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Original research

# Study of antimicrobial susceptibility to respiratory pathogens in pigs

**N. Yu. Neverkovets, V. O. Chumak, A. V. Kokariev**
*Dnipro State Agrarian and Economic University, Dnipro, Ukraine*

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Dnipro State Agrarian and Economic University, Serhii Efremov Str., 25, 49600, Dnipro, Ukraine

**Tel.:** +8067-975-84-93
**E-mail:** *never.nata@gmail.com*

[#]**Corresponding author:**
N. Yu. Neverkovets
*never.nata@gmail.com*

**Abstract.** The antimicrobial susceptibility of common respiratory pathogens of pigs including *Actinobacillus (Haemophilus) pleuropneumoniae*, *Pasteurella multocida*, *Streptococcus suis*, and *Trueperella (Arcanobacterium) pyogenes* – was studied in the samples isolated from industrial pig farms located in east and central province of Ukraine. The study was carried out with analysis the results of surveillance the microbial strains susceptibility in the 620 animals with signs of respiratory pathology. The samples were isolated and tested during 2023-2024 at the Research Center for Biosafety and Environmental Control of Agricultural and Industrial Complex Resources. To determine the sensitivity of bacteria to antibiotics, the Kirby-Bauer agar diffusion method was used. Resistance was determined against to particular antibiotic groups, the application of which is regulated by the government requirements of Ukraine. In order to identify differences in the prevalence of resistant strains to individual antibiotics dependent on the year of the study, the results were statistically analyzed with using the $\chi$ 2 criterion. The dynamics of the spread of resistance to individual antimicrobial drugs were identified. The most effective category D drugs that are allowed to be used for the treatment of animals on the first day of infection caused by the studied bacteria are drugs containing Amoxicillin. However, resistance to Amoxicillin in *A. pleuropneumoniae* isolates increased from 12% in 2023 to 18% in 2024. The consequence of the requirements breach for the rational use of antimicrobial drugs was the resistance generation in respect to category B antimicrobial drugs, which are allowed to be used for the treatment of productive animals only in the absence of effective drugs of categories C or D. Fluoroquinolone-resistant strains of *S. suis* were detected in 6%, and *A. pleuropneumoniae* in 9-18% of samples, respectively in 2024. Among the studied isolates of *P. multocida* and *T. pyogenes*, there is no observed the resistance against fluoroquinolones. The introduction of regular monitoring of antibiotic resistance will allow selecting the most effective antimicrobial treatment regimen for respiratory pathology in pigs and will contribute to the implementation of the requirements of the "One Health" concept in respect with agricultural production.

**Keywords:** antibiotic resistance; disk diffusion; monitoring.

# Дослідження чутливості до антимікробних речовин збудників респіраторних захворювань свиней

**Анотація.** Досліджено чутливість до протимікробних засобів поширених респіраторних патогенів свиней - *Actinobacillus (Haemophilus) pleuropneumoniae*, *Pasteurella multocida*, *Streptococcus suis*, *Trueperella (Arcanobacterium) pyogenes*. Робота виконана на ізолятах, одержаних від 620 тварин із ознаками респіраторної патології, які надходили протягом 2023-2024 років до Науково-дослідного центру біобезпеки та екологічного контролю ресурсів АПК. Для визначення чутливості бактерій до антибіотиків використовували метод дифузії в агар за Kirby-Bauer. Стійкість визначали до антибіотиків, використання окремих категорій яких регламентує в Україні "Порядок використання протимікробних ветеринарних лікарських засобів у ветеринарній медицині". З метою виявлення відмінностей у поширеності резистентних штамів до окремих антибіотиків залежно від року дослідження результати оброблено статистично з розрахунком критерію $\chi$2. Виявлено динаміку поширення резистентності до окремих протимікробних препаратів. Найбільш ефективними препаратами категорії Д, які дозволено використовувати для лікування тварин у перший день інфекції, викликаної досліджуваними бактеріями є препарати з Амоксициліном. Проте резистентність до Амоксициліну у ізолятів *A. pleuropneumoniae* зросла із 12% у 2023 році до 18% у 2024 році. Наслідком порушення вимог щодо раціонального використання протимікробних препаратів є поява резистентності до протимікробних препаратів категорії В, які дозволено використовувати для лікування продуктивних тварин лише за відсутності ефективних препаратів категорій С або Д. У 2024 році стійких до фторхінолонів штамів *S. suis* виявлено 6%, а *A. pleuropneumoniae* у 9-18% зразків відповідно. Серед досліджених ізолятів *P. multocida* і *T. pyogenes* резистентність до фторхінолонів відсутня. Впровадження регулярного моніторингу антибіотикорезистентності дозволить підбирати найбільш ефективну схему протимікробного лікування респіраторної патології свиней та сприятиме втіленню вимог концепції "Єдине здоров'я" у агровиробництво.

**Ключові слова:** антибіотикорезистентність; дискова дифузія; моніторинг.

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## Introduction

Antimicrobial resistance is a serious problem in the implementation of the "One Health" concept, according to which the health of people, animals and the state of the environment are interconnected. In order to combat antimicrobial resistance and reduce their use in pig farming, methods for preventing and/or treating infections are constantly being sought. The rational use of antimicrobials is the main factor in preventing the formation of resistance against them in pathogens of bacteriosis in productive animal species (Salmanov et al., 2018, The European Union summary report, 2024).

Antimicrobial resistance has been identified as a major threat to global health. The pig food chain is considered an important source of antimicrobial resistance genes (ARGs). However, there is still a lack of knowledge on the dispersion of ARGs in pig production system, including the external environment (Scicchitano et al., 2024).

The microbial composition of the upper respiratory tract undergoes significant evolution after birth and tends to stabilize after weaning. Antimicrobial treatment, airborne ammonia, diet, and flooring type are recognized environmental factors influencing the composition of the microbiome. The upper respiratory tract is dominated by the phyla *Proteobacteria* and *Firmicutes*. Reduced diversity and unbalanced microbial composition are associated with an increased risk of respiratory diseases, referred to as porcine respiratory disease complex (Pirolo et al., 2021).

Porcine respiratory disease complex is a multifactorial syndrome that causes health problems in farmed pigs and economic losses. Etiological factors can be bacteria, viruses or mycoplasmas, but housing conditions also affect the health of the animal. The severity of bronchial and pulmonary lesions depends on the combination of etiological factors and environmental conditions, which leads to pneumonia, pleuropneumonia and pleurisy. The pathogens *A. pleuropneumoniae, P. multocida, Streptococcus spp.* and *Actinomyces pyogenes* cause both specific and non-specific lesions, so it is necessary to create control and treatment programs for the farms individually (Ruggeri et al., 2020).

The microbiome of pneumonia in pigs of the finishing group is represented by a co-infection with 3-6 microorganisms, and in pigs on fattening with 4-7 microorganisms. It was found that the most common microorganisms in pneumonia in pigs are PCV2, *Glaesserella parasuis, S. suis, Mycoplasma hyorhinis and P. multocida*. Taking into the account that piglets affected with various pathogens beginning from first day of life, microbilal infections accompanied by viral co-infection as a rule (Masiuk et al., 2024).

In Ukraine, among the pathogens of infectious diseases of animals and poultry, the largest share (about 40%) of resistant ones belongs to *Escherichia coli*. The highest level of resistance of *E. coli* from car-casses and minced pork is registered to β-lactams, but there are differences depending on the region. In the Odessa region, antibiotic-resistant isolates of *E. coli* were isolated in washes in 34.7% of cases, and in the Sumy region in 53.7% of cases. The isolates have showed the greatest resistance to Ampicillin - in 67.3% and 80.6% of cases, Lincomycin in 22.4% and 52.4%, Gentamicin in 24.5% and 44.4%, but did not have re-sistance to Ofloxacin - 4.1% and 4.8%, respectively (Kasianchuk et al., 2018).

The approach to the use of antimicrobials is changing due to the accumulation of knowledge about the importance of the composition of the body microbiota, in particular on the mucosa of the upper respiratory tract. In the absence of perinatal antimicrobials (Penicillin, Streptomycin, Ceftiofur, Tulathromycin), the diversity of the nasal microbiota increases at weaning. In particular, the abundance of representatives of the genera *Prevotella* and *Lactobacillus* increases and the number of *Moraxella, Bergeyella, Neisseria* decreases, while *Haemophilus* and *Mycoplasma* decreased on one farm in Spain, but increased on another. Such changes in the composition of the microbiota were accompanied by improved health status of piglets and higher productivity in the growing phase (Correa-Fiz et al., 2019).

Results of a study of the nasal microbiome from birth to 10 weeks of life of piglets showed that there is a certain relationship between the state of the microbiome and age and characteristics of maintenance. In particular, the age of the piglets showed a correlation with 19% of individual characteristics in the microbial composition of the piglets. Stabilization of the microbiome occurred approximately 2 weeks after weaning. Although opportunistic microorganisms were present, they did not cause disease (Vlasblom et al., 2024).

According to the results of the study of antimicrobial resistance by the European Food Safety Authority, the most important pathogens affecting pig health are *E. coli, S. suis, A. pleuropneumoniae, P. multocida, G. parasuis, Bordetella bronchiseptica, Staphylococcus aureus, Staphylococcus hyicus, Brachyspira hyodysenteriae, T. pyogenes, Erysipelothrix rhusiopathiae, S. dysgalactiae, Mycoplasma hyosynoviae, Mycoplasma hyorhinis, Mycoplasma hyopneumoniae* and *Brachyspira pilosicoli*. There are significant differences in the level of resistance to individual groups of antibiotics among isolates from animals in different EEC countries (Nielsen et al., 2021).

Analysis of nearly 20.000 isolates collected from 2006 to 2016 in the United States from pigs with respiratory complex identified *P. multocida* among the bacterial pathogens in 42.3%, *S. suis* 34.6%, *A. suis* 14.7% and *Haemophilus parasuis* 8.3% of the total number of samples (Hayer et al., 2020).

Among the pathogens of respiratory diseases of pigs in the United States and Canada, *A. pleuropneumoniae, P. multocida, S. suis*, and *B. bronchiseptica* dominated (Sweeney et al., 2017).

The results of monitoring in 2021 in accordance with the State Strategy of Ukraine on reducing the risks of the formation and spread of antimicrobial-resistant strains of microorganisms showed high resistance of *Enterobacteria, Enterococcus* and *Campylobacter* isolated from pigs to antibiotics of various groups. The largest number of antibiotic-resistant commensal and zoonotic bacteria was found in pig farms in Donetsk, Kyiv, Dnipropetrovsk, Kirovohrad and Chernihiv regions (Chechet et al., 2023).

Antimicrobial drugs usually prescribed for pigs in European countries include extended-spectrum penicillins (31.2%), polymyxins (24.7%), tetracyclines (15.3%), macrolides (9.7%), pleuromutilins (4.5%), cephalosporins (4.4%), trimethoprim+sulfanamide (2.4%), lincosamides (2.1%), aminoglycosides and fluoroquinolones (1.3% each), amphenicols (0.4%) (Sarrazin et al., 2019).

Based on the experience of combating factor respiratory diseases of pigs in Ukraine, it has been established that compliance with the basic requirements of biosecurity reduces the incidence to 11.7% from 40%. Bacterial pathogens of respiratory diseases are sensitive to Levofloxacin, Cefotaxime, Ceftiocur, Florfenicol, Tilmicosin, Tiamulin and resistant to penicillin antibiotics, Erythromycin, Tetracycline (Rebenko, 2017).

The frequency of respiratory diseases increased from 11.4% to 23.7% on pig farms of Poltava and Kharkiv regions in 2019–2023. During the study period, the isolation of *Mycoplasmas* and *Pasteurellae* de-creased by 7.2 and 6.3 times, and *E. coli* and *Actinobacillus* increased by 17.7 and 8.0 times, respectively. *Staphylococci, Streptococci, Pseudomonads,* and *Proteus* were not isolated during the last year studies. The highest sensitivity (85%) was found in *S. suis* to cefazolin, doxycycline, enrofloxacin, tetracycline, and oxy-tetracycline. *E. coli* isolates were sensitive to 64%, in particular to ciprofloxacin, doxycycline, and enrofloxa-cin. *P. multocida* isolates were the most resistant, to 48% sensitivity, namely to amoxicillin, enrofloxacin, florfenicol, and danofloxacin (Voytenko et al., 2023).

The antibiotic resistance generation is due to the genetic properties of microorganisms, their acquisition of new genetic information, or due to a change in the level of expression of the bacterial cell's own genes. An important factor in controlling the spread of antibiotic resistance is the justification of dosage regimens of antibacterial drugs and their use against microorganisms. Today, the sensitivity of microorganisms to antibiotics is determined according to the recommendations of the European organization EUCAST. There is a change in the sensitivity of microflora to antibiotics, which is not covered by the screening of multidrug resistant microorganisms. Resistance to antimicrobial drugs determines important relationships in microbial communities and pathogen-animal interaction. For the purpose of monitoring, both diffusion methods with samples of antimicrobial substances and the detection of individual antibiotic resistance genes are used (da Silva et al., 2022, Chemerovska & Rublenko, 2022).

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) recently revised the def-initions of S (susceptible), I (susceptible at increased exposure), and R (resistant) categories for interpreting clinical susceptibility, recognizing the close relationship between drug exposure and susceptibility reporting. An organism is classified as “resistant” if there is a high likelihood of therapeutic failure, even when in-creased exposure to the antimicrobial agent is used (Giske et al., 2022).

The aim of present study was the surveillance of antibiotic susceptibility occurrence in common respiratory pathogens of pigs on the farms of Ukraine.

**Materials and methods**

The study included samples of nasal isolates obtained from 620 animals with signs of respiratory pathology from the farms of Kharkiv, Mykolaiv and Dnipropetrovsk regions of Ukraine, which were received during 2023-2024 at the Department of Bacteriology and Biotechnology Research Center for Biosafety and Environmental Control of Agricultural and Industrial Complex Resources.

To determine the sensitivity of microorganisms to antibiotics, the Kirby-Bauer agar diffusion method was used. For this, according to the generally accepted method, suspensions of daily cultures of microorganisms with a turbidity of 0.5 standard units according to McFarland were prepared, which were determined using a Biosan model DEN-1 densitometer. The resulting suspension was applied to Mueller-Hinton medium (for most microorganisms) and Mueller-Hinton medium with the addition of blood components and other growth supplements for the cultivation of fastidious microorganisms. For the studies, antibiotic discs manufactured by Liofilchem (Italy) and Himedia (India) were used.

The cultures were incubated at 37 0 C ± 2 0 C for 24 ± 2 hours. The diameters of the formed zones of growth inhibition were measured in millimeters. Growth inhibition around the discs is an indicator of the ability of this compound to inhibit bacterial cell proliferation (Hudzicki, 2009).

The prevalence of resistance to antimicrobial substances contained in drugs registered in Ukraine and divided into four categories - A, B, C and D according to the Procedure for the Use of Antimicrobial Drugs - was determined among the identified pathogens. The adopted rules do not allow the administration of category A drugs to productive animals in Ukraine. Therefore, the sensitivity of isolated strains of *A. pleuropneumoniae, P. multocida, S. suis, T. pyogenes* to antimicrobial substances of the following categories was determined:

- D (Amoxicillin, Doxycycline, Oxytetracycline, Spectinomycin, Tetracycline, Trimethoprim/Sulfa);
- C (Amoxicillin+clavulanic acid, Gentamicin, Lincomycin, Tiamulin, Tulathromycin, Fluorophenicol);
- B (Enrofloxacin, Marbofloxacin, Ciprofloxacin) (Poryadok, 2021).

In order to identify differences in the prevalence of resistant strains to individual antibiotics depending on the year of the study, the results were analyzed statistically. Four-field tables were used to calculate the $\chi$ 2 criterion (Pearson's $\chi$-square with Yates correction using the STATISTICA 6.0 program).

**Results**

The spectrum of antibiotic resistance to antimicrobial agents of three categories used in Ukraine was determined in isolate samples with the most common pathogens of respiratory infections in pigs.

A study of the relative abundance of resistant *A. pleuropneumoniae* strains in samples collected in 2023 and 2024 and a comparative analysis of the number of such strains showed significant differences in susceptibility to Ciprofloxacin, Tulathromycin, Spectinomycin, Trimethoprim/Sulfa, and Oxytetracycline (Fig. 1).

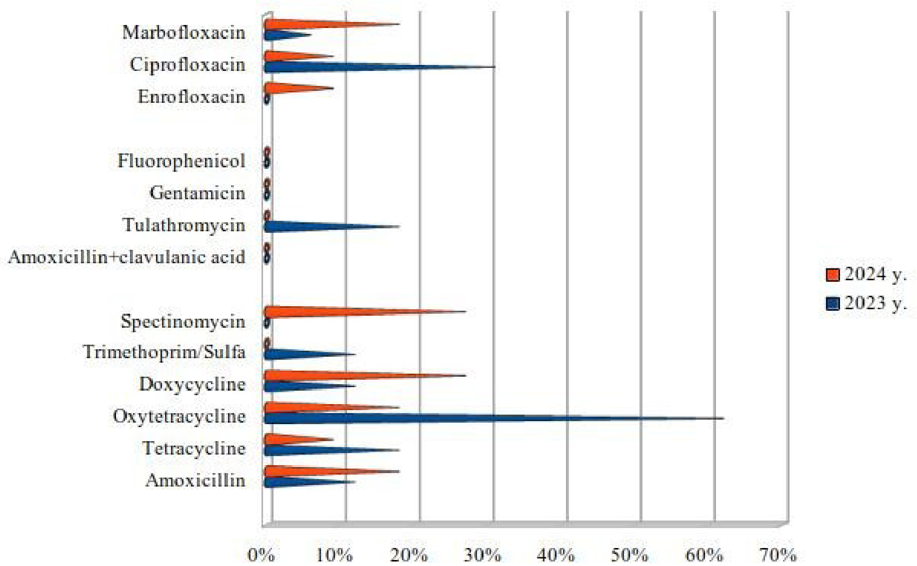


**Fig. 1.** The detected frequency of antibiotic resistant in *A. pleuropneumoniae* (% of total isolates).

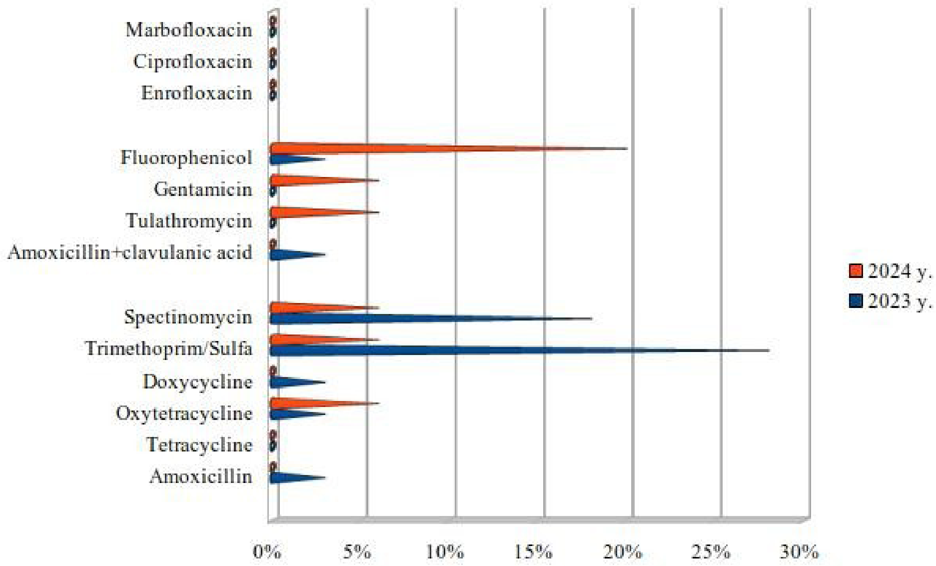


**Fig. 2.** The detected frequency of antibiotic resistant in *P. multocida* (% of total isolates).

In the samples collected in 2024, *A. pleuropneumoniae* showed a decrease in the number of resistant strains to Ciprofloxacin (from 97 to 27 samples, $\chi^2$ =45.38), Tetracycline (from 54 to 27 samples, $\chi^2$ =10.4), Oxytetracycline (from 186 to 54 samples, $\chi^2$ =121), Trimethoprim-Sulfa (from 36 to 1 sample, $\chi^2$ =36.2), Tulathromycin (from 97 to 27 samples, $\chi^2$ =54.12), but an increase to Amoxicillin (from 36 to 54 samples, $\chi^2$ =4.24), Enrofloxacin (from 1 to 27 samples, $\chi^2$ =23.41), Marbofloxacin (from 18 to 54 samples, $\chi^2$ =20.45), Doxycycline (from 36 to 81 samples, $\chi^2$ =21.5), Spectinomycin (from 1 to 81 samples, $\chi^2$ =88.15).

If in 2023, among antimicrobial drugs of category D, resistance was most often found to Oxytetracycline, and least to Spectinomycin, then in 2024, strains were most sensitive to the combination of Trimethoprim/Sulfanamide. To drugs of category C (combinations of Amoxicillin+clavulanic acid, Gentamicin and Fluorophene), sensitivity was registered in 100% of isolates. As for drugs of category B, it should be noted that more frequent cases of resistance to Enrofloxacin and Marbofloxacin were found among *A. pleuropneumoniae* isolates.

The identified features of the antibiotic resistance spectrum in *P. multocida* strains also demonstrated differences between the studied periods (according to Fig. 2).

Comparative analysis of the results obtained for the periods of 2023 and 2024 showed significant differences in the number of cases of detection of resistant strains of *P. multocida* to Amoxicillin (from 9 to 1 samples, $\chi^2$ =7.22), Doxycycline (from 9 to 1 samples, $\chi^2$ =7.22), Spectinomycin (from 54 to 18 samples, $\chi^2$ =20.45), Trimethoprim-Sulfa (from 84 to 18 samples, $\chi^2$ =51.45), but an increase in Gentamicin (from 1 to 18 samples, $\chi^2$ =16.56), Florfenicol (from 9 to 60 samples, $\chi^2$ =42.59), Tulathromycin (from 1 to 18 samples, $\chi^2$ =16.56).

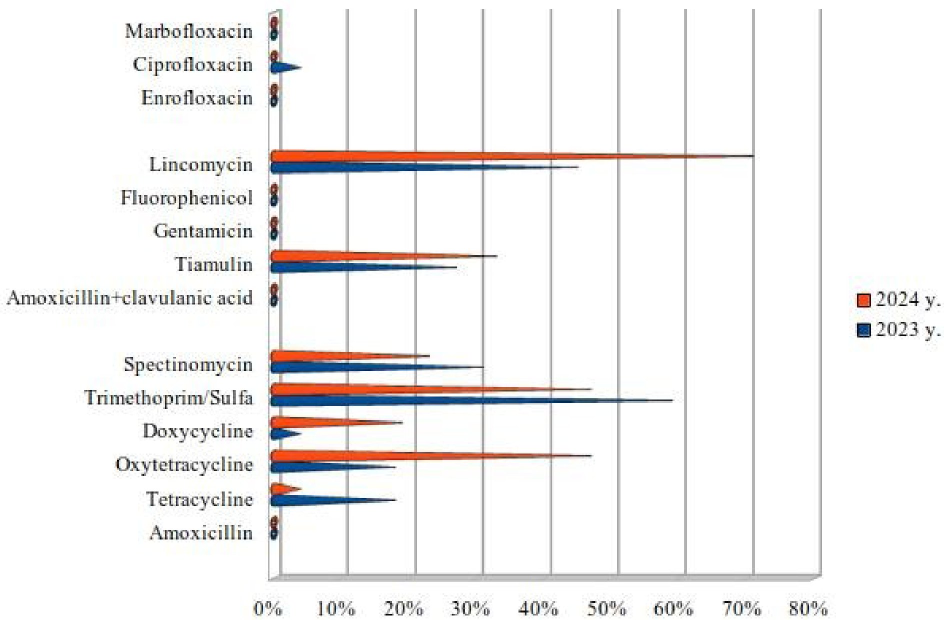


**Fig. 3.** The detected frequency of antibiotic resistant in *S. suis* (% of total isolates)

Among antimicrobial drugs of category D, resistance was most often detected in *P. multocida* to the combination of Trimethoprim/Sulfanilamide and least often – to Tetracycline, from category C most often to Fluorophenicol and least often – to the combination of Amoxicillin+clavulanic acid. As for category B, resistance to all three fluoroquinolones was not detected among *P. multocida* isolates.

The identified features of the antibiotic resistance spectrum in S. suis strains also demonstrated differences between the number of such strains in samples taken in 2023 and 2024 (Fig. 3).

Comparative analysis of samples taken in 2023 and 2024 showed different changes in the representation of antibiotic-resistant strains. In particular, the number of resistant S. suis strains to Doxycycline decreased (from 51 to 27 samples, $\chi$ 2 =8.49), but increased to Gentamicin (from 21 to 66 samples, $\chi$ 2 =27.22), Enrofloxacin (from 1 to 18 samples, $\chi$ 2 =16.56), Marbofloxacin (from 3 to 18 samples, $\chi$ 2 =9.67), Ciprofloxacin (from 3 to 8 samples, $\chi$ 2 =9.67), Oxytetracycline (from 147 to 183 samples, $\chi$ 2 =8.73), Trimethoprim-Sulfa (from 72 to 141 samples, $\chi$ 2 =34.65).

Among antimicrobial drugs of category D, *S. suis* isolates were most often resistant to Oxytetracycline, and were 100% sensitive to Amoxicillin, from category C, 100% were sensitive to the combination Amoxicillin + clavulanic acid, and 86% were resistant to Lincomycin. Regarding drugs of category B (Enrofloxacin, Marbofloxacin and Ciprofloxacin), up to 6% of *S. suis* isolates were found to be resistant to them in 2024.

The identified features of the antibiotic resistance spectrum in *T. pyogenes* strains were primarily related to high sensitivity to Amoxicillin and relatively low sensitivity to Lincomycin (Fig. 4).

The number of resistant strains of *T. pyogenes* decreased to Ciprofloxacin (from 12 to 1 samples, $\chi$ 2 =10.29), Tetracycline (from 54 to 12 samples, $\chi$ 2 =30.03), Spectinomycin (from 93 to 69 samples, $\chi$ 2 =4.87), Trimethoprim-Sulfa (from 177 to 141 samples, $\chi$ 2 =8.67), but increased to Doxycycline (from 12 to 57 samples, $\chi$ 2 =33.16), Oxytetracycline (from 54 to 141 samples, $\chi$ 2 =57.50), Lincomycin (from 135 to 213 samples, $\chi$ 2 =41.63).

Among antimicrobial drugs of category D, *T. pyogenes* isolates were most often resistant to the combination of Trimethoprim/Sulfanamide, and 100% were sensitive to Amoxicillin; from category C, 100% were sensitive to the combination of Amoxicillin+Clavulanic acid, Fluorophene, and Gentamicin, and 71% were resistant to Lincomycin, and 33% were resistant to Tiamulin. As for category B, sensitivity decreased only to Ciprofloxacin.

In general, the obtained results of the study showed the peculiarities of the spectrum of antibiotic resistance in each of the studied pathogens of respiratory complications in pigs.

**Discussion**

The study presents data on the sensitivity of pathogenic bacteria to antibiotics from the composition of antimicrobial drugs, divided into categories according to the current recommendations. The results obtained are important for the optimal selection of treatment regimens for respiratory infections in pigs. They will also prevent the emergence of antibiotic resistance. Available literature data show that resistance levels are a dynamic parameter, and therefore constant monitoring is necessary to adjust treatment regimens.

The results of resistance monitoring of S. suis strains (n=448) from pigs in Denmark during 2004-2017 showed a high level of resistance to Tetracycline (72.2-79.5% among isolates), a gradual increase in resistance to Trimethoprim-Sulfate (from 0% to 8.6%) and a decrease in resistance to Ciprofloxacin (from 4.9% to 1.8%), also, a slight level of resistance to Florfenicol (0% - 0.9%). Among *A. pleuropneumoniae* isolates, less than 1% were resistant to Ciprofloxacin, Florfenicol, Tiamulin and Tulathromycin, and 4% - 7.6% to Tetracycline (Holmer et al., 2019).

The effectiveness of antibiotic therapy in the treatment of respiratory diseases of bacterial etiology depends on the spectrum of action of the antimicrobial drug and the level of sensitivity of the pathogen or pathogens to its action. From the biological material for bronchopneumonia of weaned piglets in Lviv region, strains of *Enterococcus faecalis, S. suis* and *E. coli* were isolated. According to the level of minimum inhibitory concentration (MIC) of Amoxicillin, 81.25% of *Enterococcus* isolates and all isolated strains of Streptococcus and E. coli were either sensitive, or moderately sensitive. Determination of antibiotic sensitivity of microflora isolated from piglets with bronchopneumonia revealed that most isolates were resistant to Lincomycin (83.3%), Benzylpenicillin (56.7%), Oxytetracycline (53.3%), and most sensitive to Ceftiofur

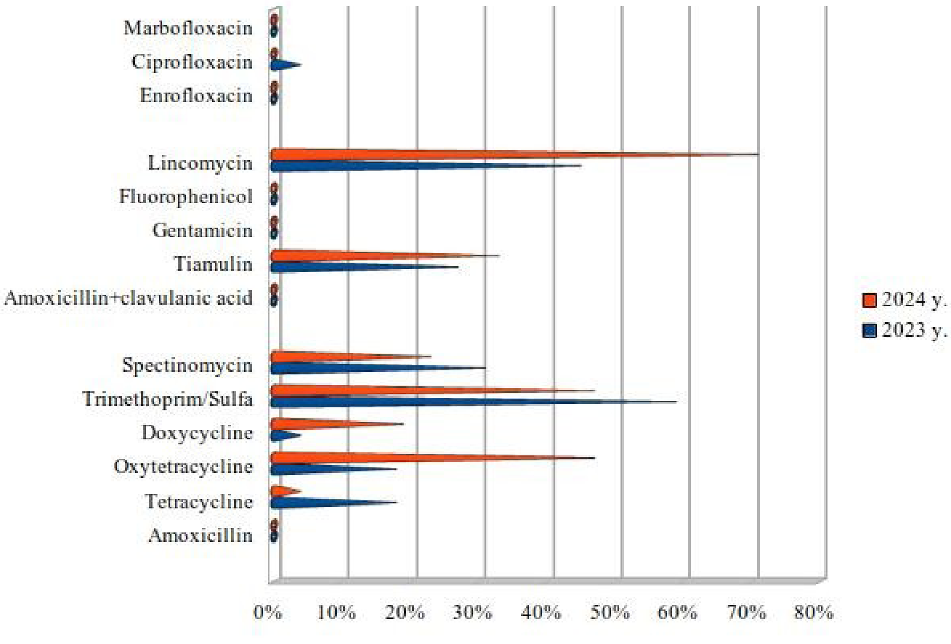


**Fig. 4.** The detected frequency of antibiotic resistant in *T. pyogenes* (% of total isolates)

(3.3%), Enrofloxacin and Gentamicin (6.7% each), Doxycycline and Streptomycin (16.7% each). The bacterial strains including *S. suis, P. multocida, B. bronchiseptica, E. coli* and *S. aureus* were isolated and identified from the nasal secretions of piglets with bronchopneumonia. The most sensitive to Fluorphenicol were *B. bronchiseptica* (all isolated strains of bacteria were sensitive - 88.2% or moderately sensitive - 11.8%) and *E. coli* (6 isolates - sensitive, 2 - moderately sensitive). Other pathogens (*S. suis, P. multocida*), causative agents of bronchopneumonia in piglets, had a high degree of sensitivity (about 75%). Half of the *S. aureus* isolates (50%) were resistant to Fluorphenicol (Stetsko et al., 2024).

*A. pleuropneumoniae* is an important respiratory pathogen of porcine pleuropneumonia (PPP). Several pathogenic members of the family Pasteurellaceae are detected into animal of all aging groups. However, the invasion of these pathogens is often observed in lungs and pleura while this infectious process is accompanied by multiple complications including pericarditis, pharyngitis, and polyarthritis.

The susceptibility of isolates, taken from the lungs of pigs with pneumonia in UK, to Ampicillin, Enrofloxacin, Erythromycin, Fluorophenicol, Sulfisoxazole, Tetracycline, Tilmicosin, Trimethoprim was determined using the disk diffusion method and minimum inhibitory concentration (MIC) analysis. Of the isolates tested, 57% were resistant to Tetracycline (MIC ≥ 4 mg/L), 20% to Ampicillin (MIC ≥ 4 mg/L), 17% to Trimethoprim (MIC ≥ 32 mg/L) and 6% to Enrofloxacin (MIC ≥ 0.25 mg/L). Only 33% of the isolates were susceptible to all antimicrobial agents tested (Bossé et al., 2017).

*A. pleuropneumoniae* isolates were found to be 100% susceptible to Ceftiofur and Fluorophenicol, over 90% to Enrofloxacin and Tulathromycin, and 0% to 6% of strains to Tetracycline in the USA and Canada (Sweeney et al., 2017).

In our studies, the highest resistance was found in A. pleuropneumoniae to tetracyclines – in 10-60% of isolates. However, no resistant strains were found to Gentamicin, Fluorophenicol, and the combination of Amoxicillin and clavulanic acid.

*P. multocida*, together with Mannheimia haemolytica (formerly *P. haemolytica*), causes pasteurellosis, which is characterized by septicemia, inflammation of the respiratory tract, or digestive tract. It is a normal inhabitant of the nasal passages of most pigs, but is often associated with viral respiratory diseases. *P. multocida* isolates were 100% susceptible to Ceftiofur, Enrofloxacin and Fluorophenecil. High susceptibility rates (over 90%) were observed to Ampicillin, Penicillin, Tilmicosin and Tulathromycin, and to Tetracycline from 22.3% to 35.3% in the USA and Canada (Sweeney et al., 2017).

In pig farms of Kharkiv, Sumy, Kherson and Odessa regions, the occurrence of gastroenteritis in suckling piglets and respiratory pathology in animals of older age groups is caused by associations of bacteria with a diverse species composition, among which P. multocida is the dominant species. *Neisseria spp., P. haemolytica, M. hyopneumoniae* were also identified (Kolchyk, 2018).

*P. multocida* isolates were not found to have resistance to fluoroquinolones, but to tetracyclines resistance was registered in 5% of isolates, to Fluorophenicol in 20%, and to the combination of Trimethoprim and Sulfanilamide in 25%.

*S. suis* is a zoonotic disease that can be transmitted to humans from pigs. It is localized in the vagina of sows, so piglets are infected at birth, and infections occur through skin wounds in suckling and weaned piglets. High susceptibility rates (over 90%) in *S. suis* to Ampicillin, Ceftiofur, and Fluorophenecil have been identified in pigs on farms in the USA and Canada (Sweeney et al., 2017). At the same time, susceptibility to Tetracycline was found in only 1.3% of isolates.

Antimicrobial resistance to 22 antibiotics was studied for 200 *S. suis* strains collected from different geographical regions of France. The majority of strains (86%) showed resistance to at least one antibiotic with low levels of resistance to fluoroquinolones, penicillins, pleuromutilins and diaminopyrimidine sulfonamides and higher levels to macrolide-lincosamides and tetracyclines. Multidrug resistance was observed in 138 strains. Statistical analysis showed a lower resistance to Trimethoprim-Sulphamethoxazole and a higher level of resistance to macrolide-lincosamides and penicillin in Brittany than in other regions of France. The studies demonstrate the need to monitor antimicrobial resistance of this zoonotic pathogen (Dechêne-Tempier et al., 2023).

Isolates of *S. suis* isolated from sick pigs of farms in Kyiv, Zhytomyr, Cherkasy, Vinnytsia regions of Ukraine. 73.4% of diseases are caused by associated pathogens, and *Streptococcus* as a monoinfection only in 26.5% of cases. During 2017-2021, *S. suis* resistance to Penicillin (2.9%), Amoxicillin (0%), Ceftriaxone (14.7%), Cephalexin (55.9%), Erythromycin (88.2%), Clindamycin (76.5%), Enrofloxacin (20.6%), Ciprofloxacin (8.8%), Tetracycline and Doxycycline (14.7% each), Gentamicin (44.1%) was detected (Savcheniuk et al., 2022).

In *S. suis* isolates isolated taken in pig farms of Kyiv, Cherkasy, Vinnytsia, Chernihiv and Poltava regions during 2017–2021 resistance was detected to Penicillin (5.3%), Amoxicillin (4.4%), Ceftriaxone (3.5%), Cephalexin (4.4%), Erythromycin (73.6%), Clindamycin (80.7%), Enrofloxacin (46.5%), Ciprofloxacin (34.2%), Tetracycline (76.3%), Doxycycline (74.6%) and Gentamicin (29.8%). During the studied period, resistance increased to beta-lactams, did not change to fluoroquinolones, Gentamicin, Erythromycin, Clindamycin, Doxycycline, Tetracycline (Tarasov et al., 2024). According to our results, *S. suis* strains had the highest resistance to Lincomycin (over 80%) and Oxytetracycline (50%), but retained sensitivity to Amoxicillin. Strains that acquired resistance to fluoroquinolones (up to 5%) were also identified.

Intensification of pig farming contributes to the manifestation of endogenous and exogenous factors that can affect animal health. Disruption of immune homeostasis is the main cause of infections caused by opportunistic microorganisms. One of these is *T. pyogenes*, which is widespread on the skin and mucous membranes, therefore causing purulent infections in pigs and other animal species. The localization or generalization of the infection depends on the immunity of the animals, their individual susceptibility and environmental factors. The causative agent *T. pyogenes* is identified as sensitive to beta-lactams and sulfonamides (Jarosz et al., 2014).

*T. pyogenes* is a causative agent of pneumonia and abscesses in pigs in Brazil, but its importance has not yet been proven, probably due to the difficulty of isolation and identification of the pathogen. All strains were susceptible to beta-lactams, Fluorophenicol, Gentamicin, Spectinomycin and Tiamulin. The highest resistance levels were observed to sulfonamides, tetracyclines and Clindamycin (Moreno et al., 2017).

*T. pyogenes* isolates from animals in Brazil were resistant to Amoxicillin (3.6%), Ampicillin (7.4%), Cephalexin (3.3%), Cefoperazone (2%), Ceftiofur (3.9%), Ciprofloxacin (2.2%), Enrofloxacin (3.6%), Fluorophene (0%), Benzylpenicillin (6.3%), Gentamicin (5.9%), Norfloxacin (10.9%), Rifampicin (4.2%), Trimethoprim-Sulphamethoxazole (49.3%) and Tetracycline (9.2%) (Ribeiro et al., 2015).

A total of 180 isolates of *T. pyogenes* obtained from slaughtered pigs raised under intensive and extensive technology were investigated for differences in antibiotic susceptibility. Low MIC90 values were obtained for Penicillin and Amoxicillin (0.008 and 0.06 μg/ml, respectively), Ceftiofur, Gentamicin and Enrofloxacin (1 μg/ml, respectively). Under intensive pig farming technology, MIC90 increases, in particular for Neomycin and Streptomycin (32 μg/ml vs. 8 μg/ml), Trimethoprim/Sulphamethoxazole (30.4/1.6 μg/ml vs. 1.90/0.10 μg/ml). These data suggest that antimicrobial susceptibility of T. pyogenes can be influenced by the herd management systems (Galán-Relaño et al., 2019).

In Japan, antimicrobial susceptibility of *T. pyogenes* isolates from pigs diagnosed with septicemia at slaughter was investigated. Strains were found to be susceptible to Ampicillin, Cefazolin, Gentamicin, and Levofloxacin. However, 70.1% of the strains were resistant to Tetracycline, 62.7% to Streptomycin, 20.9% to Erythromycin, 19.4% to Clindamycin, and 1.5% to Oxacillin and Kanamycin. No statistically significant changes in resistance were found during the observation period from 2006 to 2017 (Fujimoto et al., 2023).

In China, *T. pyogenes* was detected in samples isolated from pigs with respiratory clinical symptoms. Antibiotic susceptibility testing showed that all isolates were susceptible to fluoroquinolones and penicillins, and high levels of resistance were found to Gentamicin (77.8%), Amikacin (74.1%), Erythromycin (85.2%), and Azithromycin (85.2%) (Dong et al., 2019). According to our data, *T. pyogenes* isolates are sensitive to Amoxicillin, Gentamicin, Fluorophenicol and fluoroquinolones, and more than 50% of isolates were resistant to Lincomycin and the combination of Trimethoprim with Sulfanilamide. The obtained data partially differ from the literature, which is due to the duration of use of individual drugs in pig farming in different countries.

## Conclusion

The differences in the spectrum of resistance to antimicrobial drugs were identified among strains of bacteria *A. pleuropneumoniae, P. multocida, S. suis, T. pyogenes* on the pig farms of Ukraine during 2023 - 2024. The most effective category D antimicrobials, which are allowed to be used to treat animals on the first day of illness, are drugs with Amoxicillin. However, resistance to Amoxicillin in *A. pleuropneumoniae* strains increased from 12% in 2023 to 18% in 2024. In 2024, fluoroquinolone-resistant strains of A. pleuropneumoniae were detected in 9-18% of samples, and of *S. suis* in 6%.

The monitoring of antibiotic resistant strains dissemination could be fruitful to select the most effective antimicrobial treatment regimen for respiratory pathology in pigs and can contribute the progress into "One Health" concept implementation in Ukraine.

## Conflict of interest

The authors declare no conflict of interests.

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