Effect of colistin sulfate in the health status and bacteriological gut flora in store pigs with nonspecific gastroenteritis

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Abstract. Piglets with the nonspecific gastroenteritis received Colistin 12000000 with drinking water for 5 days is the doses 0.6 g/100 kg in Group 1 and 0.8 g/100 kg in Group 2. This provided the complete therapeutic efficacy (the clinical recovery) on Day 4 in both treatment groups. The dynamics of the erythrocytes and leukocytes suggested the recovery of the morphofunctional homeostasis after the disease in both treatment groups. After the treatment with the Colistin 12000000, the prevalence of the *Streptococcus gallolyticus* reduced by 8.3% in the Group 1. *Klebsiella pneumoniae* prevalence reduced by 33.3%. In Group 2, the prevalence of *Streptococcus gallolyticus* reduced by 41.7% after the 5-day therapy and was 25.0% at the end of the study. *Klebsiella pneumoniae* prevalence reduced twice, and it was ultimately observed in 16.7% of cases. Other bacteria presented in the washings at the baseline in both groups were no more observed at the end of the treatment. The symbiotic flora was found in both groups at the end of the experiment in a sufficient number. Thus, the efficient dose of the test drug was 0.6 g/100 kg provided the desirable clinical effect comparable to the higher dosing regimen. The drug well tolerated, with no side effects or adverse events reported throughout the study.

1 Introduction

Non-infectious diseases related to the decrease in natural resistance of animals result in significant economic losses in animal production. Such diseases can be up to 90% of all animal disease cases in the farm [5, 6]. Therewith, gastrointestinal pathologies account for about 40% of the non-infectious diseases in terms of the incidence, general spread and significance of the losses. In most of cases, they are caused by the failures related to the animal feeding and housing [2, 3].

Currently, pig industry is on the top of the most efficient segments of the agrifood industry in the terms of the meat production. However, it is characterized by the highest incidence of mass gastrointestinal pathologies due to the use of the intensive hog growing technologies. Dyspepsia and gastroenteritis are the most common gastrointestinal diseases in the store pigs. It is of the importance that the gastrointestinal diseases of various etiology cause up to 70% of mortality in the pig farms [7]. They are extremely dangerous for piglets who frequently develop severe gastrointestinal diseases related to the decrease in the systemic immune reaction and caused by the external factors, including but not limited to bacteria [8, 9]. The advanced cases include complications reflected in the dystrophic and degenerative changes in the tissues and organs [10].

Since the most gastrointestinal pathologies are underlain by the changes in the gut flora, the antibacterial drugs remain the most efficient therapies for such diseases.

Currently, there are extensive investigations aimed at the search for the antibacterial drugs efficient in the infectious inflammatory gastrointestinal diseases, and the antibiotics based on the colistin sulfate are contemplated in this regard [1]. Colistin (polymyxin E) is a cyclic polypeptide antibiotic produced by certain *Paenibacillus polymyxa* strains. This polymyxin antibiotic has a bactericidal effect against most resting and dividing Gram negative bacteria due to the changes in the structure and the function of their surface and plasma membrane. It is used in the calves, goats, poultry, sheep and pigs for the gastrointestinal infections caused by the colistin-susceptible bacteria such as *E. coli*, *Hemophilus*, and *Salmonella* [3].

However, understanding the efficacy of such drugs requires a sufficient level of the process and clinical testing. Considering the above, Agrovetzashchita NVC LLC suggests using the Colistin 12000000 in the bacterial gastrointestinal diseases in the pigs.

This study is aimed at the investigation of the efficacy of the Colistin 12000000 in the gastrointestinal pathologies in the store pigs.

This requires addressing the following task: to determine the efficacy of the Colistin 12000000 in doses 0.6 g and 0.8 g per 100 kg (5-day therapy) in the bacterial gastrointestinal infections in the store pigs.

2 Materials and methods

The clinical trial was conducted in 35-45-day piglets at the pig holding. Two treatment groups were formed, wherein each group included twelve piglets with a gastrointestinal disorder and diarrheal syndrome.

Colistin 12000000 was administered orally with water for 5 days in the dose 0.6 g per 100 kg in Group 1 and in the dose 0.8 g per 100 kg in Group 2.

During the study, the animals were housed in separate cages. The diet was the same in all groups and corresponded to the normal diet for this animal species and age.

The efficacy of Colistin 12000000 was estimated based on the clinical signs of the gastrointestinal pathology at the end of the experiment as confirmed by the bacteriological analysis and blood counts.

The bacteriological samples were taken from the rectal mucosa with a sterile swab. The blood was drawn from the vena cava cranialis with a disposable sterile vacuum system into the anticoagulant tubes and clot activator tubes. Blood and nasal washings were taken on the Screening Day 1 before the initiation of the treatment and five days after the end of the treatment.

Bacteriological analysis of the rectal swabs was performed by the primary inoculation of 5% blood agar and universal chromogenic media (BioRad). The cultures were incubated for 2 days at 37 °C. All grown microorganisms were identified using the MALDI-ToF mass spectrometry on Microflex LT (Bruker®) by the direct application.

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

Digital experimental data were processed using the descriptive statistics to test the significance of the differences in the parameters compared with Student's t-test commonly used in the biology and animal science in Microsoft Excel.

3 Results and discussion

At the baseline, the general health parameters corresponded to an acute phase of the gastrointestinal disease and dehydration due to the diarrhea. The piglets showed a depressed state with poor appetites. 25.0% of animals in the Group 1 and 16.7% in the Groups 2 completely refused the feed. 75.0% and 91.7% of animals, respectively, had an unsatisfactory appearance. The motor activity was reduced in all animals. 100% of cases showed a diarrheal syndrome. The bowel evacuation was accompanied by the tenesmus and a characteristic sitting posture. The stool was liquid, foul-smelling, with a feed and mucus impure. The body temperature was within the age-related physiological normal range. Some individuals had the temperature above 40°C.

The bacterial composition of the rectal washings from the sick piglets suggested that the disease was caused by a complex of the opportunistic microorganisms due to the general sickness related to the negative effect of a number of the process parameters.

After recording the clinical parameters and taking the biomaterial for the laboratory tests, the animals were orally administered with the antibacterial drug and water according the study regimen by the groups.

As early as on Treatment Day 1, no one animal completely refused the feed, and most animals had shown the satisfactory appetites. The motor activity increased in 83.3% of the piglets in both groups, 66.7% of the animals in the Group 1 and 75% in the Groups 2 had a satisfactory appearance. 75.0% and 83.3% of the piglets, respectively, still had the diarrhea.

On Treatment Day 2, the piglets demonstrated 4.2% decrease in their body temperature ($P<0.001$). During the rest of the treatment period, the temperature reduced by 1-2.3% vs. the baseline ($P\leq0.05$ or $P\leq0.01$).

On Treatment Day 4, 100.0% of the piglets had no diarrhea, showed good appetites, a satisfactory appearance, and a good motor activity. During the follow-up, all the parameters corresponded to the physiological normal ranges. No diarrhea signs were noted.

The body weight analysis is provided in Table 1. The treatment resulted in the intensive growth of the animals yielding a higher average daily gain vs. the gain anticipated for the process in this holding (0.3 kg in the week 1 post weaning, 0.38 kg in the week 2, and 0.34 kg average gain).

Table 1. Piglet body weight at the baseline and at the end of the study, kg.

Parameter	Treatment Group 1	Treatment Group 2
Baseline		
Σ n=12	102.6	139.8
x±Sx	8.6±0.19	11.7±0.41
10 days after the end of the treatment		
Σ n=12	180	241.8
x±Sx	15.0±0.47	20.2±0.76
ΔΣ	77.4	102
Δ x	6.5	8.5
ΔΣ, %	175.4	173.0
Δ x, %	175.4	173.0
Average daily gain	0.403	0.531

Thus, the gain in Group 1 and in Group 2 was 18.5% and 32.4% higher than the in-process gain, respectively

This suggests a steady trend of high average daily gain vs. the in-process values, indicating a high efficacy of the Colistin 12000000 in the terms of the interruption of the bacterial infection and the intrinsic rapid recovery of the healthy vital signs.

The blood count data obtained before and five days after the treatment are provided in Table 2.

The study has shown that the erythrocyte sedimentation rate (ESR) was within the normal range (2-9 mm/h). At the same time, this parameter reduced by the end of the study. This reduction was 8.5% in the

Group 1 and 25.1% in the Group 2. A low confidence with high values of the differences is due to the high intrinsic variability of the parameter.

The pigs are characterized by a sufficiently broad WBC range with a high upper level (10-22 × 109/L). The piglets had normal WBC values at the baseline in both groups. However, by the end of the study this parameter increased by 29.6% in the Treatment Group 1 (P≤0.001) and by 80.5% in the Treatment Group 2 (P≤0.001). As a result, the WBC was slightly above the norm in the Group 2**.**

Table 2. CBC dynamics.

Parameter	Group	
	Group 1	Group 2
ESR, mm/h		
Baseline	2.00±0.405	2.67±0.397
End of study	1.83±0.252	2.00±0.270
WBC, 10^9/L		
Baseline	15.96±1.663	14.05±2.348
End of study	20.34±1.257***	25.36±2.448***
RBC, 10^{12}/L		
Baseline	6.11±0.494	6.72±0.396
End of study	6.42±0.130	6.43±0.080
Hemoglobin, g/L		
Baseline	71.91±5.183	71.83±4.835
End of study	79.25±2.235***	93.03±1.024***
Hematocrit, %		
Baseline	27.09±2.527	31.97±2.333
End of study	35.61±0.988***	38.71±0.715***
Bands, %		
Baseline	1.00±0.000	1.08±0.095
End of study	0.92±0.087	2.25±0.261
Segmented, %		
Baseline	52.50±1.388	45.75±2.002
End of study	49.83±2.545	43.58±1.545
Lymphocytes, %		
Baseline	42.67±1.313	50.25±0.324
End of study	45.00±2.562***	46.58±1.732
Eosinophils, %		
Baseline	1.09±0.091	1.50±0.000
End of study	2.42±0.300	3.27±0.449***
Monocytes, %		
Baseline	2.36±0.244	1.33±0.127
End of study	1.33±0.196	1.67±0.213
Basophils, %		
Baseline	0.17±0.117	0.00±0.00
End of study	0.42±0.155	2.58±0.489

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

Note that the piglets had no significant changes in the WBC differential characteristic for an inflammatory process. In the course of the disease, the changes in the WBC differential percent values were reported. The segmented neutrophils percentage reduced in both groups vs. the baseline. The reduction was 2.67% in the Group 1 and 2.17% in the Group 2. The bands content was small in both groups. The monocyte levels were small both at the baseline and at the end of experiment in all groups. By the end of the experiment, their levels wwere similar in both groups. The lymphocytes increased by 2.33 in the Group 1 (P≤0.001), and reduced by 3.67% in Group 2. At the same time, the lymphocytes level was similar in both groups. We noted an increase in the eosinophils by 1.03% in Group 1 and by 1.77% in the Group 2 (P≤0.01). We also noted an increase in the basophils by 0.25% in Group 1 and by 2.58% in the Group 2 (P≤0.01).

RBC estimation has shown that this parameter corresponded the normal range in both groups throughout the study and varied between 6.11-6.72 × 10^{12}/L. At the same time, the baseline hemoglobin levels were significantly below the normal range. In the course of the study, it improved by 10.2% in Group 1 (P≤0.001), and by 29.5% in the Group 2 (P≤0.001). Similar changes were reported for the hematocrit. At the baseline, it was at the lower reference limit, but increased by 35.1% in the Group 1 (P≤0.001) and by 21.1% in the Group 2 (P≤0.001) by the end of the experiment.

We also registered a decrease in the ESR indicative of an improvement in the inflammatory process. The WBC rise up to the upper limit of the norm and above it with no apparent adverse changes in the WBC differential suggesting an increase in the immune reactivity. The WBC differential parameters in both groups suggest the trend to the reduction in the neutrophils, the normalization of the lymphocyte levels, increase in the eosinophils which is indicative of the decline in the acute phase processes mediated by the white blood cells.

The most significant changes were related to the erythrocytes. Rapid significant increase in the hemoglobin and hematocrit in both groups may suggest an activation of the hemopoiesis due to the elimination of the adverse effects of the infection.

Therefore, the described changes related to the erythrocytes and leukocytes suggested the recovery of the morphofunctional homeostasis after the disease in both treatment groups.

At the baseline, we estimated the susceptibility of the microorganisms isolated from the rectal washings of the piglets with the gastrointestinal pathologies to Colistin 12000000. The results are provided in the Table 3.

The choice of the piglets was based on their group affiliation, the most intensive microbial growth in their samples and the apparent colony forming effect as well as on the presence of the most dangerous pathogens.

The analysis demonstrated that the susceptibility of *E. coli* and *Pseudomonas aeruginosa* to Colistin 12000000 was 0.5 µg/mL in both groups. *Klebsiella pneumoniae* had the same susceptibility. The susceptibility of *Pasteurella aerogenes* varied within 0.5-2 µg/mL.

Table 3. Analysis of the susceptibility of the microorganisms to Colistin 12000000, µg/L.

No.	Microorganism	MIC, µg/mL
1	*E. coli*	0.5 – S*
6	*Klebsiella pneumoniae*	0.5 – S*
7	*E. coli*	0.5 – S*
15	*Pasteurella aerogenes*	0.5 – S*
16	*E. coli*	0.5 – S*

16	*Klebsiella pneumoniae*	0.5 – S*
20	*Pasteurella aerogenes*	0.5 – S*
23	*Pasteurella aerogenes*	0.5 – S*
30	*Pasteurella aerogenes*	0.5 – S*
31	*E. coli*	0.5 – S*
35	*Pasteurella aerogenes*	0.5 – S*
38	*Klebsiella pneumoniae*	0.5 – S*
44	*Pasteurella aerogenes*	2 – S*
47	*Pasteurella aerogenes*	0.5 – S*
48	*Pseudomonas aeruginosa*	0.5 – S*
58	*E. coli*	0.5 – S*
57	*E. coli*	0.5 – S*
60	*Pasteurella aerogenes*	0.5 – S*

**S; susceptible; *R: resistant*

Thus, Colistin 12000000 was efficient against both Gram positive and Gram-negative flora. All the bacteria studied were susceptible to this drug which allows its use in the various bacterial infections. Of special note is the fact that the growth of *Escherichia coli*, one of the main causative agents of the infectious inflammatory diseases, was efficiently inhibited at the drug concentration of 0.5 µg/mL.

When estimating the species composition of the microbial flora in the nasal washings, the most common microorganisms found in both groups included: *E. coli, Streptococcus gallolyticus, Klebsiella pneumoniae.*

Table 4. Rectal microbial flora dynamics.

Microbial flora	Baseline		End of study	
	n	%	N	%
Total number of the piglets	12	100	12	100
Treatment Group 1				
E. coli	11	91.7	12	100.0
Streptococcus gallolyticus	8	66.7	7	58.3
Klebsiella pneumoniae	5	41.7	1	8.3
Pasteurella aerogenes	3	25.0	0	0.0
Enterococcus faecalis	2	16.7	0	0.0
Proteus mirabilis	1	8.3	0	0.0
Streptococcus suis	1	8.3	0	0.0
Acinetobacter towneri	1	8.3	0	0.0
Rothia nasimurium	1	8.3	0	0.0
Lactobacillus salivarius	0	0.0	5	41.7
Lysinibacillus fusiformis	0	0.0	3	25.0
Enterococcus hirae	0	0.0	3	25.0
Lactobacillus johnsonii	0	0.0	2	16.7
Acinetobacter radioresistens	0	0.0	1	8.3
Lysinibacillus boronitolerans	0	0.0	1	8.3
Treatment Group 2				
E. coli	12	100.0	12	100.0
Streptococcus gallolyticus	8	66.7	3	25.0
Klebsiella pneumoniae	4	33.3	2	16.7
Proteus mirabilis	1	8.3	1	8.3
Enterococcus villorum	1	8.3	0	0.0
Enterococcus faecalis	1	8.3	0	0.0
Aerococcus viridans	1	8.3	0	0.0
Citrobacter freundii	1	8.3	0	0.0
Streptococcus hyovaginalis	1	8.3	0	0.0
Pasteurella aerogenes	1	8.3	0	0.0
Streptococcus orisratti	1	8.3	0	0.0
Enterococcus hirae	0	0.0	8	66.7
Lysinibacillus fusiformis	0	0.0	3	25.0
Lactobacillus salivarius	0	0.0	1	8.3
Lactobacillus johnsonii	0	0.0	1	8.3
Staphylococcus saprophyticus	0	0.0	1	8.3
Bacillus megaterium	0	0.0	1	8.3
Comamonas kerstersii	0	0.0	1	8.3
Corynebacterium stationis	0	0.0	1	8.3

Escherichia coli is a rod-shaped opportunistic bacterium living and growing in the absence of the oxygen, mainly in the animal and human intestine. Some its strains belong to the symbiotic flora but can cause the enteric infections in adverse conditions.

Streptococcus gallolyticus is a Gram-positive opportunistic pathogen which can cause bacteriaemia and is predominantly found in the large intestine.

Klebsiella pneumoniae is a Gram-negative rod-shaped immobile capsule-forming bacteria belonging to *Klebsiella* genus which can also cause bacteriaemia and various organ system-related diseases.

Therefore, all three most common bacteria isolated from the intestine are opportunistic microorganisms, with *Klebsiella pneumoniae* having the most apparent pathogenicity.

In the Treatment Group 1, *E. coli* was found in 91.7% of the cases; *Streptococcus gallolyticus* in 66.7%; *Klebsiella pneumoniae* in 41.7%; *Pasteurella aerogenes* in 25%; *Enterococcus fae*calis in 16.7%; and *Proteus mirabilis, Streptococcus suis, Acinetobacter towneri* were found in 8.3% of the cases.

In the Treatment Group 2, *E. coli* was found in all cases; *Streptococcus gallolyticus* in 66.7%, *Klebsiella pneumoniae* in 33.3% of the cases. Other bacterial species (*Proteus mirabilis, Enterococcus villorum, Enterococcus faecalis, Aerococcus viridians, Citrobacter freundii, Streptococcus hyovaginalis, Pasteurella aerogenes, Streptococcus orisratti*) were found in single cases.

After the 5-day therapy, the prevalence of *Streptococcus gallolyticus* in the Group 1 reduced by 8.3% and ultimately was 58.3%. *Klebsiella pneumoniae* prevalence reduced by 33.3%, this bacterium was only found in one case, while the number of *E. coli* increased by 8.3%. Other previously isolated bacteria were no more found. At this time point, new bacteria were isolated, including *Lactobacillus salivarius* (41.7%); *Lysinibacillus fusiformis* and *Enterococcus hira*e (25.0%); *Lactobacillus johnsonii* (16.7%); *Acinetobacter radioresistens, Lysinibacillus boronitolerans* and *Lysinibacillus boronitolerans* (8.3%).

In the Group 2, prevalence of *Streptococcus gallolyticus* reduced by 41.7% and was 25.0% five days after the 5-day therapy. *Klebsiella pneumoniae* prevalence reduced twice, and it was ultimately registered in 16.7% of the cases. *E. coli* number remained unchanged. Other previously isolated bacteria were no more registered, except for the *Proteus mirabilis* (8.3%). New bacterial species were found, including *Enterococcus hirae* (66.7%); *Lysinibacillus fusiformis* (25.0%); *Lactobacillus salivarius, Lactobacillus johnsonii, Staphylococcus saprophyticus, Bacillus megaterium, Comamonas kerstersii, Corynebacterium stationis* (8.3%).

In both cases, Colistin 12000000 had no effect on *E. coli* that may be due to either high antibiotic resistance or symbiotic nature and a good sustainability of this microorganism in the gut flora. Given the good animal recovery dynamics when treated with the antibacterial drug, this bacterium likely had no key role as an etiological factor of the disease.

It is worth noting that among the new isolated microorganisms, *Lactobacillus salivarius, Lactobacillus johnsonii* and *Enterococcus hirae* are symbiotic species naturally presented in the intestinal lumen. Their presence is a positive parameter characterizing the digestive function. The amount of these microorganisms was highest at the end of the experiment. Other newly isolated bacteria can be considered commensal species, and some of them may be opportunistic ones. However, their amount was insignificant. Increase in the activity and emergence of new bacterial species after the treatment suggests their presence in the process premise environment and cannot be considered as an adverse factor as the animals completely recovered by this time point.

4 Conclusion

The study has shown that Colistin 12000000 has a high therapeutic efficacy in the infectious and inflammatory gastrointestinal pathologies caused by the resident opportunistic bacterial flora in the store pigs.

Oral administration of the drug in the piglets with the nonspecific gastroenteritis for 5 days in the doses 0.6 g/100 kg or 0.8 g/100 kg provided the complete therapeutic efficacy (the clinical recovery) on the Day 4 in both treatment groups. The dynamics of the erythrocytes and leukocytes suggest the recovery of the morphofunctional homeostasis after the disease in both treatment groups. After use of Colistin 12000000 in a dose 0.6 g/100 kg for 5 days, the prevalence of *Streptococcus gallolyticus* reduced by 8.3%. *Klebsiella pneumoniae* prevalence reduced by 33.3%. After the 5-day therapy with Colistin 12000000 in a dose 0.8 g/100 kg, the prevalence of *Streptococcus gallolyticus* reduced by 41.7% and was 25.0% at the end of the study. *Klebsiella pneumoniae* prevalence reduced twice, and it was ultimately registered in 16.7% of the cases. The other bacteria presented in the washings at the baseline in both groups were no more observed at the end of the treatment. The symbiotic flora was found in both groups at the end of the experiment in a sufficient number.

Thus, the efficient dose of the test drug was 0.6 g/100 kg as provided the desirable clinical effect comparable to the higher dosing regimen.

The drug well tolerated, with no side effects or adverse events reported throughout the study.

The results of the study of the efficacy of Colistin 12000000 give ground for recommending this drug dissolved in the drinking water for the treatment of the gastrointestinal diseases in the pigs with the diarrheal syndrome in the oral dose 0.6 g/100 kg for 5 days.

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