The Comparative Characteristics of *Actinobacillus pleuropneumonia* Strains Resistance and Local Epidemiology State in Ukraine

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Abstract. *Various serotypes of Actinobacillus pleuropneumonia are the causative agents of porcine pleuropneumonia and lead to significant economic loss in the whole world. Effective antibiotic therapy requires data on individual serotype resistance and its coinfection variants. The aim of the present study was to disclose A. pleuropneumonia epidemiology of swine farming in Ukraine during 2022–2024. The isolates from 16 provinces of Ukraine were analyzed with RT-PCR and microbial culture techniques to identify the serotype and its antibiotic resistance. The co-occurrence of four and five serotypes was observed only in two provinces. Besides, the infections caused by one serotype were observed in 79% cases, while coinfection cases of two and three A. pleuropneumoniae serotypes were detected in 21% of all studied regions. Totally, 12 different serotypes were identified in all locations. Serotypes 8 and 2 were most prevailing and together accounted for 51% of all cases. The results on antibiotic resistance demonstrated significant diversity with respect to various antibiotic classes. However, isolated A. pleuropneumoniae strains exhibited high sensitivity to both β-lactams and quinolones while the highest resistance was detected to macrolides and lincosamides. In spite of a large spread of both serotypes 8 and 2, serotype 2 resistance to tetracyclines was significantly low – 2.8%. In contrast, serotype 8 resistance to tetracyclines made up 68.5%. Besides, contrasting sensitivity to tetracycline was detected for serotypes 8 and 2 at 13.9% and 80.6%, respectively. Obtained data showed that most isolated strains were sensitive to cephalosporines, fluoroquinolones, and β-lactams while these strains exhibited high resistance to macrolides and lincosamides. In addition, intermediate resistance was detected to tetracyclines. Together, observed results evidence that multi-resistant A. pleuropneumoniae strains to macrolides, lincosamides, and tetracyclines could be generated in Ukraine in the recent years.*

Introduction

Respiratory diseases of infectious etiology in pigs are the actual problem in the world (Przyborowska-Zhalniarovich et al., 2021; de Almeida et al., 2025; Kwan et al., 2025). Besides, recent reports evidence the rising risk of swine respiratory infection on the farms in Ukraine (Derevyanko and Ayshpur, 2023; Garkavenko et al., 2024). *Actinobacillus pleuropneumoniae* is detected in almost 50% of pneumonia cases observed during the fattening pig period (Kokariev et al., 2023). This microorganism causes highly contagious disease named actinobacillus pleuropneumonia (APP) or porcine pleuropneumonia (PPP). APP provokes significant economic costs to pig farms due to high mortality, decrease in productivity, and significant expenses for the infection treatment and preventive measures (Stringer et al., 2022; Malcher et al., 2024; Sjölund et al., 2025). Nowadays, 19 serotypes of *A. pleuropneumoniae* are described in literature and identified as the strains which vary in virulence and spreading capacity in different countries (Arnal Bernal et al., 2024; Seakamela et al., 2024).

One strategy to reduce the incidence of APP on swine farms includes both local therapeutic measures using antibacterial drugs and total anti-epizootic measures, based on vaccination of pigs against APP (Sjölund et al., 2025; Loera-Muro and Angulo, 2018; Deng et al., 2025; Hyun Park et al., 2025). An efficient antibiotic therapy, which depends directly on the sensitivity of *A. pleuropneumoniae* to antibacterial drugs, is to eliminate local manifestations of APP (Blondeau and Fitch, 2024; Brenciani et al., 2024). Special features are reported in relation to the fact that *A. pleuropneumoniae* strains exhibit the potential to acquire resistance against antibiotics and can generate multi-resistant strains. Multi-resistance is considered as a factor which can extremely complicate the process of treatment and recovery of the herd in the near years (Somogyi et al., 2023; Paulina and Dawid, 2025). The most effective APP monitoring method in the herd is the immunization with homologous vaccines (Xie et al., 2017; Thu Dao et al., 2020). It deserves to be mentioned that within the herd there can be several simultaneously circulating APP strains, which significantly complicates monitoring of the disease (Cuccato et al., 2014).

Currently, the results on APP monitoring and the identification of *A. pleuropneumoniae* have been reported in relation to commercial swine herds in Ukraine (Kokariev et al., 2023). Besides, the data on

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the sensitivity to antibiotics have been published in a recent report (Neverkovets et al., 2025). However, there are no reports with respect to genetic variability of *A. pleuropneumoniae* within different regions of Ukraine. Apart from this, the features of sensitivity and resistance of widespread serotypes to different antibacterial compounds are not considered. In this regard, the study of *A. pleuropneumoniae* genetic biodiversity, resistance, and coinfection variances is both novel and timely in Ukraine as well as other countries.

The aim of the study was to find out *A. pleuropneumonia* epidemiology of swine farming in Ukraine, assess coinfection variances, and analyze the resistance level with respect to various antibiotic classes among isolated serotypes.

Material and methods

General information

The laboratory diagnostic tests and data analysis were carried out during 2022–2024 at the Biosafety-Center R&D Department of Dnipro State Agrarian and Economic University (Dnipro, Ukraine). The samples of tissues were collected from pigs from 33 farms located in 16 different provinces of Ukraine. In total, 177 samples for PCR and 154 samples for culture microbiology were selected. Almost all the farms involved in the present study were assessed as APP-positive enterprises.

Ethical considerations

The present research was carried out in accordance with the framework of the "General Ethical Principles of Animal Experiments", which have been approved by the National Congress of Bioethics held in Kyiv (2001), and in line with the provisions of the European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes (ETS No. 123; https://www.coe.int/en/web/cdcj/laboratory-animals). The experimental protocols on the animal sampling were approved by the Local Ethics Committee for Animal Experiments of Dnipro State Agrarian and Economic University (DSAEU), Ukraine (Protocol No. 07-032022).

Sample collection

The fragments of lungs with visible pathological-anatomical signs of APP complications were isolated from the pigs that died across different herds (5–7 samples individually were separated from each herd). Lung tissue samples were immediately cooled and then transported to the laboratory within 8 hours of collection.

RT-PCR analysis

Each sample of the pulmonary tissue from foci of injury was homogenized individually using FastPrep-24 device. Extraction of nucleic acids (NA) from homogenates was carried out using the "BioExtract Premium Mag" reagent kit and the automated NA extraction "KingFisher Duo" Prime purification system (Thermo Fisher Scientific, USA).

Genome specificity of *A. pleuropneumoniae* serotypes was identified via polymerase chain reaction (RT-PCR) and its genotyping carried out using 19 serovars in NA samples immediately after extraction. All RT-PCR analyses were carried out using commercial tests «EXOPOL» (Spain). The amplification and detection of results was performed using "CFX 96" Touch RT-PCR Detection System (BioRad, USA). Extraction of NA and PCR was carried out according to the recommendations of test-kit producer.

Microbiological culture analysis

Identification of *Actinobacillus pleuropneumoniae* was carried out using the bacteriological culture method by seeding microorganisms into a dense growth medium containing blood and NAD^+ (BioMerieux, France). Primary cultures were incubated at a temperature of $37^0 \pm 2^0$ C in the 5% CO_2 atmosphere for 24 hours (Jorgensen & Turnidge, 2015). The identification of microorganisms was carried out using MALDI-TOF technology. In total, 154 *A. pleuropneumoniae* containing samples were identified and separated by the culture method.

The antibiotic sensitivity of the isolated APP strains was detected using the disc-diffusion method according to Kirby-Bauer. Commercial antibiotic-containing discs were applied to assess both resistance and sensitivity to various antibiotics belonging to β-lactams, fluoroquinolones, aminoglycosides, tetracyclines, macrolides, lincosamides, pleuromutilins, sulfonamides, and amphenicols (Bioanalyse, Turkey; Liofilchem, Italy; Himedia, India). The measurement of zone diameters after incubation and disc application was carried out automatically using SCAN 500 device (Interscience, France).

Statistical analysis

Statistical analysis of the obtained data on the serotype ratio, sensibility and resistance of isolated *A. pleuropneumoniae* strains to various antibiotics was carried out using one-way analysis of variance (ANOVA) with StatView 5.0 (SAS Institute Inc., USA). Observed results were expressed as mean ± standard error of mean (M ± SEM). Statistical comparisons were performed after verification for the normality distribution and the difference between the general variances. The graphs were created using GraphPad Prism 9 software (GraphPad Software, USA). *P* values less than 0.05 were considered statistically significant.

Results

The obtained results showed genetic diversity of *A. pleuropneumoniae* serotypes identified in the samples selected from big farms in Ukraine. In addition, the APP identification results demonstrated significant differences in the spread of separate serotypes as well as variability in terms of the levels of resistance and sensitivity of *A. pleuropneumoniae* to antibacterial drugs.

Epidemiological analysis

The results of an epidemiological study showed the complexities of regional peculiarities in terms of *A. pleuropneumoniae* serotype allocation as well as the coinfection variances at least in 16 regions of Ukraine (Fig. 1).

The obtained results undoubtedly evidence that different *A. pleuropneumoniae* serotypes circulated among farming pigs in all provinces of Ukraine that were included into the present study. Only four provinces were found to have a single APP strain presence. On the whole, 12 different serotypes of *A. pleuropneumoniae,* namely, 1, 2, 3, 5, 6, 7, 8, 9/11, 12, 13, 17, 18, were identified (Fig. 2).

Serotypes 2 and 8 of *A. pleuropneumoniae* were detected as the most common among farming pigs in different Ukraine regions. Together these serotypes were identified in pig herds located in 8 regions included in the study. Serotypes 1, 7, 9/11, and 18 were less spread, and registered in 3–4 regions of Ukraine. Serotypes 3, 5, 6, 12, 13, and 17 were identified in 1–2 regions of the country.

Unlike aforementioned serotypes detected in Ukraine, *A. pleuropneumoniae* serotypes 4, 10, 14, 15, and 16 reported in other countries were not even once observed.

The highest diversity in terms of *A. pleuro-*

Fig. 1. The allocation of *A. pleuropneumoniae* strains in Ukraine provinces identified in 2022–2024. Numbers represent specific serotypes. Numbers within a region indicate *A. pleuropneumoniae* serotypes identified in herds located in that corresponding area.
Note: the regions where APP genotyping assay was not carried out are marked in grey.

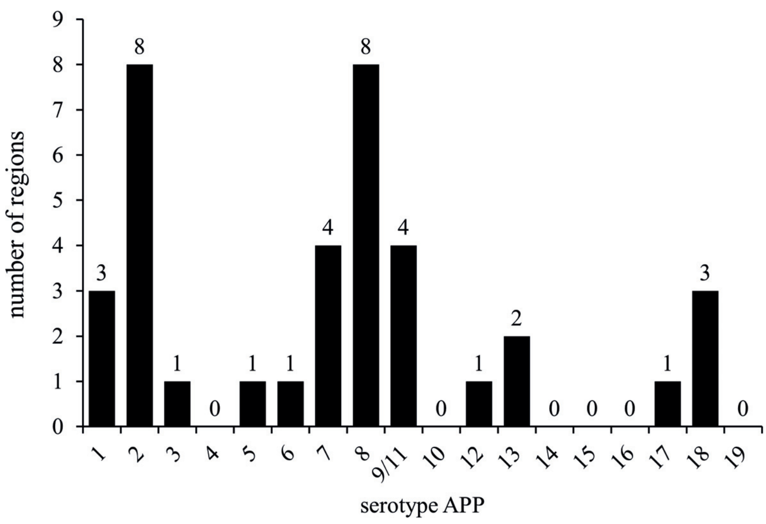

Fig. 2. The number of Ukraine regions in which several *A. pleuropneumoniae* serotypes were identified as present or absent.
Note: absence of a corresponding serotype is marked as “0”.

pneumoniae serotypes was observed in Dnipropetrovsk and Odessa regions. Particularly, only in Dnipropetrovsk province, five serotypes were identified in colocation. The presence of four serotypes together was observed in Odessa province. Serotype 2 and 8 collocations were identified in these regions. However, in Dnipropetrovsk province, serotypes 1, 5, and 9/11 were observed, while serotypes 7 and 18 were detected in Odessa province (Fig. 3).

The coinfection cases of 2 or 3 different serotypes of *A. pleuropneumoniae* were observed in 63% of the investigated regions in Ukraine. The presence of only one serotype was identified in 25% of all provinces included in the study.

The results on *A. pleuropneumoniae* strains ratio demonstrated the prevailing spread of serotypes 2 and 8 in all provinces as well as among all identified strains. Together, these two serotypes represented 51% of the total APP-positive isolates identified in the study (Fig. 4).

Serotypes 9/11, 7, 1, 18, and 13 taken together made up 39% of all identified strains in the present study and were observed as less spread *A. pleuropneumoniae* strains. Other serotypes, including 3, 5, 6, 12, and 17, were characterized as a restricted spread group of *A. pleuropneumoniae* strains in Ukraine. The total of aforementioned strains did not exceed 10% of all identified cases.

The analysis of coinfection cases demonstrated that mono-infection was prevailing in comparison to the sum of all other coinfection variances. Cases involving the identification of a single APP serotype within a herd accounted for 79% of all observed cases (Fig. 5).

The results evidence that the predominant finding among identified *A. pleuropneumoniae* cases was the presence of a single serotype, with mono-infection cases accounting for 79% of all herds involved in the study. The presence of two or three serotypes was observed in 21% of all herds.

The coinfection number accompanied by serotypes 2 and 8 involved in disease initiation accounted for 43% of all identified coinfection cases. In common, obtained results evidence that 12 serotypes circulated in Ukraine during 2022–2024 while serotypes 2 and 8 were prevailing among spread isolates. Other known serotypes, including 4, 10, 14, 15, and 16, were not observed in pig farming in Ukraine.

Sensitivity and resistance of A. pleuropneumoniae serotypes to antibiotics

The study of sensitivity of *A. pleuropneumoniae* strains to various antibiotic groups demonstrated circulation of several serotypes which exhibited resistance to three or more groups of antibiotics. The number of aforementioned strains was equal to about 32% of the total serotype number detected in Ukraine.

The highest degree of sensitivity to antibiotics of both β-lactams and quinolones group were detected

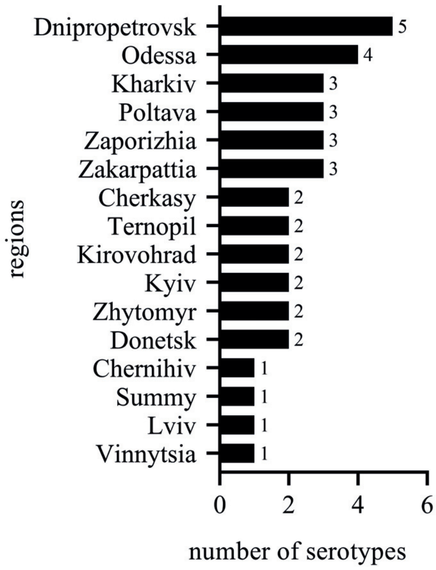

Fig. 3. Diversity rate of *A. pleuropneumoniae* serotypes in different regions of Ukraine
Note: the number of collocated serotypes is depicted on the right side of a corresponding column.

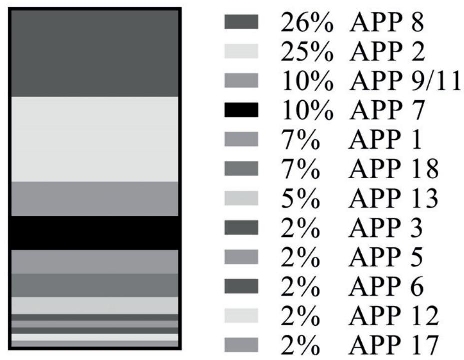

Fig. 4. Total ratio of detected *A. pleuropneumoniae* serotypes

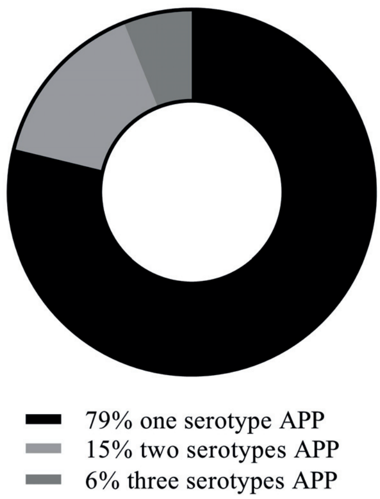

Fig. 5. The percentage of mono-infection and coinfections of *A. pleuropneumoniae* observed by the number of serotypes

in *A. pleuropneumoniae* strains with a similar level (Figs. 6–7). The total number of sensitive isolates to β-lactams was 88.3 ± 5.27%, and to quinolones – 83.9 ± 2.69%.

The results of microbiological culture study of isolated *A. pleuropneumoniae* serotypes showed a relatively high sensitivity and a complete absence of resistance to antibacterial drugs of β-lactams in the serotype 2 strains. Sensitivity of serotype 8 strains and other serotypes to β-lactams did not significantly differ, ranging within 86–87%. The number of *A. pleuropneumoniae* serotype 8 resistant strains and other serotypes was equal to 7.41 % and 11.11%, accordingly.

The analysis of sensitivity to quinolones showed insignificant differences between various *A. pleuropneumoniae* serotypes where the sensitivity level was within 83–89 %. However, among the *A. pleuropneumoniae* serotype 8 isolates, the frequency of resistant forms was twice as high as that observed in serotype 2 as well as other related serotypes.

The results on *A. pleuropneumoniae* strain sensitivity to aminoglycosides demonstrated significantly lower sensitivity levels in comparison with quinolone exposure (Fig. 8).

The maximal resistance level was observed for serotype 8 that accounted for about 30% of all isolated strains. Relatively low resistance levels were demonstrated by serotype 2 and other serotypes that exhibited resistance within the range of 5–14% (Fig. 8). In spite of the presence of resistant forms among serotype 2 strains in respect to aminoglycosides, the level of sensitivity to this group was equal to almost 90%. The obtained results showed that isolated *A. pleuropneumoniae* serotype 2 strains possessed the highest sensitivity to aminoglycosides in comparison with other serotypes. Serotype 8 as well as other serotypes demonstrated significantly lower levels of sensitivity ranging within 63–68%.

The most substantial differences in both sensitivity

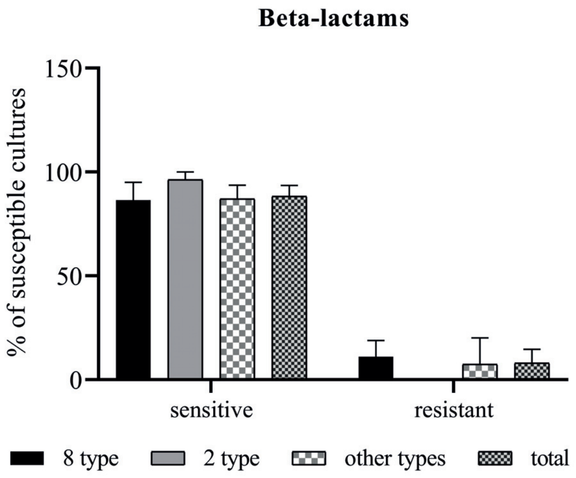

Fig. 6. Sensitivity and resistance of *A. pleuropneumoniae* strains to β-lactams

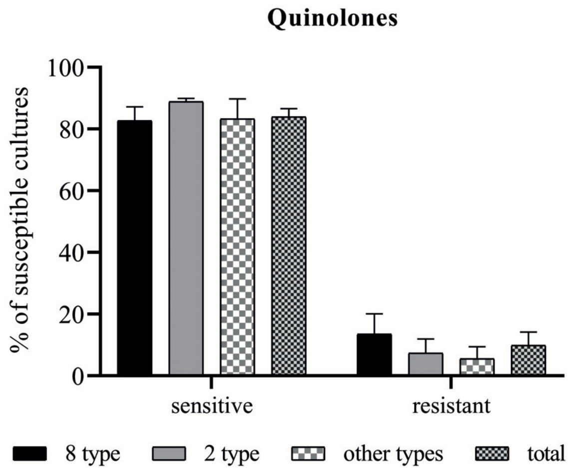

Fig. 7. Sensitivity and resistance of *A. pleuropneumoniae* strains to quinolones

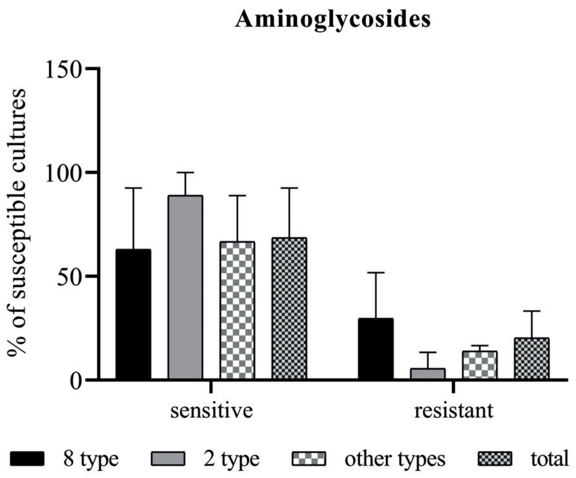

Fig. 8. Sensitivity and resistance of isolates of *A. pleuropneumoniae* to aminoglycosides

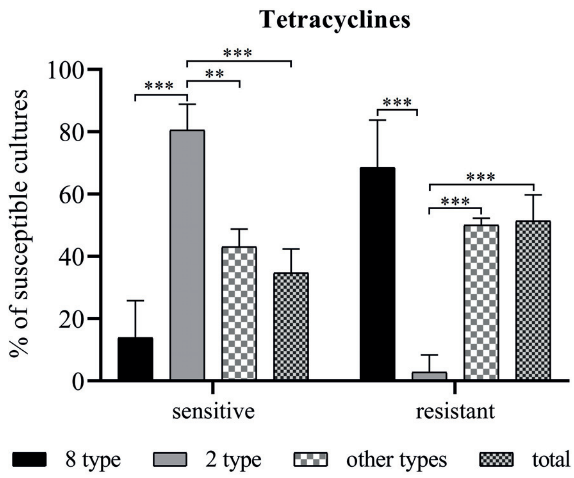

Fig. 9. Sensitivity and resistance of isolates of *A. pleuropneumoniae* to tetracyclines. Significance of differences in both sensitivity and resistance of serotype 2 in comparison with serotype 8, other, and total groups: ** – $P < 0.01$, *** – $P < 0.001$

and resistance were detected between isolated *A. pleuropneumoniae* serotypes 2 and 8 when exposed to the tetracyclines group (Fig. 9).

The observed data showed reciprocal differences in sensitivity and resistance to tetracyclines where serotype 2 exhibited the lowest sensitivity and maximal resistance. In contrast, serotype 8 demonstrated the lowest resistance potential and maximal sensitivity to tetracyclines treatment. The mean percentage of resistant serotype 8 strains to tetracyclines was 68.52%. However, the percentage of isolated sensitive strains did not exceed 14%, which approaches total values.

In spite of these findings, there were no observed significant differences between sensitivity and resistance in the groups of total serotypes and other serotypes. Isolated *A. pleuropneumoniae* strains grouped as the total cohort exhibited a slightly varied sensitivity of 34.7 ± 7.69%. The percentage of strains resistant to tetracyclines was observed above 50%. Thus, sensitivity and resistance in other strains, excluding serotypes 2 and 8, did not significantly differ from total values.

The percentage of *A. pleuropneumoniae* serotype 2 strains resistant to tetracyclines was extremely low and did not exceed 2.8%. This level was significantly lower than the same data of serotype 8 strains (68.5%, $P \leqslant 0.001$). On the other hand, above 80% of *A. pleuropneumoniae* serotype 2 strains were sensitive to tetracyclines, which is significantly higher in comparison with the same parameter of serotype 8 strains ($P \leqslant 0.001$).

A relatively low level of sensitivity was detected in all analyzed strains (2 and 8) and strain groups ("other" and "total") exposed to macrolides. The sensitivity of serotype 8 to macrolides was equal to 32%. Meanwhile, resistance of serotype 8 strains to macrolides accounted for 57% (Fig. 10).

Thus, the results evidence that all isolated strains and groups developed both sensitivity and resistance to macrolides without significant differences.

The comparative analysis of sensitivity among all *A. pleuropneumoniae* isolates showed that the highest level was observed in the treatment with 5 antibiotics including florfenicol, amoxicillin+clavulanic acid, ceftiofur, gentamicin, and marbofloxacin belonging to amphenicols, *β-lactams, aminoglycosides, and fluoroquinolones*. Besides, sensitivity levels of more than 89% were demonstrated by 27% of all isolates (Fig. 11).

From 50% to 80% of all *A. pleuropneumoniae* strains were sensitive to six antibiotics – ciprofloxacin, enrofloxacin, amoxicillin, tulathromycin, trimetho-

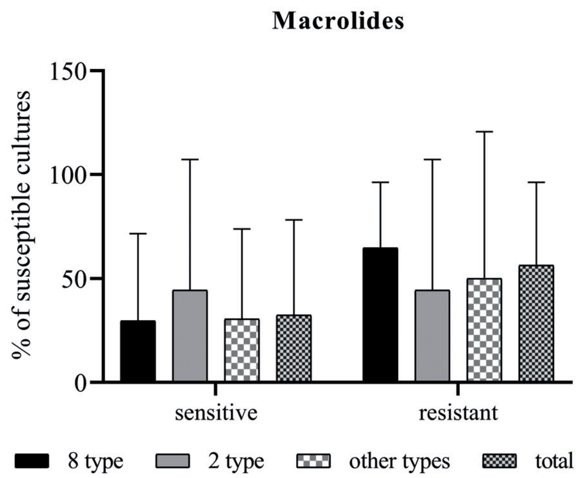

Fig. 10. Sensitivity and resistance of *A. pleuropneumoniae* isolates to macrolides

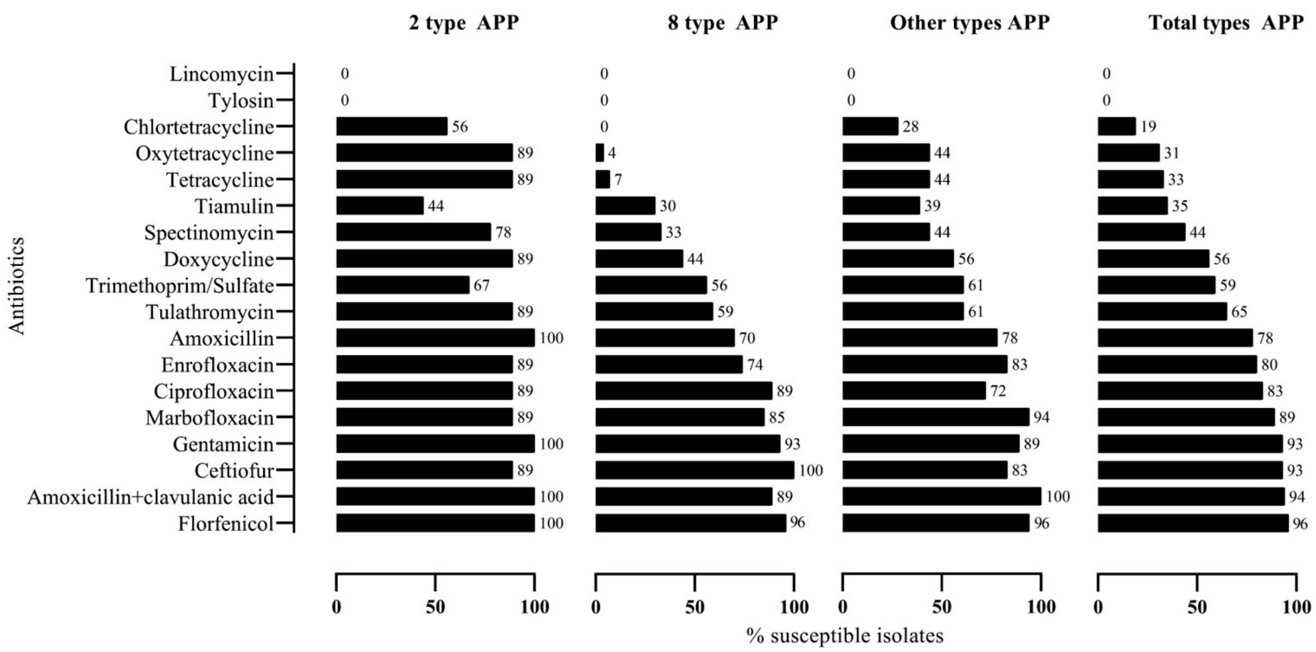

Fig. 11. The sensitivity of *A. pleuropneumoniae* isolates to the antibiotics panel

prim/sulfate and doxycycline belonging to macrolides, β-lactams, sulfonamides, and tetracyclines. The *A. pleuropneumoniae* strains sensitive to aminoglycosides, pleuromutilins, and tetracyclines were identified as having the lowest prevalence in Ukraine. However, the isolated strains of *A. pleuropneumoniae* did not demonstrate sensitivity to lincomycin and tylosin belonging to lincosamides and macrolides.

The obtained results showed that 89–100% isolates of serotype 2 were sensitive to 12 of 18 antibiotics. In contrast, isolates of serotype 2 were not sensitive to lincomycin and tylosin.

Among *A. pleuropneumoniae* serotype 8 strains, 89–100% isolates were sensitive only to 5 of 18 antibiotics. This level was 2.4 times lower than the same values for serotype 2 strains. At the same time, no isolates of serotype 8 demonstrated sensitivity to chlortetracycline. Only 4–7% of serotype 8 strains were sensitive to tetracyclines.

The group of other serotypes of *A. pleuropneumoniae,* except 8 and 2, demonstrated the level of sensitivity to antibiotics similar to overall indicators and did not differ significantly from them. The results on *A. pleuropneumoniae* resistance levels proved the presence of isolates resistant to the effect of almost all applied antibacterial drugs (Fig. 12).

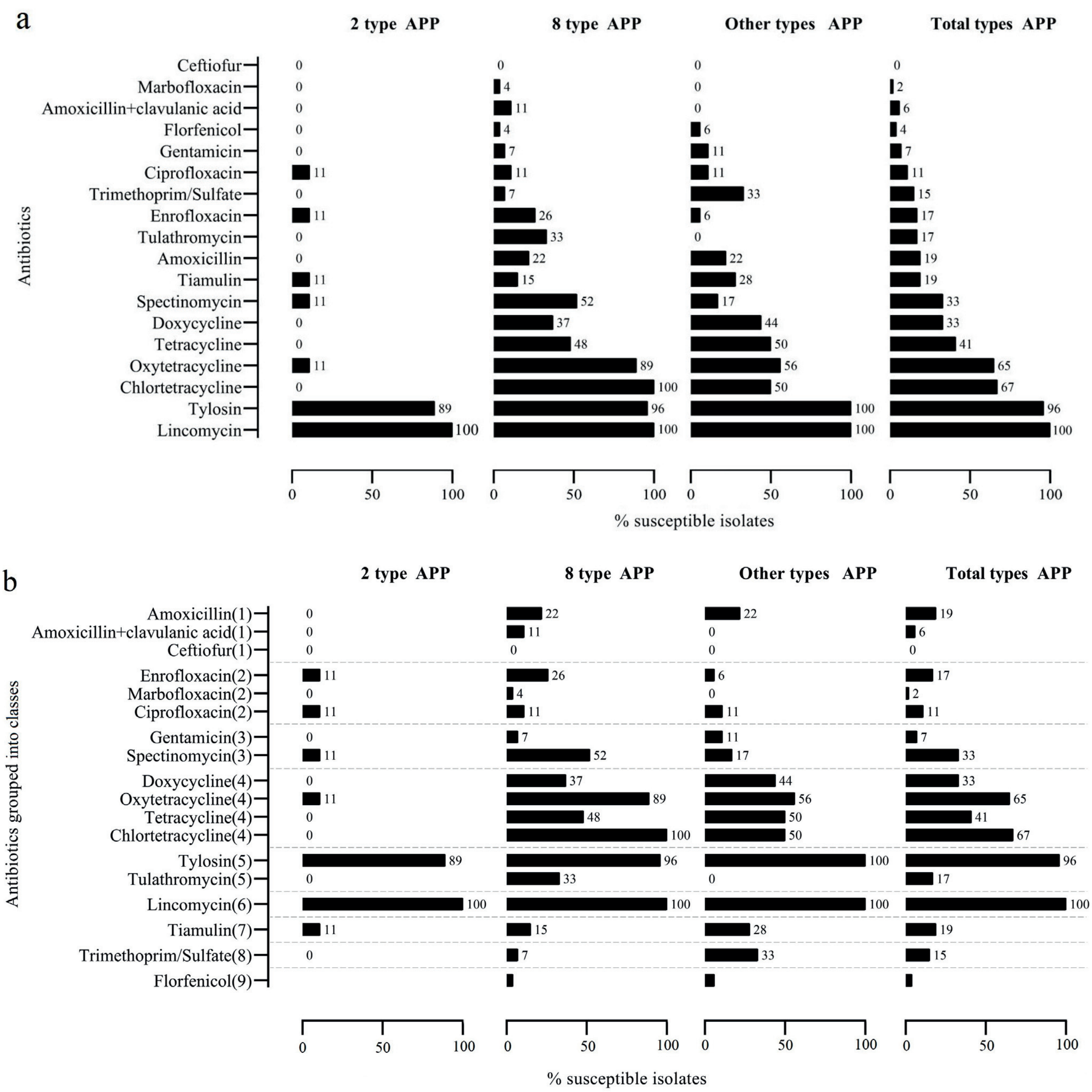

Fig. 12. Resistance of *A. pleuropneumoniae* isolates to various antibiotics grouped with respect to susceptibility increase (a) and grouped into classes (b). The classes are marked with specific numbers: 1 – β-lactams; 2 – fluoroquinolones; 3 – aminoglycosides; 4 – tetracyclines; 5 - macrolides; 6 – lincosamides; 7 – pleuromutilins; 8 – sulfonamides; 9 – amphenicols

Isolated *A. pleuropneumoniae* serotype 8 strains demonstrated various resistance levels to 17 of 18 antibiotics. In contrast, isolated serotype 2 strains exhibited resistance only to 7 antibiotics. The group of other serotypes developed resistance to 14 antibiotics. Besides, the whole group of isolated strains was absolutely resistant to lincosamides (lincomycin) and almost 96% of strains were resistant to macrolides (tylosin). Varying resistance rates were detected in all serotype groups with respect to macrolides while resistance was high to tylosin and low to tulathromycin. Similar differences were observed with respect to aminoglycosides where resistance was low to gentamycin and significantly higher to spectinomycin. These findings evidence that resistance monitoring should be constructed with both comparative analysis for individual antibiotics and antibiotic classes (Fig. 12).

Almost all isolated serotype 2 strains exhibited no resistance to tetracyclines. Meanwhile, about 50% of isolated serotype 8 strains, similarly to other grouped serotypes, were resistant to this antibiotic class.

As a special feature of all isolated strains, complete absence of resistance to cephalosporines was observed. However, moderate sensitivity to cephalosporines was observed in several strains of various serotypes excluding type 8. Besides, all *A. pleuropneumoniae* isolates exhibited no resistance to fluoroquinolones and β-lactams, except definite isolates of serotype 8.

In general, the results demonstrated unique differentiation with respect to *A. pleuropneumoniae* serotype distribution in the provinces of Ukraine. In addition, obtained data showed high diversity of both sensitivity and resistance of isolated *A. pleuropneumoniae* strains to various antibiotic classes.

Discussion

APP is a globally widespread disease of farming swine, which is caused by different strains of *A. pleuropneumoniae*. Due to its variability, this microorganism has a large number of serotypes. It was previously thought that only 15 serotypes of *A. pleuropneumoniae* exist (Soto Perezchica et al., 2023). However, over time, in the world the serotypes which were not subjected to typing started to be detected. In recent years, detailed molecular and genetic studies have confirmed the presence of serotypes 16, 17, 18 and 19 in several regions including North America and West Europe (Bossé et al., 2018; Stringer et al., 2021). At the same time, the isolates different from already known 19 serovars continue to be registered, which indicates the evolution of these bacteria (Angen et al., 2025). Thus, today 19 antigen different *A. pleuropneumoniae* serotypes are known.

Existing published data concerning epidemiology of APP considering serovars of bacteria are limited and fragmented. In Taiwan, serovars 15, 5, 1, 7 and 2 were found present (Kwan et al., 2025). In Poland, a serological study was carried out where *A. pleuropneumoniae* was identified by serovar 2 and separate serogroups (3, 6, 8 and 1, 9, 11); based on these results, regional epidemiological variations were established (Paulina and Dawid, 2025). The reports on epidemiology of individual *A. pleuropneumoniae* serotypes as well as coinfection variances on swine farms in Ukraine are practically absent. Hence, the results on APP serotype diversity and coinfection variances in Ukraine are presented for the first time.

The results of the present analysis showed a region-dependent and relatively wide spread of *A. pleuropneumoniae* among productive swine herds in Ukraine. In swine with *A. pleuropneumoniae* induced pneumonia, only one serovar of *A. pleuropneumoniae* was detected in the lung tissue in three of four cases, while in every fourth case, the presence of two or three different serovars of *A. pleuropneumoniae* was identified.

The obtained results showed that in 16 regions of Ukraine at least 12 different serovars of *A. pleuropneumoniae* circulate, among which serovars 2 and 8 are the most spread. Serovars 1, 7, 9/11, and 18 are moderately spread. Besides, serovars 17 and 18, which were reported in the last decades, were identified in the present study. The results are in concordance with data on *A. pleuropneumoniae* serovar ratio in England reported earlier where serotype 8 was prevailing (Li et al., 2016). The results could be linked to the translocation of the aforementioned serovars to Ukraine via fattening pigs to improve swine breed genetics. A similar mechanism appears to be the most likely causative factor, based on the potential of *A. pleuropneumoniae* to colonize swine tonsils and ensure prolonged persistence of the infectious agent (Soto Perezchica et al., 2023). Thus, our results concerning this fact deserve special attention similarly to previous reports on the serovar 8 sequence (Bossé et al., 2018).

The genotypes including 4, 10, 14, 15, and 16 were not detected in any samples. It can be due to their absence or critically low presence on swine farms in Ukraine.

Studying the sensitivity of bacteria to antibacterial drugs in the world is an important task to maintain the health of both humans and animals (Ho et al., 2025). Assessing *A. pleuropneumoniae* sensitivity to antibiotics is no exception since this microorganism exhibits the potential to generate resistant forms like many others (Guo et al., 2021). Due to mutations, *A. pleuropneumoniae* strains can become resistant to various antibiotics (Wang et al., 2010). Recently, a detailed mechanism of gene translocation between bacteria *A. pleuropneumoniae* and members of *Enterobacteriaceae* family has been reported (Xu et al., 2024). These findings can clarify the significant impact of resistant forms of *A. pleuropneumoniae* on farming swine health.

Analysis of the data obtained regarding the wide range of sensitivity and resistance of *A. pleuropneumoniae* isolates showed the presence of isolates resistant to three or more classes of antibiotics. These findings confirm *A. pleuropneumoniae* potential to generate multi-resistant strains. Furthermore, the detection of strain resistance and dissemination can be a suitable method to predict the risks of APP incidence caused by resistant strains via coinfection.

Recent reports are devoted to the study of resistance as a rule. In spite of this, several research studies were additionally focused on the phenotypic and genotypic differences of *A. pleuropneumoniae* strains in a course of antibiotic resistance assessing. Particularly, Guarneri and coauthors (2024) have described that serovar 9/11 exhibited closely related resistance to florfenicol and enrofloxacin, while serovar 5 developed high resistance to trimethoprim/sulfamethoxazole. Besides, *A. pleuropneumoniae* strains isolated in Italy exhibited the most common resistance to tetracyclines and β-lactams (Guarneri et al., 2024). In spite of these findings, our results showed high resistance to β-lactams and relatively low resistance to tetracyclines (Fig. 12b). Furthermore, our study results demonstrated that all isolates of *A. pleuropneumoniae* were resistant to lincomycin and almost 96% of isolates were resistant to tylosin. Thus, our findings evidenced that at least part of isolates exhibited multi-resistance signs. Furthermore, complete absence or critically low sensitivity to beta-lactams, fluoroquinolones, and aminoglycosides evidenced that all identified serotypes exhibited resistance minimum to these antibiotic classes. Besides, serovar 2 strains developed resistance to the aforementioned classes as well as to tetracyclines that could reflect the shift to multi-resistant changes.

Special attention is drawn to the results on reciprocal differences in resistance to tetracyclines exhibited by serovars 2 and 8. Similar differences may be due to the variable genetics of these serovars. On the one hand, serotype 8 causes significant clinical signs and pushes veterinarians to apply antibiotics more often. On the other hand, recent results on complete genome sequence of *A. pleuropneumoniae* serotype 8 have shown low genetic variation in the strains isolated in various countries as well as in respect with reference strain 405 (Bossé et al., 2021). The data obtained in our study are in accordance with recent reports and evidence that the conservative features of serotype 8 strains could be the cause of prevalence of this serotype in Ukraine.

Recently, *A. pleuropneumoniae* serotype 12 field strains have been described in Chile. Atypical field strains possessed both capsule genes of serotype 12 and LPS genes belonging to serotype 15 (Vincent et al., 2025). These strains possess an unusual composition of toxin genes, carry capsule genes belonging to serotype 12 but produce LPS with genes belonging to serotype 15. Furthermore, these strains showed close phylogenetic relationships to atypical strains detected in Canada, Japan, and USA. These results confirm the high risk of new strain generation including multi-resistant forms of *A. pleuropneumoniae*. The complex character of antibiotic resistance observed in Ukraine across various serotypes could be a driving factor to push genetic diversity of *A. pleuropneumoniae* in several provinces affected by APP. Serotype 12 spread in Ukraine was assessed as minimal among all identified serotypes. In view of high variability of serotype 12 strains, low detectable numbers of this serovar could be dependent on the restricted ability of commercial kits to detect atypical serotypes.

The analysis of colocation of 12 serotypes identified in Ukraine demonstrates a significant diversity of strains circulating on pig farms. The diversity of colocations could be dependent on the geographic location as well as the unique climate features in every province of Ukraine.

Taking into the account recently published reports, our study is the first to demonstrate biodiversity of *A. pleuropneumoniae* serotypes and their individual features in respect to both sensitivity and resistance against most applicable antibiotics in swine farming. Furthermore, the observed results can expand the current ideas on the APP spread, epidemiological features, and circulation on swine farms located in separate regions of Ukraine as well as in the pig industry globally.

Conclusions

The results show that APP coinfection cases exhibited an individual serotype profile in every province. Besides, maximal diversity of *A. pleuropneumoniae* serotypes were found in two regions only, which could be related to a specific geographic location and climate of these areas. Among all, serotypes 8 and 2 were most prevalent and serotypes 17 and 18 were identified as relatively new for Ukraine.

The results on high efficiency of ceftiofur, marbofloxacin, and amoxicillin+clavulanic acid use against *A. pleuropneumoniae* suggest that several cephalosporines, fluoroquinolones, and β-lactams can be prospective chemicals to restrict the current APP spread. In contrast, tetracyclines exhibited clear inverse differences in resistance/sensitivity ratio with respect to serotypes 2 and 8. Despite this, a ratio for "other" and "all" grouped serotypes was almost equal suggesting intermediate resistance to tetracyclines. Additionally, high resistance was observed for tylosin and lincomycin. The present study demonstrated the risk of *A. pleuropneumoniae* multi-resistant strain generation to macrolides, lincosamides, and tetracyclines in Ukraine recent years. Further research is needed to investigate details of antibiotic resistance of all identified strains combined with sequence analysis.

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