The effectiveness of various antibacterial drugs in the treatment of swine respiratory diseases

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Abstract. The polyethiological nature of pig respiratory diseases involves the use of antibacterial drugs of the widest possible spectrum of action. The success of the whole range of therapeutic measures will largely depend on the choice of antibacterial drugs. This article presents the results of a clinical (field) study evaluating the effectiveness of azithromycin, florfenicol and amoxicillin in respiratory diseases of pigs under conditions of contamination with associates of highly resistant pathogens (\textit{Mycoplasma spp.}, Hemolytic coagulase-positive \textit{Staph.aureus}, non-hemolytic Lac-coli fermenting \textit{E. hemolytic Enterococcus faecalis}, \textit{Klebsiella pneumoniae}, \textit{Klebsiella oxytoca}, \textit{Morganella morganii}, \textit{Pseudomonas aeruginosa}, \textit{Proteus mirabilis}, \textit{Enterobacter aerogenes}, \textit{Enterobacter cloacae}). The study included 90 crossbred piglets 100-110 days old with pronounced clinical signs of respiratory diseases. In the course of the work, the dynamics of changes in the state of piglets was assessed: the rate of disappearance of clinical signs, the average daily weight gain, the presence of associates of highly resistant microorganisms after a course of treatment, the mortality rate and the number of adverse events and side reactions. The results of the study revealed the formation of multidrug resistance in a number of pathogens. Preparations based on florfenicol and azithromycin showed higher therapeutic efficacy in the treatment of respiratory diseases of piglets under conditions of contamination of associates of highly resistant pathogens.

1. Introduction
The problem of porcine respiratory diseases, or CRPS (porcine respiratory disease complex), is still one of the most urgent. The main feature of CRPS in farms with a high livestock density is the presence of associates of various pathogens (1-4). For example, under conditions of contamination with mycoplasma (in particular, \textit{M. hyopneumoniae}), the susceptibility of animals to other bacterial pathogens increases, which is important to consider when choosing an AB agent (5,6).

Despite significant steps towards the development and practical implementation of methods of specific prophylaxis (vaccination), the overwhelming majority of veterinarians consider exclusively broad-spectrum antibacterial agents (ABA) as etiopropic therapy for CRPS (7-9) with high bioavailability and a short course of treatment, which allow you to quickly stop the spread of infection, prevent massive mortality and weight loss (gain in live weight), and also reduce labor costs during group treatments (7,10). At the same time, selection of multidrug-resistant forms of bacterial pathogens is often provoked by prolonged and unjustified use of ABA (11-13). The task of preventing and/or overcoming the problem of multidrug resistance, as a rule, can only be dealt with by radical approaches (up to the destruction of the entire livestock) or new ABA with an ultra-wide spectrum of action.
action and an original mechanism for its implementation. The search for such ABA and confirmation of their effectiveness are always associated with a large amount of research work.

The aim of this study was to evaluate the therapeutic efficacy of three antimicrobial drugs based on different antibacterial drugs in the treatment of CRPS (table 1).

| Table 1. Main characteristics of the selected antibacterial drugs. |
|---------------------------------------------------------------|
| **No** | **A drug** | **Amoxicillin** | **Azilon** | **Respol** |
|--------|-----------|----------------|-----------|-----------|
| 1.     | Active substance | amoxicillin | azithromycin | florfenicol |
| 2.     | AB group | penicillins | macrolides | amphenicols |
| 3.     | Time to reach max concentration in blood plasma | 1-2 hours | 30-60 minutes | 30-90 minutes |
| 4.     | Time to maintain therapeutic concentration in the body | 48 hours | up to 72 hours (in the lungs, macrophages up to 120 hours) * | 48 hours |
| 5.     | Features of kinetics | fast and even distribution in organs and tissues | * accumulates mainly in the focus of inflammation | fast and even distribution in organs and tissues |
| 6.     | Activity against mycoplasmas | not | Yes | Yes |
| 7.     | The interval between injections | 48 hours | 24 hours | 48 hours |

Note: * hereinafter veterinary medicinal product based on 15% amoxicillin trihydrate.

2. Materials and methods

The work was carried out on the basis of a pig-breeding farm LLC "Agrosoyuz" (Smolensk region) according to PKI RE / AZ-R-01/2020. The study included 90 crossbred piglets (4-breed crossing: Russian White × Duroc × Yorkshire × Pietrain) 100-110 days of age with signs of acute rhinotracheitis and tracheobronchitis, which were randomly divided into 3 groups of 30 animals each. All piglets were immunized according to the schedule of vaccination and immunization on the farm, they did not use antibacterial drugs with florfenicol and azithromycin for 30 days, and other antibacterial drugs for 7 days before the start of the study. Vaccination was not available at the time of the study.

Treatment regimen in groups:

- **Respol group**: Respol drug (LLC "AleksAnn" for the New Group company) at a dose of 1 ml / 20 kg of animal weight (40 mg of florfenicol / 1 kg) intramuscularly once a day, twice a day with an interval of 48 hours;
- **Azilon group**: Azilon drug (LLC "AleksAnn" for the New Group company) at a dose of 1 ml / 20 kg of animal weight (5 mg azithromycin / kg) intramuscularly once a day, twice with an interval of 24 hours;
- **Amoxicillin group**: a drug based on 15% Amoxicillin trihydrate at a dose of 1 ml / 10 kg of animal weight (15 mg of active ingredient / kg) intramuscularly three times with an interval of 48 hours.

The conditions of keeping (group in boxes) and feeding in all groups were identical.

In order to isolate the causative agents of the disease on the first day before the start of treatment, 10 piglets of animals from each group (No. 1-10 group Amoxicillin; No. No. 11-20 group Respol; No. No. 21-30 group Azilon) were selected material (washings from the nasal cavities) for bacteriological research with the determination of antibiotic sensitivity and PCR diagnostics for mycoplasmosis (performed at LLC "Chance Bio").

Blood samples for general clinical analysis (CBC) were taken from 10 animals of each group on the first day before the start of treatment (Day 1) and on the 7th day of the experiment (Day 7). The
samples were delivered to the laboratory of "Chance Bio" LLC within 24 hours from the moment of blood sampling in a thermo container equipped with refrigeration elements ensuring a temperature not higher than 8 °C.

The dynamics of the recovery of piglets within 7 days from the start of treatment, the average daily weight gain during the observation period (7 days), mortality, and the dynamics of changes in the CBC indicators were assessed. Additionally, we took into account the number of adverse events and side reactions that occurred.

Statistical analysis of the data was performed using the Prism 7.01 software using conventional methods. Fisher's exact method was also used for intergroup comparisons.

3. Results
At the time of group formation, all pigs showed symptoms of acute rhinotracheitis and tracheobronchitis: cough, shortness of breath, nasal discharge. The diagnosis was made comprehensively on the basis of anamnesis, clinical examination data, taking into account the results of microbiological studies and CBC.

Microbiological research results.

Microbiological analysis of the material (washings from the nasal cavity) revealed that the disease was caused by a mixed infection (table 2); *Mycoplasma spp.* was identified (PCR diagnostics) in all samples. It is known that with a decrease in the body's resistance, opportunistic *E. coli* can manifest its pathogenic potential, therefore, in this work, it was considered as a potential pathogen.

| No | Isolated microorganisms                                    | Frequency of occurrence in samples |
|----|------------------------------------------------------------|-----------------------------------|
| 1  | *Mycoplasma spp.* (PCR diagnostics)                        | 100%                              |
| 2  | hemolytic coagulase-positive *Staphylococcus aureus*       | 60%                               |
| 3  | hemolytic *Enterococcus faecalis*                          | 66.7%                             |
| 4  | non-hemolytic *Lac*-fermenting *Escherichia coli*          | 56.7%                             |
| 5  | *Klebsiella pneumoniae*                                    | 43.3%                             |
| 6  | Morganella morganii                                        | 16.7%                             |
| 7  | Enterobacter cloacae                                       | 13.3%                             |
| 8  | Enterobacter aerogenes                                     | 10%                               |
| 9  | *Pseudomonas aeruginosa*                                  | 6.7%                              |
| 10 | *Proteus mirabilis*                                        | 6.7%                              |
| 11 | *Klebsiella oxytoca*                                       | 3.3%                              |

The dissemination of the material by the isolated microorganisms was different. Most often, in large quantities (from 104 CFU to 106 CFU and more), 4 infectious agents were detected in the samples: *hemolytic coagulase-positive Staph. aureus*, *hemolytic Enterococcus faecalis*, *Klebsiella pneumoniae*, and *non-hemolytic Lac-fermenting E. coli* (figure 1).

Antibiotic sensitivity of isolated microorganisms.

The isolated microorganisms had different sensitivity to florfenicol, azithromycin and amoxicillin. Thus, *non-hemolytic Lac-fermenting E. coli, Klebsiella pneumoniae, Morganella morganii were resistant to amoxicillin, and hemolytic Enterococcus faecalis* was resistant to florfenicol and azithromycin (table 3).
Figure 1. Dominant infectious agents of the economy.

| No | Microorganism                                      | Amoxicillin | Azithromycin | Florfenicol |
|----|----------------------------------------------------|-------------|--------------|-------------|
| 1  | hemolytic coagulase-positive Staph. aureus          | 77.8%       | 94.4%        | 94.4%       |
| 2  | hemolytic Enterococcus faecalis                    | 85%         | 0%           | 0%          |
| 3  | Klebsiella pneumoniae                              | 0%          | 100%         | 100%        |
| 4  | non-hemolytic Lac-fermenting E. coli               | 0%          | 58.8%        | 58.8%       |
| 5  | Morganella morganii                                | 0%          | 100%         | 100%        |
| 6  | Enterobacter aerogenes                             | 0%          | 100%         | 100%        |

Note: a total of 30 samples were taken.

It should be noted that Enterobacter cloacae, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella oxytoca isolated in significant amounts in a total of 1/3 of the samples were resistant or insensitive to all selected antibiotics.

In five samples (No. 1,12,16,26,27) all isolated microorganisms were insensitive to amoxicillin, and only in one (No. 14) to florfenicol and two (No. 14, 18) to azithromycin.

Dynamics of changes in clinical signs.
The general condition of the piglets before the start of treatment was satisfactory. The animals were observed to have cough, nasal discharge, shortness of breath. On the 5th day in the Amoxicillin group, 5 piglets retained symptoms of the disease, in the Azilon and Respol groups - in 2 in each. By the 7th day, there were no signs of the disease in any of the pigs in the Azilon and Respol groups; in the Amoxicillin group - in 28 out of 30 (table 4). After the end of the study, two piglets from the Amoxicillin group required additional treatment.

Table 4. Dynamics of recovery of piglets.

| No | Groups       | DV in the preparation | Number of pigs in a group, head | Number of piglets without signs of respiratory disease | Palo, head | Forced slaughter head |
|----|--------------|------------------------|---------------------------------|--------------------------------------------------------|------------|-----------------------|
| 1  | Respol florfenicol | 30                     | 28                              | 93,3 % Day 5 head 30                                  | 100        | 0                     |
| 2  | Azilon azithromycin | 30                     | 28                              | 93,3 % Day 7 head 30                                  | 100        | 0                     |
| 3  | Amoxicillin amoxicillin | 30                     | 25                              | 83,3 Day 7 head 28                                   | 93,3       | 0                     |

Thus, the therapeutic efficacy in the Azilon and Respol groups was 100%, in the Amoxicillin group - 93.3% (figure 2).

Average daily gain.

Piglets of each group were weighed before the start (Day 1) and after the end of treatment (Day 7). The smallest increase in live weight was recorded in the Amoxicillin group (table 5), which may be due to the fact that on the 7th day in this group, two piglets still had signs of the disease. The average
daily weight gain in the Azilon and Respol groups was 29 g and 19 g higher than in the Amoxicillin group, respectively.

Table 5. Results of control weighings are requested (total body weight per group).

| No | Groups                | Total live weight, kg | Average daily weight gain, g. | Difference compared to the Amoxicillin group, g. |
|----|-----------------------|-----------------------|-------------------------------|-----------------------------------------------|
|    | before treatment      | after treatment       |                               |                                               |
|    | Day 1                 | Day 7                 |                               |                                               |
| 1  | Respol (n = 30)       | 1090                  | 1211                          | 576                                           |
|    |                       |                       |                               | +19                                           |
| 2  | Azilon (n = 30)       | 1142                  | 1256                          | 586                                           |
|    |                       |                       |                               | +29                                           |
| 3  | Amoxicillin (n = 30)  | 1153                  | 1270                          | 557                                           |
|    |                       |                       |                               | -                                             |

CBC results.
Analysis of the results of the KLA showed that only in the Amoxicillin group the leukocyte content of pigs increased (almost to the upper limit of the norm). In the other two groups, the number of leukocytes did not change significantly, but the proportion of lymphocytes in the animals in the Respol group increased (table 6). All other indicators were within the physiological norm or did not deviate from it clinically significantly.

Table 6. Dynamics of changes in hematological parameters in piglets of different groups (before and after treatment).

| Indicators          | Respol before | Respol after | Azilon before | Azilon after | Amoxicillin before | Amoxicillin after | Units | Units          |
|---------------------|---------------|--------------|---------------|--------------|--------------------|--------------------|-------|----------------|
| Erythrocytes        | 6.9±0.21      | 6.9±0.41     | 7.4±0.23      | 6.6±0.03     | 7.3±0.22           | 7.4±0.51           | 5.0-10.0 | *10⁹/l       |
| Hemoglobin          | 112.0±4.12    | 112.2±6.26   | 112.2±2.63    | 107.0±0.0    | 113.3±6.69         | 113.7±5.78         | 100-160 | g/l           |
| Hematocrit          | 37.6±1.41     | 37.6±2.07    | 38.2±1.04     | 35.6±0.50    | 38.7±2.16          | 38.1±2.17          | 32.0-50.0 | %            |
| Leukocytes          | 17.1±2.03     | 16.0±3.01    | 19.0±2.61     | 18.6±5.15    | 13.9±1.14          | 19.9±3.27          | 7.0-20.0 | *10⁹/l       |
| Stab                | 0±0           | 0±0          | 0±0           | 1.0±1.0      | 0±0                | 0±0                | 3-7    | %             |
| Segmented           | 42.0±1.81     | 34.6±2.44    | 44.2±5.49     | 50.0±8.0     | 37.7±4.17          | 37.3±3.33          | 28-45  | %             |
| Eosinophils         | 6.8±2.78      | 2.0±0.31     | 3.0±1.3       | 1.5±1.5      | 6.7±1.45           | 0.7±0.81           | 0-6    | %             |
| Basophils           | 1.8±0.37      | 0±0          | 1.4±0.60      | 0±0          | 3.0±1.53           | 0.7±0.67           | 0-1    | %             |
| Monocytes           | 1.6±0.68      | 0±0          | 0.8±0.58      | 0±0          | 1.0±0.58           | 0±0                | 2-6    | %             |
| Lymphocytes         | 47.8±3.77     | 63.4±2.25*   | 50.6±6.05     | 47.5±7.5    | 51.7±1.86          | 61.3±3.71          | 40-70  | %             |
| Platelets           | 374.6±29.6    | 389.6±50.2   | 437±43.6      | 342.5±93.5  | 318.3±32.9         | 335.0±39.5         | 120-720 | *10⁹/l       |

Note: * p <0.05 (intragroup comparison)

Adverse reactions and side effects. During the study period, no adverse reactions and / or side effects were noted in any of the groups.
4. Discussion
The results of the microbiological study confirmed that CRPS is caused by an associate of pathogenic and opportunistic microorganisms; in this case: mycoplasma and predominantly hemolytic coagulase-positive Staph. aureus, hemolytic Enterococcus faecalis, Klebsiella pneumoniae, as well as non-hemolytic Lac-fermenting E. coli, which acquires pathogenic properties under conditions of reduced resistance. The penicillin antibiotic amoxicillin a priori does not work on Mycoplasma spp. and penicillin-forming strains of Klebsiella spp. In addition, in this farm, opportunistic E. coli strains acquired 100% resistance to amoxicillin. In fact, amoxicillin was ineffective under these conditions against 3 out of 5 dominant pathogens. The effectiveness of the amoxicillin-based treatment regimen was 93.3%.

Florfenicol and azithromycin are known to be active against Mycoplasma spp. Found in 100% of the samples in this study. In addition, unlike amoxicillin, Klebsiella spp, Morganella morganii, Enterobacter aerogenes were sensitive to these antibiotics. The isolated E. coli strains were resistant or insensitive to these antibiotics in 41.2% of cases; hemolytic Enterococcus faecalis was resistant in 100% of cases. That is, the Respol and Azilon preparations (containing florfenicol and azithromycin, respectively) had an effect on 4 out of 5 dominant pathogens, which correlates with a noticeable increase in the average daily weight gain and higher treatment efficiency in the corresponding groups of animals.

It is interesting to note that the post-antibiotic effect of azithromycin provides high therapeutic efficacy even after 2 injections with an interval of 24 hours. It can be assumed that the features of the pharmacokinetics of this antibiotic create conditions for the fastest stabilization of the piglets' condition, which is accompanied by an increase in feed conversion and more active weight gain: the increase in live weight in the Azilon group was the greatest.

I would like to draw your attention to the fact that some of the isolated pathogens (Enterobacter cloacae, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella oxytoca), in total occurring in 1/3 of the samples, were insensitive to all selected antibiotics. This indicates a high potential for a significant spread of these pathogenic microorganisms, which should be taken into account by farm specialists when choosing treatment regimens in the future.

5. Conclusion
The results of microbiological screening of the farm during the study revealed a stable tendency towards the formation of multidrug resistance in a number of CRPS pathogens. Preparations based on florfenicol (Respol, LLC "AleksAnn", RF) and azithromycin (Azilon, LLC "AleksAnn”, RF) showed pronounced therapeutic efficacy (100%) in the treatment of CRPS in piglets under conditions of confirmed contamination with associates of highly resistant pathogens.

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