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Serotyping and antimicrobial resistance of *Actinobacillus pleuropneumoniae* isolates from fattening pigs in Poland from 2019 to 2024

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Abstract

Background *Actinobacillus pleuropneumoniae* is a prevalent respiratory pathogen causing substantial economic losses in swine production worldwide. The bacterium's ability to rapidly develop antimicrobial resistance (AMR) poses a significant challenge to effective treatment and control. In Poland, limited data on *A. pleuropneumoniae* serotype distribution and AMR patterns hinder evidence-based treatment strategies. This study examined the serotype diversity and AMR patterns of *A. pleuropneumoniae* isolates from porcine pleuropneumonia outbreaks in northeastern Poland between 2019 and 2024, providing crucial information for regional veterinary practices and antimicrobial stewardship efforts.

Results Analysis of 119 isolates from 67 farms demonstrated the predominance of serotype 2 (65.5%), followed by serogroups 3, 6, 8 (18.5%) and 1, 9, 11 (15.1%). This distribution differs from recent trends in other European countries, suggesting regional epidemiological variations. High resistance rates were observed for tylosin (55.5%), gentamicin (36.1%), doxycycline (32.8%), and sulfamethoxazole/trimethoprim (26.1%). Multidrug resistance fluctuated between 14.3% and 21.9% over the study period, with no clear linear trend. From 2022 onwards, strains exhibiting resistance to seven or more antimicrobials, including cephalosporins, emerged, marking a significant shift in resistance profiles. Temporal analysis revealed diverse resistance patterns, with significant increases in some antimicrobials (e.g., sulfamethoxazole/trimethoprim, $p=0.001$) and stability in others (e.g., tetracycline, $p=0.890$). Notably, several antimicrobials, including florfenicol and colistin, maintained 100% efficacy against all isolates throughout the study period.

Conclusions The findings highlight the dynamic nature of AMR development in *A. pleuropneumoniae* and underscore the need for ongoing surveillance in the region. The emergence of highly resistant strains, particularly those resistant to cephalosporins, raises concerns about future treatment options. These results can guide evidence-based treatment strategies and enhance antimicrobial stewardship efforts in regional swine production. Furthermore, the study emphasizes the importance of local AMR data in guiding antimicrobial use policies and the need for a coordinated approach to combat AMR in veterinary medicine.

Keywords Bacterial disease, Porcine pleuropneumonia, Swine respiratory disease, Antimicrobial resistance, Serotype distribution, Multidrug resistance

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Introduction

Actinobacillus pleuropneumoniae (*A. pleuropneumoniae*) is a primary pathogen responsible for porcine pleuropneumonia, a significant respiratory illness affecting pigs of various ages. Characterized by fibrinous pleuritis and hemorrhagic pneumonia with necrotic lung lesions, the disease often results in high mortality and economic losses [1–3]. This impact underscores the need for a detailed understanding of the pathogen's epidemiology and its resistance patterns to effectively treat the outbreaks of disease in endemically infected farms. *A. pleuropneumoniae* exhibits serotype diversity, with 19 known serotypes differentiated by capsular polysaccharides and cell wall lipopolysaccharides [4, 5]. Each serotype carries distinct virulence factors leading to a range of clinical manifestations [1, 3, 6]. Regional variation in serotype prevalence has been observed over time. For instance, serotype 2 was previously predominant in Korea, but more recent data indicate shifts, with serotype 5 becoming the most common [7, 8]. In Europe, serotypes 9, 2, and 7 currently dominate among clinical pig isolates [3, 9–12].

The economic burden of *A. pleuropneumoniae* infections is significant, as it leads to increased mortality, reduced feed efficiency, and slower growth rates in pigs [3, 13]. To mitigate these impacts, rapid diagnosis and prompt treatment are essential [3, 8, 14]. Antimicrobials are currently the primary treatment for acute outbreaks, and their use is often guided by regional antimicrobial resistance (AMR) patterns in swine pathogens [15]. However, the global rise in AMR poses a challenge to effective treatment strategies. For instance, in Korea, susceptibility to florfenicol declined sharply from over 94% in 2006 to just over 50% by 2010, illustrating how AMR patterns can vary significantly over time and from one region to another [8].

The issue of AMR in *A. pleuropneumoniae* is part of a broader trend in both human and veterinary medicine. The European Food Safety Authority (EFSA) reported an average antimicrobial consumption of 92.6 mg/kg in food-producing animals in the EU between 2019 and 2021, with penicillins and tetracyclines being the most commonly sold [16]. In Poland, the 2021 consumption was notably higher at 175.5 mg/kg, ranking second among EU countries [16]. The widespread and often indiscriminate use of antimicrobials contributes to the increase in AMR, posing a public health threat through the potential transfer of resistant bacteria from animals to humans [17].

AMR is a growing issue among various bacterial species, yet comprehensive data on resistance patterns in many pathogens remain scarce [18–23]. The EFSA has thus initiated mandatory AMR monitoring in zoonotic

pathogens, as well as a coordinated strategy for monitoring AMR in diseased animals [17, 19, 24]. This initiative is crucial for understanding the evolution of resistance and for informing stewardship efforts to preserve antimicrobial efficacy.

Efforts to reduce antimicrobial use in agriculture, while essential, must be balanced with the need to control animal pathogens like *A. pleuropneumoniae*. The European Commission (EC) has implemented measures to combat AMR, including the restriction of last-resort antimicrobials in food-producing animals as per Regulation (EC) No 2019/627 [25]. However, unintended consequences, such as the selection of resistant pathogens, highlight the complexity of managing AMR in animal production systems [3, 13, 17, 26–28].

Effective treatment of *A. pleuropneumoniae* relies on knowledge of the antimicrobial susceptibility patterns of circulating strains. These patterns can vary significantly between serotypes and over time, necessitating continuous surveillance [9, 12, 14, 29]. In Poland, however, there is limited data on AMR in *A. pleuropneumoniae* [27, 30], indicating a critical gap in understanding regional resistance trends.

Our study aims to fill this knowledge gap by examining the AMR profiles of *A. pleuropneumoniae* isolates from pigs in northeastern Poland over a six-year period (2019–2024). The objectives include analyzing temporal trends in resistance to individual antimicrobials and exploring correlations between resistances. This research is vital for informing clinical management and guiding antimicrobial stewardship in swine production, ultimately contributing to efforts to mitigate the spread of AMR.

Results

Collected isolates and serotyping

Over the course of six years, from 2019 to 2024, a total of 119 isolates of *A. pleuropneumoniae* were collected, each representing a unique outbreak in fattening pigs on 67 farms in northeastern Poland. These outbreaks were active at the time of sample collection and analysis. The annual distribution of outbreaks showed a dynamic pattern, with 23 in 2019, 8 in 2020, 16 in 2021, 23 in 2022, 31 in 2023, and 18 in 2024. It is important to note that data was collected during a partial year, which may explain the lower number of outbreaks for that year. Most farms (70.1%, 47/67) submitted samples from a single outbreak, while 14.9% (10/67) experienced two outbreaks, 7.5% (5/67) had three outbreaks, and another 7.5% (5/67) reported more than three outbreaks during the study period (Table 1).

Serotyping revealed that serotype 2 was the most prevalent (65.5%, 78/119), followed by serogroups 3, 6, 8 (18.5%, 22/119), and serogroups 1, 9, 11 (15.1%, 18/119).

Table 1 Number of outbreaks of *Actinobacillus pleuropneumoniae* infections on 67 farms during the study period (2019–2024)

No. of outbreaks	Year					
	2019	2020	2021	2022	2023	2024
1	11	6	14	13	12	14
2	3	1	1	2	2	2
3	2	0	0	2	1	0
> 3	0	0	0	0	3	0
total	23	8	16	23	31	18

Eight farms experienced recurrent outbreaks with the same serotype (app2) over study period, while 12 farms had outbreaks caused by different serotypes each year

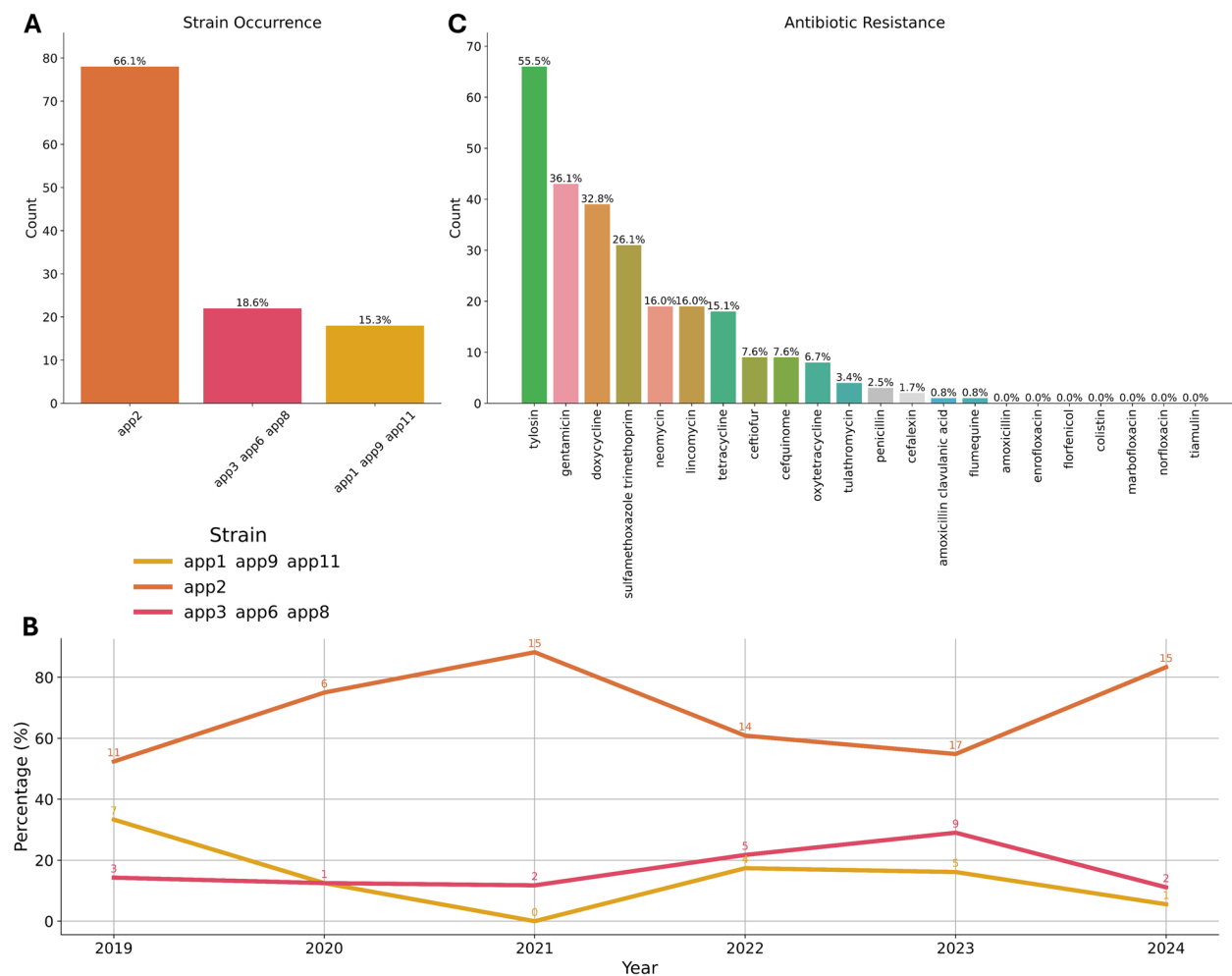


Fig. 1 Distribution and antimicrobial resistance patterns of *A. pleuropneumoniae* isolates from northeastern Poland, 2019–2024. **A** The bar chart illustrates the strain occurrence, showing the distribution of *A. pleuropneumoniae* serotypes among 119 isolates. **B** The bar graph depicts antibiotic resistance rates, displaying the percentage of isolates resistant to various antimicrobial agents. **C** The line graph illustrates temporal trends, showing the frequency of strain diagnosis over the study period (2019–2024). The graph demonstrates annual distribution of serotypes, revealing fluctuations in prevalence, with serotype 2 consistently dominant throughout the five-year period

Table 2 Yearly antimicrobial resistance rates by agent and class from 2019 to 2024 among *Actinobacillus pleuropneumoniae* isolates

Antimicrobial class	Antimicrobial agent	Number of resistant isolates per year						
		2019	2020	2021	2022	2023	2024	Total
Beta-lactams: Basic penicillins	Amoxicillin	0	0	0	0	0	0	0
	Penicillin	0	0	0	1	1	1	3
Beta-lactams: Penicillins with beta-lactamase inhibitors	Amoxicillin/ clavulanic acid	0	0	1	0	0	0	1
Beta-lactams: 1st and 2nd generation cephalosporins	Cefalexin	0	1	0	0	1	0	2
Beta-lactams: 3rd and 4th generation cephalosporins	Ceftiofur	0	0	0	4	3	2	9
	Cefquinome	0	1	0	3	4	1	9
Sulfonamides	Sulfamethoxazole/ trimethoprim	0	0	1	15	9	6	31
Fluoroquinolones	Enrofloxacin	0	0	0	0	0	0	0
	Flumequine	0	0	0	0	0	1	1
	Marbofloxacin	0	0	0	0	0	0	0
	Norfloxacin	0	0	0	0	0	0	0
Amphenicols	Florfenicol	0	0	0	0	0	0	0
Aminoglycosides	Gentamicin	7	5	7	13	5	6	43
	Neomycin	10	0	0	0	2	7	19
Polymyxins	Colistin	0	0	0	0	0	0	0
Lincosamides	Lincomycin	1	1	2	5	6	4	19
Tetracyclines	Doxycycline	2	4	4	10	11	8	39
	Oxytetracycline	2	1	1	1	1	2	8
	Tetracycline	3	2	3	4	2	4	18
Pleuromutilins	Tiamulin	0	0	0	0	0	0	0
Macrolides	Tulathromycin	2	0	0	2	0	0	4
	Tylosin	11	6	14	15	10	10	66

The occurrence and distribution of *A. pleuropneumoniae* serotypes are shown in Fig. 1A, and the annual distribution of serotypes is presented in Fig. 1B.

Antimicrobial susceptibility

Antimicrobial susceptibility testing was performed on all 119 isolates against 22 antimicrobial agents (Table 2). Seven antimicrobials (amoxicillin, colistin, enrofloxacin, florfenicol, marbofloxacin, norfloxacin, and tiamulin) showed 100% susceptibility across all isolates. The highest resistance rates were observed for tylosin (55.5%), gentamicin (36.1%), doxycycline (32.8%), and sulfamethoxazole/trimethoprim (26.1%). Moderate resistance levels were observed for lincomycin and neomycin (both 16.0%), as well as tetracycline (15.1%). Ceftiofur and cefquinome showed 7.6% resistance each, while oxytetracycline had 6.7% resistance. Low resistance rates (<5%) were observed for tulathromycin (3.4%), penicillin (2.5%), cefalexin (1.7%), amoxicillin/clavulanic acid (0.8%), and flumequine (0.8%). The distribution of resistance rates for various antimicrobial agents is illustrated in Fig. 1C (Table 2).

The analysis of antibiotic resistance profiles across these serotypes revealed distinctive patterns. Serotype 2 exhibited an average resistance of 9.0%, with notably high

resistance to tylosin (56.4%), gentamicin (33.3%), and doxycycline (28.2%). Serogroups 3, 6, 8, which showed an average resistance of 14.3%, demonstrated elevated resistance to tylosin (59.1%), doxycycline (45.5%), and sulfamethoxazole-trimethoprim (36.4%). Meanwhile, serogroups 1, 9, 11 displayed an average resistance of 11.1%, with moderate resistance to tylosin (44.4%) and gentamicin (44.4%), alongside a intermediate resistance to doxycycline (38.9%).

Temporal trends in antimicrobial resistance

The proportion of *A. pleuropneumoniae* isolates resistant to at least one antimicrobial agent fluctuated over the study period: 56.5% in 2019, 100% in 2020, 87.5% in 2021, 69.6% in 2022, 41.9% in 2023, and 66.7% in 2024 (Fig. 2).

The seasonal decomposition analysis of the three serotypes reveals distinct trends over the study period (Fig. 1, Supplementary Figs. 1–3). No significant seasonal patterns were observed for serotype distribution over time. The residuals across all serotypes suggest that while the model captures the overall trends, additional factors may be influencing these patterns that are not fully explained by the seasonal decomposition.

Antimicrobial resistance development was analyzed by seasonal decomposition and annual trend analysis,

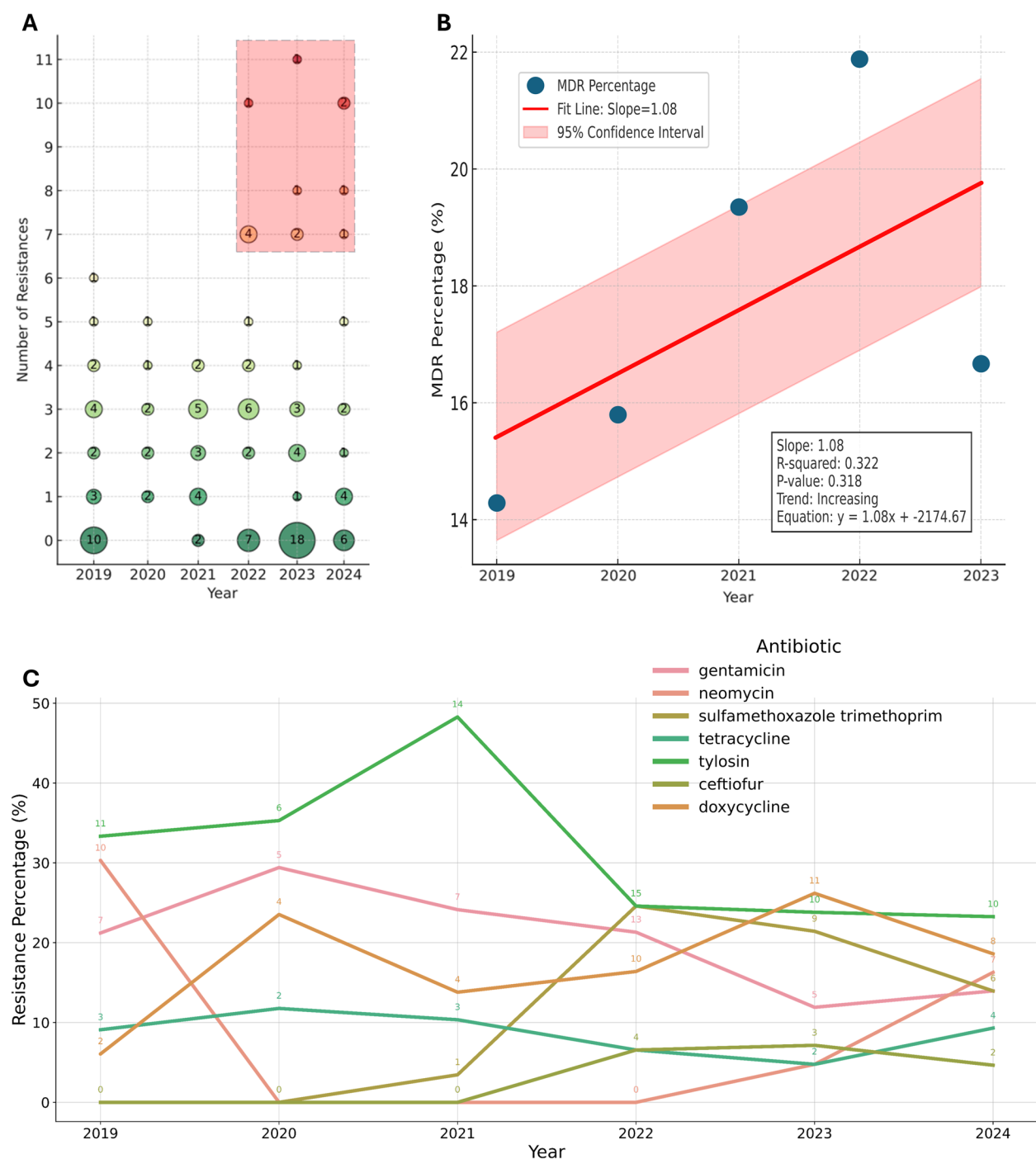


Fig. 2 Temporal trends in antimicrobial resistance patterns of *A. pleuropneumoniae* isolates from northeastern Poland, 2019–2024. **A** The bubble chart displays the distribution of isolates by the number of antimicrobials they are resistant to over the study period. The size of each bubble represents the number of isolates with that specific resistance profile. **B** The scatter plot with a linear regression line illustrates the trend in the number of multi-resistant isolates over time. The red line represents the fitted linear model, with the shaded area showing the 95% confidence interval. **C** The line graph depicts the antibiotic resistance trends over time for seven key antibiotics. Each line represents a different antibiotic, showing the number of resistant isolates for each year from 2019 to 2024

revealing diverse patterns among the antimicrobial drugs studied (Fig. 2, Supplementary Figs. 4–9). Sulfamethoxazole/trimethoprim resistant isolates increased significantly from 2021 to 2022 ($p=0.001$). A significant number of isolates was resistant against doxycycline ($p=0.024$) with a gradual increase over the study period, aligning with its high seasonal variability (Fig. 2).

Multidrug resistance

Multidrug resistance (MDR), defined as resistance to three or more antimicrobial classes [31], was observed in varying proportions across the years: 14.3% in 2019, 15.8% in 2020, 19.4% in 2021, 21.9% in 2022, 16.7% in 2023, and 15.4% in 2024. The most common pattern of multi antimicrobial agent resistance involved resistance to macrolides (tylosin, $n=56$) and tetracyclines (doxycycline, $n=37$), frequently in combination with aminoglycosides (gentamicin, $n=43$) and/or sulfonamides (sulfamethoxazole/trimethoprim, $n=30$) (Table 3).

Table 3 Prevalence of multi antimicrobial agent resistance patterns among *Actinobacillus pleuropneumoniae* isolates from northeastern Poland, 2019–2024

Resistance pattern	No. of resistant isolates	Percentage (%)
GEN-TYL	40	33.61
DO-TYL	32	26.89
TMPsulfa-TYL	28	23.53
DO-GEN	25	21.01
DO-GEN-TYL	23	19.33
GEN-TMPsulfa	23	19.33
DO-TMPsulfa	22	18.49
GEN-TMPsulfa-TYL	21	17.65
DO-TMPsulfa-TYL	20	16.81
NEO-TYL	18	15.13
DO-GEN-TMPsulfa	18	15.13
LIN-TYL	17	14.29
DO-GEN-TMPsulfa-TYL	16	13.45
TET-TYL	16	13.45
DO-LIN	16	13.45
GEN-LIN	15	12.61
GEN-LIN-TYL	15	12.61
LIN-TMPsulfa-TYL	14	11.76
LIN-TMPsulfa	14	11.76
GEN-TET	14	11.76

Multi antimicrobial agent resistance is defined as resistance to two or more antimicrobials. The table presents 20 most prevalent patterns identified in the study, using standardized abbreviations for clarity

Abbreviations: AMC amoxicillin clavulanic acid, AMX amoxicillin, CEF ceftiofur, CFQ cefquinome, CFX cephalixin, COL colistin, DO doxycycline, ENR enrofloxacin, FFC florfenicol, FLU flumequine, GEN gentamicin, LIN lincomycin, MAR marbofloxacin, NEO neomycin, NOR norfloxacin, OTC oxytetracycline, PEN penicillin, TET tetracycline, TIA tiamulin, TMP sulfa trimethoprim/ sulfamethoxazole, TUL tulathromycin, TYL tylosin

Notably, from 2022 onwards, *A. pleuropneumoniae* strains resistant to seven or more antimicrobials, including cephalosporins, tended to emerge in resistance profiles ($p=0.051$). The distribution of isolates by the number of antimicrobials they are resistant to over the study period is shown in Fig. 2A (Fig. 2, Table 3).

The percentage of multidrug-resistant (MDR) isolates increases by an estimated 1.08% per year (Fig. 2B). This finding was not statistically significant ($p=0.318$). The R-squared value of 0.322 indicates that the model explains approximately 32.2% of the variability in the MDR percentage. The correlations between resistances to different antibiotics are visualized in Fig. 3, which presents a phi correlation matrix with significance levels for the observed resistance patterns in *A. pleuropneumoniae* isolates.

Discussion

In this retrospective study, we analyzed the serotypes and AMR profiles of 119 *A. pleuropneumoniae* isolates isolated from outbreaks over six years (2019–2024) in 67 farms located in northeastern Poland, a significant pig production region. Our findings revealed that serotype 2 was predominant (65.5%), followed by serogroups 3, 6, 8 (18.5%) and 1, 9, 11 (15.1%). This serotype distribution pattern demonstrates notable concordance with contemporary epidemiological trends observed throughout European countries, where serotype 2 maintains predominance [3].

Contemporary surveillance data from Hungary indicates the dominance of serotypes 2 and 13, with prevalence rates of 39.5% and 15.4%, respectively [31]. In Italy, recent nationwide surveillance conducted by Guarneri et al. (2024) demonstrated that the majority of isolates belonged to serotypes 9/11 (39.2%) and 2 (28.1%) [28], while a concurrent regional study by Cuccato et al. (2024) in Piedmont farms revealed predominant serotypes 2 (45.3%) and 6 (24.5%) [32]. Finnish epidemiological investigations by Halli et al. (2020) and Haimi-Hakala et al. (2017) have established infections with serotype 2 as a significant etiological factor in chronic pleurisy among slaughter pigs [32, 33]. Historical data from Belgium, as reported by Maes et al. (2001), indicated serotypes 2, 3, and 9 as the most frequently isolated strains from porcine specimens [34].

Notably, Switzerland presents a unique epidemiological profile, with *A. pleuropneumoniae* serotype 2 considered eradicated since 2003 [35, 36]. The serotype distribution in England and Wales demonstrates distinct epidemiological patterns compared to continental Europe, with serotype 8 showing clear predominance and relatively lower frequencies of serotypes 2, 6, 7, and 12 [37]. This

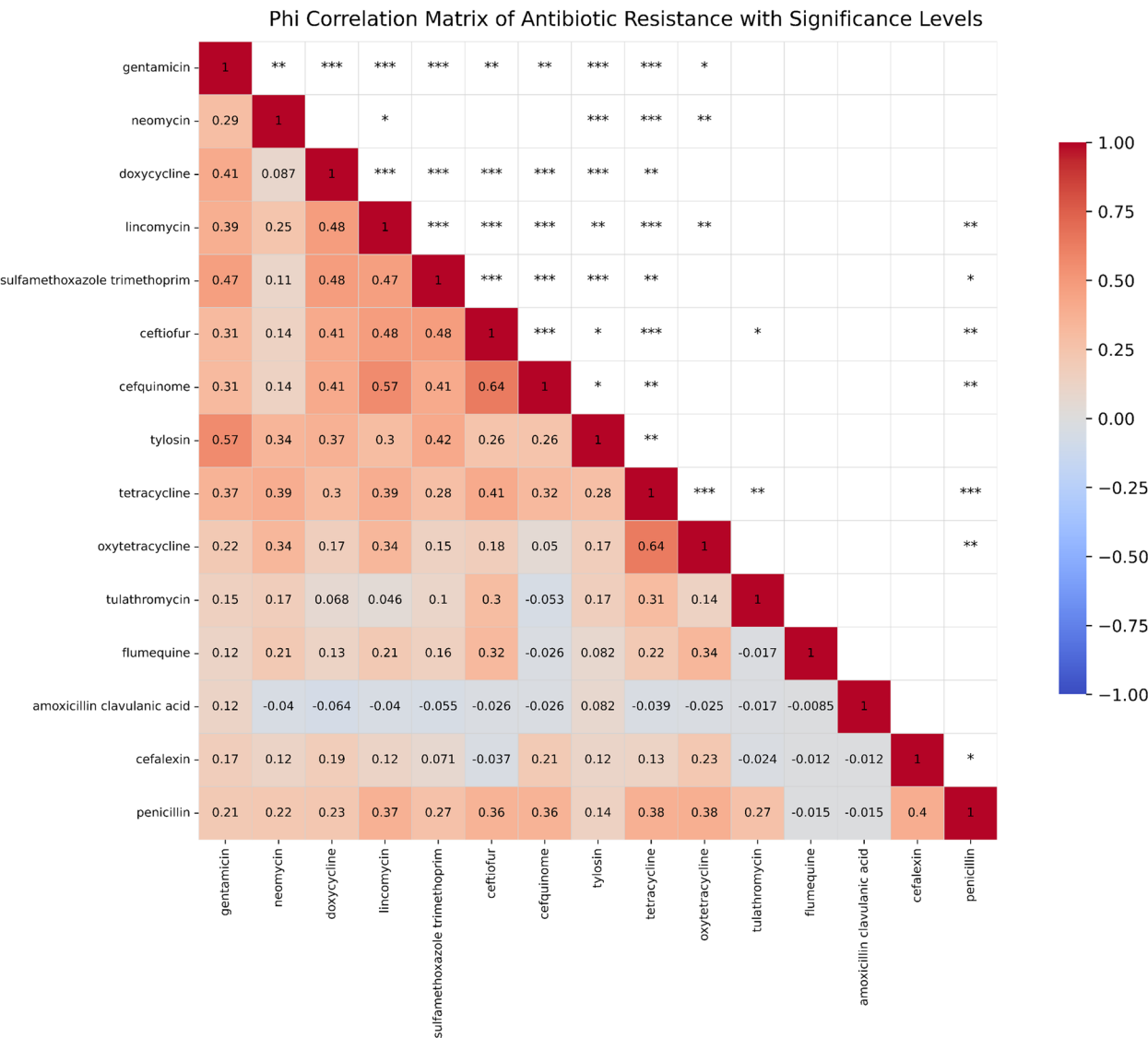


Fig. 3 Phi Correlation Matrix of Antibiotic Resistance with Significance Levels in *A. pleuropneumoniae* isolates from northeastern Poland, 2019–2024. The heatmap illustrates the strength and direction of correlations between resistances to different antibiotics. The lower triangle of the heatmap displays the phi correlation coefficients, with a color gradient ranging from dark blue (–1.00, perfect negative correlation) through white (0, no correlation) to dark red (1.00, perfect positive correlation). The upper triangle contains the corresponding *p*-values, with statistical significance levels indicated by asterisks: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001. Each cell provides both numerical and visual representations of the relationships between resistance profiles of different antibiotics, emphasizing statistically significant correlations

pattern shows remarkable similarity to North American epidemiological data, where *A. pleuropneumoniae* serotype 8 consistently emerges as the predominant strain within the 3/6/8/15 cross-reacting group in clinical cases [38]. The marked predominance of serotype 2 in our investigation suggests sustained regional persistence of this particular serotype in Poland, aligning with historical epidemiological patterns while simultaneously highlighting distinct geographical variations in strain distribution across European territories.

Regarding antimicrobial susceptibility, our study uncovered a complex pattern of resistance across various antimicrobial classes. High resistance rates were observed for doxycycline, aligning with reports from Taiwan [39], Spain [11], and Italy [28]. Interestingly, lower resistance rates were noted for tetracycline and oxytetracycline, suggesting differential selection pressures within this antibiotic class [10, 40]. This variation may reflect differences in the usage patterns of specific tetracycline derivatives in veterinary practice, underscoring the

importance of monitoring individual antibiotics rather than antibiotic classes as a whole.

Although resistance to penicillin remained low among our isolates, the observed resistance to third-generation cephalosporins, such as ceftiofur and cefquinome, is concerning. This trend mirrors findings from Italy [28] and Spain [19], indicating potential emerging resistance to critically important antimicrobials. These developments underscore the necessity for ongoing surveillance and cautious use of these antimicrobials to preserve their efficacy.

While cephalosporins generally maintained low frequencies of resistance over time, the slight increase observed for cefquinome is notable and warrants attention. A similar trend in cephalosporin resistance was noted by Vanni et al. (2012), suggesting that resistance to these important antimicrobials may be gradually emerging and should be closely monitored [12]. A relatively high proportion of isolates was resistant to macrolides and aminoglycosides, consistent with previous reports [7, 12, 28–30, 35].

Additionally, our study found a relatively low number of isolates resistant to fluoroquinolones (enrofloxacin, flumequine), aligning with data from Spain and Italy [12, 19]. In contrast, countries such as Taiwan and Korea have reported increasing frequencies of resistance to fluoroquinolones [7, 9, 39], possibly due to different antimicrobial usage patterns in veterinary practice. These variations emphasize the need for region-specific AMR data to guide antimicrobial use policies effectively.

Notably, resistance to tulathromycin was rare, whereas a significant proportion of isolates exhibited resistance to tylosin. This finding contrasts with the low macrolide resistance reported in Italian. It also corroborates the assertion by Vilaró et al. (2020, 2023) that the evolution of antimicrobial susceptibility must be assessed on a case-by-case basis, as generalizations across drug classes and bacterial species are not universally applicable [19, 38].

Our study found a relatively low number of isolates resistant to polymyxin antibiotics (colistin) and amphenicols (florfenicol), aligning with most studies reporting full susceptibility or very low levels of resistance to florfenicol [10, 12, 17, 33, 38, 41, 42]. Exceptions include studies from Korea [7, 8], which reported resistance rates of 34.3% and 43.1%, respectively, and an Italian study that observed approximately 23% resistance [28]. The authors of these studies speculated that the widespread use of florfenicol for treating porcine respiratory disease complex (PRDC) in these regions may have contributed to increased resistance in *A. pleuropneumoniae*. Given that amphenicols are categorized as "veterinary critically important antimicrobial agents" for food-producing animals and belong to "Category C" according to

the European Medicines Agency [43], monitoring AMR trends for amphenicols worldwide is imperative to preserve their efficacy.

Similarly, we found a relatively low number of isolates resistant to pleuromutilins (tiamulin), consistent with previous studies [28]. An exception is the Italian study by Vanni et al. (2012), which reported 32.7% of isolates resistant to tiamulin [12]. This discrepancy underscores regional differences in antimicrobial usage and resistance patterns, highlighting the importance of local surveillance data.

Resistance to aminoglycosides was also high, particularly against gentamicin, with resistance rates varying across serotypes, reaching 44.4% in serogroups 1, 9, 11. This aligns with previous studies [17, 40, 44], suggesting widespread resistance to this class of antibiotics in *A. pleuropneumoniae* isolates. Such resistance may reflect selective pressure from the frequent use of gentamicin in veterinary practice, highlighting the need for alternative treatment strategies and ongoing surveillance.

Our analysis demonstrated fluctuating trends in resistance over the study period. An emergence of strains resistant to seven or more antimicrobials could not be proven in this study, which might be due to sample size limitations.

The high resistance rates to commonly used antimicrobials such as tetracyclines and tylosin suggest that these drugs should not be employed as first-line treatments without prior susceptibility testing. This approach is crucial to ensure effective therapy and to mitigate the further development of resistance. Conversely, the low resistance to penicillin indicates it could be considered a first-choice treatment in this region, aligning with recommendations from countries with similar resistance profiles [30]. Tailoring antimicrobial use based on local AMR patterns can enhance treatment outcomes and contribute to antimicrobial stewardship efforts.

It is important to acknowledge the limitations of our study. Our research was confined to northeastern Poland, and different patterns may exist in other regions of the country. Additionally, the lack of information on the history of the sampled animals is a limitation that could have influenced our findings, particularly regarding observed antimicrobial resistance patterns. Similar limitations were noted in other studies [28], emphasizing the need for comprehensive data collection in future research to better understand the epidemiology of *A. pleuropneumoniae* and its resistance patterns. Moreover, a major limitation of the study was the use of methods that were not state-of-the-art for serotyping (capsular gene PCR) and antimicrobial resistance testing (microdilution method for determining the minimum inhibitory concentration). As a result, the findings should be interpreted with caution to avoid overinterpretation.

Conclusion

In conclusion, our study provides the first analysis of *A. pleuropneumoniae* AMR profiles in Poland. The observed diverse resistance patterns and their temporal fluctuations underscore the necessity for ongoing surveillance and the importance of antimicrobial stewardship in swine production. These findings can inform evidence-based treatment strategies, guiding veterinarians in selecting effective antimicrobials while minimizing the risk of promoting resistance. The complex nature of AMR development highlights the critical need for continued research and adaptive strategies in managing this pressing issue in animal health and food production.

Material and methods

Bacterial strains and serotyping

A total of 119 clinical isolates of *A. pleuropneumoniae* were collected over six years (2019–2024) from a commercial laboratory in Gietrzwałd, Poland. The laboratory receives samples from 67 swine farms located in north-eastern Poland. All isolates were obtained from the lungs of fattening pigs, aged 14 to 22 weeks, that had died from acute respiratory diseases. These samples were selected to ensure accurate representation of the clinical presentation of *A. pleuropneumoniae* in the region. The isolates were cultured on CHROMagar Acinetobacter plates (Graso Biotech, Poland) and incubated at 37 °C with 5% CO₂ for 18–24 h. Serotyping was conducted using a rapid slide agglutination test with specific antisera, as described by Nielsen (1986) [45]. This method differentiates serotypes based on capsular polysaccharide antigens. Immediate subculturing ensured the viability of isolates for subsequent antimicrobial susceptibility testing, facilitating the prompt assessment of the AMR profiles in these clinical samples.

Antimicrobial susceptibility testing

Antimicrobial susceptibility was determined using the disk diffusion method on chocolate Mueller–Hinton agar, in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines for veterinary pathogens [46]. A panel of 22 antimicrobial agents representing 10 classes was tested, including β -lactams [amoxicillin (10 μ g), amoxicillin/clavulanic acid (30 μ g), penicillin (10 μ g), cefalexin (30 μ g), ceftiofur (30 μ g), cefquinome (30 μ g)]; tetracyclines [doxycycline (30 μ g), oxytetracycline (30 μ g), tetracycline (30 μ g)]; fluoroquinolones [enrofloxacin (5 μ g), marbofloxacin (5 μ g), norfloxacin (10 μ g), flumequine (30 μ g)]; phenicols [florfenicol (30 μ g)]; aminoglycosides [gentamicin (10 μ g), neomycin (30 μ g)]; macrolides and lincosamides [tylosin (30 μ g), tulathromycin (30 μ g), lincomycin (2 μ g)]; pleuromutilins [tiamulin (30 μ g)]; polymyxins [colistin (10 μ g)]; and

potentiated sulfonamides [sulfamethoxazole/trimethoprim (23.75 μ g/1.25 μ g)]. Plates were incubated at 37 °C with 5% CO₂ for 18–24 h. Inhibition zone diameters were measured and interpreted according to CLSI-established breakpoints for *A. pleuropneumoniae* [46]. Isolates were categorized as susceptible, intermediate, or resistant. For statistical analysis, intermediate isolates were grouped with resistant ones, following approaches used in previous studies [12, 28].

Quality control

Quality control was ensured by testing the reference strain *A. pleuropneumoniae* ATCC 27090, as recommended by CLSI guidelines [46]. This reference strain validated the accuracy and reliability of the antimicrobial susceptibility testing procedures, ensuring consistent and precise results across all assays.

Statistical analysis

Statistical analyses were conducted using Python 3.12 within the JupyterLab 4.3 environment. Descriptive statistics were computed using the Pandas library (version 2.2.2). Associations between resistances to different antimicrobial agents were assessed using chi-square tests to identify statistically significant relationships among resistance patterns. Phi correlation coefficients were calculated to quantify the strength of association between pairs of antibiotics, with coefficients interpreted as weak (0.1–0.3), moderate (0.3–0.5), or strong (>0.5). Limitations of the phi coefficient, such as sensitivity to uneven marginal distributions, were considered in the interpretation.

MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, as standardized by Magiorakos et al. (2012) [31]. This definition is consistent with those used in studies of swine pathogens [12, 29]. The Cochran-Armitage trend test was employed to detect trends in the prevalence of MDR isolates over time, suitable for assessing changes in binomial proportions throughout the study period. Additionally, post-hoc pairwise comparisons between specific years were performed using the chi-square test to identify significant differences in MDR prevalence between individual time points. Changes in MDR prevalence were also assessed using linear regression analysis, estimating annual rates of change based on the recalculated MDR percentages. The slope of the regression line quantifies the annual percentage increase in MDR isolates. Statistical significance was set at $p < 0.05$ for all analyses, and results are presented with 95% confidence intervals to facilitate interpretation.

Temporal trends in antimicrobial resistance were further evaluated using logistic regression models,

with the year of isolation as the independent variable and resistance status as the dependent variable. This method allowed assessment of changes in resistance probabilities over the study period, providing insights into the dynamics of antimicrobial resistance patterns in *A. pleuropneumoniae*.

Seasonal decomposition of time series data was performed using the Statsmodels library (version 0.14.0) to separate observed resistance trends into long-term trends, seasonal variations, and residuals. This approach facilitated an understanding of factors influencing resistance patterns over time.

For data visualization, including line plots, bar charts, and heatmaps, the Matplotlib (version 3.7.1) and Seaborn (version 0.12.2) libraries were utilized. In presenting statistical results, *p*-values are reported to three decimal places for precision. In figures, significance levels are indicated using symbols: *** for $p < 0.001$, ** for $p < 0.01$, and * for $p < 0.05$, enhancing readability and facilitating interpretation of the data.

Abbreviations

<i>A. pleuropneumoniae</i>	<i>Actinobacillus pleuropneumoniae</i>
AMR	Antimicrobial resistance
EFSA	European Food Safety Authority
EU	European Union
WHO	World Health Organization
EMA	European Medicines Agency
EC	European Commission
PRDC	Porcine respiratory disease complex
CLSI	Clinical and Laboratory Standards Institute
MDR	Multidrug resistance
EFT	Ceftiofur
GEN	Gentamicin
LIN	Lincomycin
N	Neomycin
OT	Oxytetracycline
PEN	Penicillin
TET	Tetracycline
TMPsulfa	Trimethoprim/sulphamethoxazole
TYL	Tylosin

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12917-025-04504-6>.

Supplementary Material 1: Supplementary Figure 1. Seasonal Decomposition of *A. pleuropneumoniae* Serogroup 1, 9, 11 Prevalence in Northeastern Poland, 2019–2024. The figure presents a time series decomposition of the occurrence of Serogroup 1, 9, 11 over the study period. (A) The top panel shows the observed data, representing the raw count of isolates diagnosed for this strain group (absolute numbers). (B) The second panel displays the overall trend, showing the smoothed proportion of isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in the occurrence of this strain group. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

Supplementary Material 2: Supplementary Figure 2. Seasonal Decomposition of *A. pleuropneumoniae* Serogroup 2 Prevalence in Northeastern Poland, 2019–2024. The figure presents a time series decomposition of the occurrence of Serogroup 2 over the study period. (A) The top panel shows the observed data, representing the raw count of isolates diagnosed for

this strain group (absolute numbers). (B) The second panel displays the overall trend, showing the smoothed proportion of isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in the occurrence of this strain group. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

Supplementary Material 3: Supplementary Figure 3. Seasonal Decomposition of *A. pleuropneumoniae* Serogroup 3, 6, 8 Prevalence in Northeastern Poland, 2019–2024. The figure presents a time series decomposition of the occurrence of Serogroup 3, 6, 8 over the study period. (A) The top panel shows the observed data, representing the raw count of isolates diagnosed for this strain group (absolute numbers). (B) The second panel displays the overall trend, showing the smoothed proportion of isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in the occurrence of this strain group. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

Supplementary Material 4: Supplementary Figure 4. Seasonal Decomposition of Tylosin Resistance in *A. pleuropneumoniae* Isolates from Northeastern Poland, 2019–2024. The figure presents a time series decomposition of tylosin resistance over the study period. (A) The top panel shows the observed data, representing the raw count of isolates resistant to tylosin. (B) The second panel displays the overall trend, showing the smoothed proportion of resistant isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in tylosin resistance occurrence. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

Supplementary Material 5: Supplementary Figure 5. Seasonal Decomposition of Gentamicin Resistance in *A. pleuropneumoniae* Isolates from Northeastern Poland, 2019–2024. The figure presents a time series decomposition of gentamicin resistance over the study period. (A) The top panel shows the observed data, representing the raw count of isolates resistant to gentamycin. (B) The second panel displays the overall trend, showing the smoothed proportion of resistant isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in gentamycin resistance occurrence. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

Supplementary Material 6: Supplementary Figure 6. Seasonal Decomposition of Doxycycline Resistance in *A. pleuropneumoniae* Isolates from Northeastern Poland, 2019–2024. The figure presents a time series decomposition of doxycycline resistance over the study period. (A) The top panel shows the observed data, representing the raw count of isolates resistant to doxycycline. (B) The second panel displays the overall trend, showing the smoothed proportion of resistant isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in doxycycline resistance occurrence. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

Supplementary Material 7: Supplementary Figure 7. Seasonal Decomposition of Sulfamethoxazole-Trimethoprim Resistance in *A. pleuropneumoniae* Isolates from Northeastern Poland, 2019–2024. The figure presents a time series decomposition of sulfamethoxazole-trimethoprim resistance over the study period. (A) The top panel shows the observed data, representing the raw count of isolates resistant to sulfamethoxazole-srimethoprim. (B) The second panel displays the overall trend, showing the smoothed proportion of resistant isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in sulfamethoxazole-srimethoprim resistance occurrence. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

Supplementary Material 8: Supplementary Figure 8. Seasonal Decomposition of Neomycin Resistance in *A. pleuropneumoniae* Isolates from Northeastern Poland, 2019–2024. The figure presents a time series

decomposition of neomycin resistance over the study period. (A) The top panel shows the observed data, representing the raw count of isolates resistant to neomycin. (B) The second panel displays the overall trend, showing the smoothed proportion of resistant isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in neomycin resistance occurrence. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

Supplementary Material 9: Supplementary Figure 9. Seasonal Decomposition of Ceftiofur Resistance in *A. pleuropneumoniae* Isolates from Northeastern Poland, 2019–2024. The figure presents a time series decomposition of ceftiofur resistance over the study period. (A) The top panel shows the observed data, representing the raw count of isolates resistant to ceftiofur. (B) The second panel displays the overall trend, showing the smoothed proportion of resistant isolates over time. (C) The third panel illustrates the seasonal component, highlighting cyclical patterns in ceftiofur resistance occurrence. (D) The bottom panel shows the residuals, representing the variation not explained by the trend or seasonal components (dimensionless).

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Authors' contributions

Conceptualization: PP; Data curation: PP, DT; Formal analysis: DT; Funding acquisition: PP; Investigation: PP; Methodology: PP, DT; Project administration: PP, DT; Resources: PP; Software: DT; Supervision: PP, DT; Validation: DT, PP; Visualization: DT; Roles/Writing—original draft: PP; Writing—review & editing: PP, DT.

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Data availability

None of the data was deposited in an official repository. All the data obtained in the present research are presented in this manuscript. The data that support the study findings are available from the authors upon request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no competing interests.

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