimalium, Pasteurella aerogenes.*

After treatment by Florfenicol 40% antibacterial drug, prevalence of *E. coli* increased up to 91.7% in Group 1 and to 83.3% in Group 2. *Rothia nasimurium* also remained active. By the end of the experiment, its prevalence reduced to 50% in Group 1 and remained unchanged in Group 2. *Klebsiella pneumoniae*

prevalence reduced by 25% in Group 1 and remained unchanged in control Group 2. In other cases, the antibacterial drug caused a frank depression of microbial flora. Thus, Group 1 had 50% reduction in prevalence of *Streptococcus suis* 50% (down to 8.3%; and 41.7% reduction in *Staphylococcus haemolyticus* (also down to 8.3%).

Other bacteria, namely *Staphylococcus chromogenes, Staphylococcus hominis*; *Acinetobacter gerneri, Lactobacillus johnsonii, Staphylococcus warneri, Staphylococcus cohni, Moraxella canis, Bacillus megaterium,* were not found at the end of the experiment.

By the end of the study, new microbial species were grown: *Enterococcus faecalis (50%); Acinetobacter lwoffii (41.7%); Acinetobacter johnsonii (33.3%); Enterococcus gallinarum, Arthrobacter histidinolovorans, Aerococcus viridians (16.7%); Bacillus mycoides, Citrobacter freundii, Staphylococcus sciuri, Acinetobacter calcoaceticus, Aeromonas caviae, Enterobacter cloacae, Lysinibacillus fusiformis, Acinetobacter towneri, Staphylococcus saprophyticus, Providencia alcalifaciens (8.3%).* These new microorganisms belong to obligate flora present in the process area of the facility.

In Group 2, the proportion of *Streptococcus suis* also reduced by 50%, down to 8.3%, and *Staphylococcus haemolyticus* was not found at the end of the experiment. By the end of the study, prevalence of *Enterococcus casseliflavus* reduced from 2 cases (16.7%) to 1 case (8.3%); *Aeromonas caviae* and *Stapylococcus saprophyticus* were also found in one case per group (8.3%) each; *Acinetobacter lwoffii* representation increased by 16.7%. *Staphylococcus haemolyticus, Staphylococcus cohni, Staphylococcus aureus, Streptococcus hyovaginalis* were not found at the end of the experiment.

At this time point, we registered new microbial species which were not found previously: *Enterococcus faecalis (41.7%); Acinetobacter gerneri (25%); Acinetobacter johnsonii, Acinetobacter radioresistens, Enterococcus gallinarum, Myroides odoratimimus (16.7%); Wautersiella falsenii, Proteus hauseri, Bacillus mycoides, Citrobacter koseri, Morganella morganii, Acinetobacter baumanii, Acinetobacter guillouiae, Enterobacter asburiae, Acinetobacter baylyi, Vagococcus fluvialis (8.3%),* see Table 2.

Table 2. Dynamics of bacterial flora in nasal washings

	Day 0		Day 9	
	n	%	n	%
Total number of animals	12	100	12	100.0
Treatment group 1				
E. coli	5	41.7	11	91.7
Rothia nasimurium	11	91.7	6	50.0
Streptococcus suis	7	58.3	1	8.3
Klebsiella pneumoniae	6	50.0	3	25.0
Staphylococcus haemolyticus	6	50.0	1	8.3
Streptococcus hyovaginalis	2	16.7	0	0.0
Acinetobacter gerneri	1	8.3	0	0.0
Staphylococcus chromogenes	2	16.7	0	0.0
Lactobacillus johnsonii	1	8.3	0	0.0
Staphylococcus warneri	1	8.3	0	0.0
Staphylococcus hominis	2	16.7	0	0.0
Staphylococcus cohni	1	8.3	0	0.0
Moraxella canis	1	8.3	0	0.0
Bacillus megaterium	1	8.3	0	0.0
Acinetobacter lwoffii	0	0.0	5	41.7
Bacillus mycoides	0	0.0	1	8.3
Citrobacter freundii	0	0.0	1	8.3
Staphylococcus sciuri	0	0.0	1	8.3
Enterococcus faecalis	0	0.0	6	50.0
Aerococcus viridans	0	0.0	2	16.7
Acinetobacter calcoaceticus	0	0.0	1	8.3
Aeromonas caviae	0	0.0	1	8.3
Acinetobacter johnsonii	0	0.0	4	33.3
Enterobacter cloacae	0	0.0	1	8.3
Arthrobacter histidinolovorans	0	0.0	2	16.7
Lysinibacillus fusiformis	0	0.0	1	8.3
Acinetobacter towneri	0	0.0	1	8.3
Staphylococcus saprophyticus	0	0.0	1	8.3
Providencia alcalifaciens	0	0.0	1	8.3
Enterococcus gallinarum	0	0.0	2	16.7
Treatment group 2				
E. coli	3	25.0	10	83.3
Rothia nasimurium	8	66.7	8	66.7
Klebsiella pneumoniae	4	33.3	4	33.3
Streptococcus suis	7	58.3	1	8.3
Enterococcus casseliflavus	2	16.7	1	8.3
Aeromonas caviae	1	8.3	1	8.3
Stapylococcus saprophyticus	1	8.3	1	8.3
Acinetobacter lwoffii	1	8.3	2	16.7
Staphylococcus haemolyticus	4	33.3	0	0.0
Staphylococcus cohni	3	25.0	0	0.0
Staphylococcus aureus	2	16.7	0	0.0
Streptococcus hyovaginalis	3	25.0	0	0.0
Enterococcus hirae	1	8.3	0	0.0
Moraxella canis	1	8.3	0	0.0
Acinetobacter baumanii	1	8.3	0	0.0
Pseudomonas fulva	1	8.3	0	0.0
Aerococcus viridans	1	8.3	0	0.0
Staphylococcus muscae	1	8.3	0	0.0
Staphylococcus chromogenes	1	8.3	0	0.0
Staphylococcus xylosus	1	8.3	0	0.0
Staphylococcus hominis	1	8.3	0	0.0
Moraxella pluranimalium	1	8.3	0	0.0
Pasteurella aerogenes	1	8.3	0	0.0
Acinetobacter johnsonii	0	0.0	2	16.7
Enterococcus faecalis	0	0.0	5	41.7
Acinetobacter radioresistens	0	0.0	2	16.7
Wautersiella falsenii	0	0.0	1	8.3
Proteus hauseri	0	0.0	1	8.3
Bacillus mycoides	0	0.0	1	8.3
Enterococcus gallinarum	0	0.0	2	16.7
Citrobacter koseri	0	0.0	1	8.3
Acinetobacter gerneri	0	0.0	3	25.0
Morganella morganii	0	0.0	1	8.3
Acinetobacter baumanii	0	0.0	1	8.3
Myroides odoratimimus	0	0.0	2	16.7
Acinetobacter guillouiae	0	0.0	1	8.3
Enterobacter asburiae	0	0.0	1	8.3
Acinetobacter baylyi	0	0.0	1	8.3
Vagococcus fluvialis	0	0.0	1	8.3

4 Conclusion

The objective findings obtained indicate that Florfenicol 40% has demonstrated high therapeutic efficacy in

infectious inflammatory respiratory pathologies in post-weaned piglets caused by resident opportunistic flora which was supported by the animal clinical status, assessment of nasal washings, and blood counts.

When treated by the drug, complete recovery was achieved on day 4 after the final administration of the test and reference drugs. As a result, prevalence of *Klebsiella pneumoniae* reduced by 25%, *Streptococcus suis* by 50%, and *Staphylococcus haemolyticus* by 41.7%. The findings obtained in the reference group were comparable to the test group.

Therapy with Florfenicol 40% and reference antibacterial drug aids in normalization of WBC differential increase hemoglobin and hematocrit levels, reduce platelet count in animals with respiratory pathologies. WBC differential parameters in both groups suggest the trend to reduction in neutrophils, normalization of lymphocyte levels, increase in eosinophils and monocytes which is indicative of decline in acute phase processes mediated by while blood cells. Rapid significant increase in hemoglobin and hematocrit in the test group may indicate an activation of hemopoiesis due to elimination of infection. Animals with respiratory pathologies showed leukocytosis reversal. In general, this picture suggests recovery of piglets from the respiratory pathology.

Considering the above, we recommend using Florfenicol 40% in acute infectious inflammatory respiratory pathologies in store pigs caused by opportunistic resident flora, in two doses of 1 mL per 30 kg of the body weight 48 hours apart. The drug well tolerated with no adverse events.

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