

RESEARCH

Open Access



Serovars and antimicrobial resistance profiles of *Actinobacillus pleuropneumoniae* isolates from clinical-case pigs in Taiwan

Wei-Fan Kwan¹, Yanwen Li², Janine T. Bossé², Ming-Tang Chiou^{3,4,5}, Hsien-Jen Chiu³, Paul R. Langford², Preben Mortensen^{6*} and Chao-Nan Lin^{3,4,5*}

Abstract

Background Pleuropneumonia remains an important challenge to swine production worldwide. The causative agent, *Actinobacillus pleuropneumoniae* serovar prevalence in countries can change over time. Few Taiwanese *A. pleuropneumoniae* prevalence studies have been published, and recent large-scale investigations remain notably scarce. This study aimed to identify the serovars and antimicrobial sensitivity of 96 current Taiwanese *A. pleuropneumoniae* clinical isolates.

Results Among the 99 *A. pleuropneumoniae* determinations, 40 (40.4%) were serovar 15, 29 (29.3%) were serovar 5, 15 (15.2%) were serovar 1, 11 (11.1%) were serovar 7, and 4 (4.0%) were serovar 2. Therefore serovar 15 was the most predominant between 2015 and 2022. Resistance of the isolates was minimal to ceftiofur (3.2%), cephalexin (5.2%), cephalothin (6.3%) and enrofloxacin (9.3%), moderate to gentamicin (28.4%), and high for florfenicol, amoxicillin and ampicillin (45.1–68.4%).

Conclusions This study updates the serovar and antimicrobial sensitivity patterns of Taiwanese clinical *A. pleuropneumoniae* isolates, showing that serovars 15, 5, 1, 7, and 2 collectively predominate in clinical outbreaks. Local *A. pleuropneumoniae* isolates exhibited high resistance to antimicrobials currently used in the Taiwanese pig production, such as amoxicillin, ampicillin, and florfenicol. However, there was low resistance to cephalexin, cephalothin, tilmicosin and lincospectin (injectable only) which can be recommended for current use in Taiwan, considering both efficacy and public health risks. Notably, isolates of the same serovar collected from the same farm on the same day occasionally displayed distinct resistance patterns, emphasizing the complex dynamics of antimicrobial resistance. Our data will help veterinarians and producers refine vaccination and treatment protocols, highlighting the importance of ongoing surveillance to respond effectively to evolving resistance trends.

Keywords *Actinobacillus pleuropneumoniae*, Diseases, Serotyping, Serovar, Antimicrobial susceptibility testing (AST), Antimicrobial resistance (AMR)

*Correspondence:
Preben Mortensen
preben.mortensen@ceva.com
Chao-Nan Lin
cnlin6@mail.npust.edu.tw

Full list of author information is available at the end of the article



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License, which permits any non-commercial use, sharing, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if you modified the licensed material. You do not have permission under this licence to share adapted material derived from this article or parts of it. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by-nc-nd/4.0/>.

Background

Actinobacillus pleuropneumoniae is a Gram-negative, rod-shaped, facultative anaerobe that causes porcine pleuropneumonia [1], a high-mortality respiratory disease that results in substantial economic losses to the worldwide pig production [2]. Strains are classified as NAD-dependent (biovar 1) and NAD-independent (biovar 2), which is determined by possession of a truncated or full length *nadV* gene, respectively [3].

Epidemiologically, serovar identification is crucial for detecting the introduction of new, potentially more virulent serovars, which can change in a geographical location over time. It also helps in determining the appropriate bacterin vaccine, as cross-protection between serovars is lacking [4]. If a serovar-independent *A. pleuropneumoniae* vaccine is not chosen, all relevant serovars must be covered [5]. Based on capsular loci, nineteen serovars are known [6].

The last published survey of slaughter pigs in Taiwan identified serovars 1, 2, 3, 5, 7 and 8 between 2002–2007 [7]. Serotyping by slide agglutination used in this investigation can give misleading results, because of cross-reactivity between serovars, particularly inside serogroups [4, 8]. Serovar 1 (131/211 = 65.4%) was the most predominant followed by serovar 2 (72/211 = 34.1%) [7].

Vaccination and antimicrobials are used to prevent and treat, respectively, *A. pleuropneumoniae* infection in the field [2]. Most *A. pleuropneumoniae* outbreaks occur in finishing pigs, and care is required to avoid antimicrobial residues in food. In addition, *A. pleuropneumoniae* antimicrobial resistance (AMR) is considered a burgeoning clinical problem [9]. Despite the recognized variability in serovars and escalating concerns over AMR, detailed insights into the distribution of serovars and AMR profiles of *A. pleuropneumoniae* across and within farms in Taiwan remain scarce.

This study aimed to bridge these gaps by investigating the serovar and AMR profiles of *A. pleuropneumoniae* isolates from diseased pigs, sourced across various farms. Three primary gaps motivated this investigation: first, the need to update serovar and resistance trends using a larger sample size; second, the availability of advanced molecular techniques (e.g., multiplex PCR) that allow for more precise serovar characterization; and third, the novel observation that isolates of the same serotype, even when recovered from the same farm on the same day, can exhibit different resistance patterns.

Results

Age distribution of *A. pleuropneumoniae* isolates

Ninety six *A. pleuropneumoniae* isolates from clinical-case pigs were analyzed. Pigs less than 4 weeks of age, those of 5–12 weeks, and 13–20 weeks accounted for

1.0% (1/96), 14.6% (14/96) and 71.9% (69/96) of the isolates, respectively. Pigs older than 21 weeks accounted for 12.5% (12/96) of the isolates.

Serovar distribution of *A. pleuropneumoniae* isolates

Multiplex PCR typing of the 96 study isolates revealed three with dual-serotype banding patterns (1 + 5, 15 + 7, and 5 + 7). Counting each serotype individually yielded 99 determinations in total. Serovar 15 predominated (40/99, 40.4%), followed by serovar 5 (29/99, 29.3%), serovar 1 (15/99, 15.2%), serovar 7 (11/99, 11.1%), and serovar 2 (4/99, 4.0%). Accordingly, serovar 15 predominated in Taiwanese farms during 2015–2022. Therefore, serovar 15 was the most predominant between 2015 and 2022. No other serovars were identified (Fig. 1A). Serovars 15, 1 and 2 were the most prevalent circulating during 2015–2016. After 2019, only two serovar 1 isolates were identified (both in 2020) (Fig. 1B).

Antimicrobial sensitivity testing (AST) of *A. pleuropneumoniae* isolates

Table 1 summarises the resistance profiles. The highest resistance rates were recorded for streptomycin (94.7%), oxytetracycline (88.3%), apramycin (86.0%), kanamycin (68.8%), ampicillin (68.4%) and doxycycline (67.7%). Moderate resistance was observed to amoxicillin (60.0%), florfenicol (45.1%), trimethoprim/sulfamethoxazole (32.3%) and gentamicin (28.4%). In contrast, resistance remained low (< 15.0%) to tilmicosin (13.9%), lincospectin (11.8%), enrofloxacin (9.3%), cephalothin (6.3%), cephalexin (5.2%) and ceftiofur (3.2%).

The serovar and AMR patterns of *A. pleuropneumoniae* isolates from different pigs at the same farms

Table 2 provides a comprehensive summary of the serovar distribution and AMR patterns observed among *A. pleuropneumoniae* isolates collected from the same pig farms across multiple sampling events. In several farms, the serovar remained consistent across multiple sampling dates, yet the AMR profiles varied considerably. For instance, in Farm 3, serovar 5 was detected in both 2021 and 2022; however, the AMR pattern shifted from APR-DOX-FFC-GEN-KAN-OTC-STR in 2021 to FFC-OTC in 2022. Furthermore, some farms exhibited multiple serovars over time. For example, Farm 4 had serovar 15 in 2018 and 2019, and serovar 5 in 2020; Farm 6 had isolates of both serovars 5 and 15 on the same sampling date. In Farm 25, although serovar 7 was predominant during 2018–2021, isolates representing “15 plus 7” and “5 plus 7” were identified in 2020 and 2021, respectively. Additionally, in several farms the AMR patterns of multiple isolates (of the same serovar) collected during the same outbreak were nearly identical (e.g., Farms 5, 23, 25, 40,

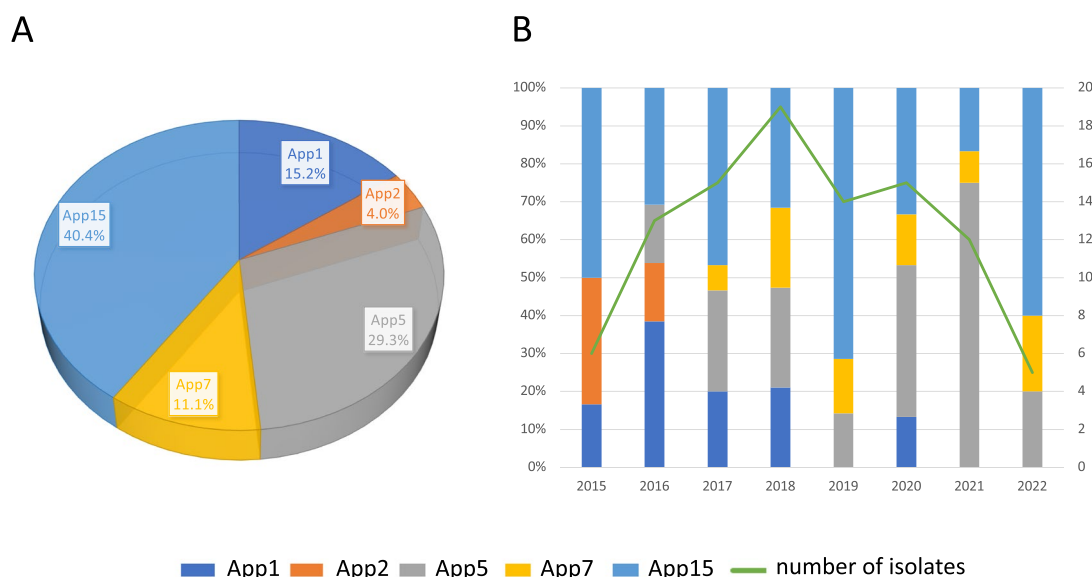


Fig. 1 Serovar (A) and annual distribution (B) of *A. pleuropneumoniae* serovars in Taiwan between 2015–2022

Table 1 Resistance of *A. pleuropneumoniae* isolates to 16 antimicrobial agents. S = sensitive; I = intermediate; R = resistant

Antimicrobials	No.	S	I	R	Resistance (%)
Ceftiofur	94	82	9	3	3.2 %
Cephalexin	96	81	10	5	5.2 %
Cephalothin	96	77	13	6	6.3 %
Enrofloxacin	86	71	7	8	9.3 %
Lincospectin	85	72	3	10	11.8 %
Tilmicosin	79	64	4	11	13.9 %
Gentamicin	95	39	29	27	28.4 %
Trimethoprim/Sulfamethoxazole	96	49	16	31	32.3 %
Florfenicol	91	46	4	41	45.1 %
Amoxicillin	90	31	5	54	60.0 %
Doxycycline	96	15	16	65	67.7 %
Ampicillin	95	27	3	65	68.4 %
Kanamycin	93	10	19	64	68.8 %
Apramycin	86	3	9	74	86.0 %
Oxytetracycline	94	7	4	83	88.3 %
Streptomycin	95	2	3	90	94.7 %

and 41), with notable exceptions observed in other Farms (e.g., 4, 34, 35, 37, 38 and 39).

Discussion

A. pleuropneumoniae is a particularly troublesome pathogen that induces fibrino-hemorrhagic pleuropneumonia and sudden death in growing and finishing pigs. Pigs aged 13 to 20 weeks accounted for the highest detection rate (69/96; 71.9%), while only one case was found in suckling

piglets. The percentage of growing pigs (13–20 weeks of age) that were greatly affected may be due to the changes in management and environment when pigs were moved from nursery to finishing houses. In addition, insufficient protection might be due to an incomplete vaccination program or cross-protection of *A. pleuropneumoniae* vaccines. For example, just one-shot vaccination instead of the required two-shot, or improper timing of vaccination in relation to colostral antibodies should be estimated on the individual farm for optimal first vaccination, or killed bacterins only protect against a specific or limited set of serovars. In Taiwan, pigs often have respiratory problems in nurseries due to multiple pathogen infection [11] and challenging ventilation and climatic conditions. However, 14.6% of cases were recorded in nurseries (5–12 weeks old) in the present study, suggesting that early infection of *A. pleuropneumoniae* is relevant in Taiwan.

A. pleuropneumoniae serovar prevalence shows considerable geographic variation. European epidemiological surveys show that serovars 4, 5 and 17 are predominant in Portugal [12]; Serovars 2, 13, 9 and 16 were the most frequent in Hungary [13]; serovar 8 in Norway and the United Kingdom [14, 15]; and serovar 2 in Germany [16]. Asia-Pacific epidemiological surveys show that serovar 2 is this most predominant in Japan [17], and serovars 1, 5, 7 and 15 are the most common in Australia [18]; The previous Taiwan 2002–2007 slaughter pig survey identified, using slide agglutination, serovars 1, 2 and 5 [7]. Serovar 1 (131/211 = 65.4%) was the most predominant followed by serovar 2 (72/211 = 34.1%) [7]. However, the cross-reactivity

Table 2 Detailed sampling information, serovar distribution, and antimicrobial resistance patterns of 96 *A. pleuropneumoniae* isolates from Taiwanese pig farms

Farm	Year	Date	Isolate Number	Farm location ^b	APP serovar	Resistance patterns (R) ^c
1	2020	18-Jun	109–1516 ^a	Miaoli	15	AMO-AMP-APR-DOX-FLM-KAN-OTC-SP-STR-TMP/SMX
2	2018	23-Aug	107–1939	Changhua	1	AMO-AMP-APR-ENR-FFC-FLM-OTC-SP-STR
3	2021	1-Sep	110–4297 ^a	Yunlin	5	APR-DOX-FFC-GEN-KAN-OTC-STR
	2022	11-Feb	111–1120	Yunlin	5	FFC-OTC
4	2018	25-Jun	107–1493	Yunlin	15	AMO-AMP-APR-KAN-OTC-SP-STR-TIL
	2019	2-Jan	108-010	Yunlin	15	AMO-AMP-APR-CEF-CTN-DOX-KAN-OTC-SP-STR-TIL
	2019	2-Jan	108-011	Yunlin	15	AMO-AMP-APR-CFX-DOX-KAN-OTC-SP-STR-TIL
	2019	6-Mar	108–305	Yunlin	15	AMP-APR-KAN-OTC-SP-STR-TIL
	2020	15-Jul	109–1855 ^a	Yunlin	5	APR-DOX-GEN-KAN-OTC-SP-STR
5	2017	4-Apr	106–658	Yunlin	15	AMO-AMP-APR-DOX-FFC-GEN-KAN-OTC-SP-STR
	2017	4-Apr	106–659	Yunlin	15	AMO-AMP-APR-DOX-FFC-GEN-KAN-OTC-SP-STR
	2017	4-Apr	106–660	Yunlin	15	AMO-AMP-APR-DOX-FFC-KAN-OTC-SP-STR
6	2021	13-Jul	110–3168	Yunlin	5	APR-DOX-FFC-OTC-STR
	2021	13-Jul	110–3170	Yunlin	15	APR-DOX-FFC-OTC-STR
7	2020	4-Dec	109–3686	Yunlin	5	APR-FFC-SP
	2021	9-Jun	110–2648	Yunlin	5	APR-FFC-OTC
8	2018	1-Jun	107–1323	Yunlin	5	AMO-AMP-APR-CFX-CTN-FFC-FLM-GEN-KAN-OTC-SP-STR-TMP/SMX
	2018	17-Aug	107–1911	Yunlin	5	AMP-APR-CFX-CTN-FFC-OTC-SP-STR-TMP/SMX
9	2020	31-Dec	109–4083	Yunlin	1	AMP-DOX-ENR-FFC-FLM-OTC-SP-STR
10	2017	7-Dec	106–2390 ^a	Yunlin	15	AMO-AMP-APR-CEF-FLM-KAN-OTC-SP-STR-TIL
11	2017	13-Dec	106–2430	Yunlin	15	AMO-AMP-APR-DOX-KAN-OTC-SP-STR
12	2021	12-Aug	110–3761	Yunlin	15	AMO-AMP-APR-DOX-KAN-OTC-STR
13	2020	8-Apr	109–746	Yunlin	15	AMO-AMP-APR-KAN-OTC-SP-STR
14	2021	27-Jul	110–3494	Yunlin	5	AMP-APR-DOX-ENR-GEN-KAN-OTC-STR
15	2020	12-Aug	109–2196	Yunlin	5	APR-OTC-SP-STR
16	2021	10-Mar	110–1142	Yunlin	5	APR-DOX-OTC-SP
17	2017	5-Jan	106-062	Yunlin	1	AMO-AMP-FLM-LS-OTC-SP-STR-TMP/SMX
18	2017	31-Oct	106–2153	Yunlin	5	APR-FFC-OTC-SP-STR
19	2019	4-Dec	108–2713 ^a	Yunlin	15	AMO-AMP-APR-DOX-FFC-KAN-OTC-STR
20	2019	14-Aug	108–1505	Yunlin	15	AMO-AMP-APR-DOX-KAN-OTC-SP-STR-TIL
21	2019	5-Dec	108–2717	Yunlin	15	AMO-AMP-APR-DOX-FFC-KAN-OTC-STR
22	2016	4-Jan	105-007	Yunlin	15	AMO-AMP-DOX-ERY-GEN-KAN-OTC-SP-STR-TET
23	2022	12-Jan	111–245	Tainan	15	APR-DOX-FFC-KAN-OTC-STR-TMP/SMX
	2022	12-Jan	111–246	Tainan	15	APR-DOX-FFC-KAN-OTC-STR-TMP/SMX
	2022	12-Jan	111–247	Tainan	15	APR-DOX-FFC-KAN-OTC-STR-TMP/SMX
24	2019	7-Jan	108-032	Tainan	5	APR-DOX-GEN-KAN-OTC-SP-STR-TMP/SMX
25	2018	13-Jul	107–1613	Kaohsiung	7	AMO-AMP-APR-FLM-KAN-SP-STR-TMP/SMX
	2018	21-Sep	107–2091	Kaohsiung	7	AMO-AMP-APR-DOX-FLM-KAN-OTC-SP-STR
	2019	11-Nov	108–2415 ^a	Kaohsiung	7	AMO-AMP-APR-FFC-FLM-STR
	2020	1-Mar	109–520 ^a	Kaohsiung	15 plus 7	AMO-AMP-APR-CTN-FFC-FLM-SP-STR
	2021	28-Jan	110–450	Kaohsiung	5 plus 7	AMO-AMP-APR-FFC-SP-STR
26	2017	21-Dec	106–2528	Kaohsiung	1	AMO-AMP-DOX-FFC-FLM-OTC-SP-STR-TMP/SMX
27	2021	19-Mar	110–1316	Kaohsiung	5	APR-DOX-OTC-SP-STR
28	2020	18-Aug	109–2291	Kaohsiung	5	APR-OTC-SP
29	2017	16-Jan	106–114	Kaohsiung	7	AMO-AMP-APR-DOX-FFC-SP-STR
30	2018	6-Dec	107–2618	Kaohsiung	1 plus 5	AMO-AMP-APR-FLM-LS-OTC-SP-STR-TMP/SMX
31	2015	16-Apr	104–362-6	Pingtung	1	APR-FFC-GEN-KAN-LS-OTC-STR
	2016	8-Mar	105–361	Pingtung	1	AMO-AMP-APR-ENR-GEN-KAN-LS-OTC-STR
32	2018	14-Mar	107–672	Pingtung	1	AMP-FLM-LS-OTC-SP-STR-TMP/SMX
	2020	20-Aug	109–2316 ^a	Pingtung	5	AMO-AMP-APR-DOX-FFC-OTC-SP-STR-TIL
33	2016	18-Oct	105–1699	Pingtung	5	APR-ERY-GEN-KAN-SP-STR
34	2016	8-Apr	105–536	Pingtung	2	CEF-ERY-GEN-KAN-SP
	2016	8-Apr	105–537	Pingtung	2	AMO-AMP-APR-DOX-ERY-KAN-OTC-STR

Table 2 (continued)

Farm	Year	Date	Isolate Number	Farm location ^b	APP serovar	Resistance patterns (R) ^c
35	2017	23-May	106–1075	Pingtung	15	AMO-AMP-DOX-FFC-KAN-OTC-STR
	2017	18-Jul	106–1591	Pingtung	15	AMO-AMP-DOX-FFC-KAN-OTC-SP-STR-TMP/SMX
	2018	26-Jan	107–293 ^a	Pingtung	15	AMP-APR-FFC-GEN-KAN-OTC-SP-STR-TMP/SMX
	2018	26-Jan	107–294 ^a	Pingtung	15	AMP-APR-CFX-CTN-DOX-FFC-GEN-KAN-OTC-SP-STR-TMP/SMX
	2018	12-Mar	107–651	Pingtung	15	AMP-APR-DOX-FFC-KAN-OTC-STR-TMP/SMX
36	2018	31-Oct	107–2323	Pingtung	7	AMO-AMP-APR-DOX-FFC-OTC-SP-STR
	2018	13-Nov	107–2430	Pingtung	7	AMO-AMP-APR-DOX-FFC-OTC-SP-STR-TIL-TMP/SMX
37	2015	23-Nov	104–1451	Pingtung	15	AMO-AMP-CFX-CTN-DOX-ERY-GEN-KAN-SP-STR-TET-TMP/SMX
	2015	23-Nov	104–1452	Pingtung	15	DOX-ERY-KAN-OTC-STR-TET
38	2018	25-Apr	107–1024	Pingtung	5	APR-FLM-OTC-SP-STR
	2021	29-Mar	110–1515	Pingtung	5	APR-DOX-ENR-FLM-GEN-KAN-OTC-SP-STR
39	2016	6-May	105–711	Pingtung	5	ERY-KAN-SP-STR-TET
	2017	15-May	106–1032	Pingtung	5	APR-DOX-FLM-GEN-KAN-OTC-SP-STR-TMP/SMX
	2017	15-May	106–1033	Pingtung	5	APR-DOX-FLM-GEN-KAN-OTC-SP-STR
	2017	15-May	106–1034	Pingtung	5	APR-DOX-FLM-GEN-KAN-OTC-SP-STR
40	2019	25-Jan	108–119 ^a	Pingtung	15	AMO-AMP-APR-DOX-FFC-KAN-OTC-SP-STR-TMP/SMX
	2020	14-Apr	109–795	Pingtung	15	AMO-AMP-FFC-KAN-OTC-STR
	2020	14-Apr	109–796	Pingtung	15	AMO-AMP-FFC-KAN-OTC-STR
41	2016	18-Mar	105–413	Pingtung	15	AMO-AMP-DOX-ERY-KAN-OTC-SP-STR-TET-TMP/SMX
	2016	18-Mar	105–414	Pingtung	15	AMO-AMP-DOX-ERY-KAN-OTC-SP-STR-TET-TMP/SMX
42	2018	2-Nov	107–2356	Pingtung	5	APR-OTC-SP-STR
43	2015	3-Dec	104–1512	Pingtung	2	ERY-GEN-KAN-SP-STR
44	2015	17-Jan	104-069	Pingtung	2	AMO-AMP-APR-DOX-ENR-KAN-LS-STR
45	2017	10-Jan	106-080	Pingtung	1	AMO-AMP-FLM-LS-OTC-STR-TMP/SMX
46	2016	14-Mar	105–392	Pingtung	1	AMO-AMP-DOX-ERY-FLM-GEN-KAN-LS-OTC-SP-STR-TET-TMP/SMX
47	2018	18-Jan	107–118 ^a	Pingtung	1	AMP-APR-DOX-FFC-GEN-KAN-OTC-SP-STR
48	2018	18-Jan	107–131	Pingtung	15	AMP-DOX-KAN-OTC-SP-STR
49	2016	15-Mar	105–398	Pingtung	1	AMO-AMP-APR-DOX-GEN-LS-OTC-STR-TMP/SMX
50	2022	17-Jan	111–813	Pingtung	7	AMO-AMP-APR-DOX-FFC-OTC-STR
51	2018	2-Jul	107–1537	Pingtung	15	AMO-AMP-APR-DOX-KAN-OTC-SP-STR-TMP/SMX
52	2019	3-Jul	108–1185	Pingtung	7	AMO-AMP-APR-DOX-OTC-SP-STR
53	2020	13-Jul	109–1803 ^a	Pingtung	1	AMO-AMP-APR-DOX-ENR-FFC-FLM-GEN-KAN-OTC-SP-STR-TIL-TMP/SMX
54	2020	3-Dec	109–3669 ^a	Pingtung	7	AMO-AMP-APR-DOX-FFC-OTC-SP-STR
55	2016	15-Mar	105–397	Pingtung	1	AMO-AMP-APR-DOX-LS-OTC-STR-TMP/SMX
56	2020	22-Jul	109–1970 ^a	Pingtung	5	APR-DOX-FLM-KAN-OTC-SP-STR
57	2016	9-May	105–731	Pingtung	1	AMP-DOX-ENR-ERY-FLM-GEN-KAN-OTC-SP-STR-TET-TIL-TMP/SMX
58	2019	24-Oct	108–2187	Pingtung	15	AMO-AMP-APR-DOX-FLM-KAN-STR-TMP/SMX
59	2016	18-Jul	105–1130	Pingtung	15	APR-DOX-ERY-KAN-OTC-SP-STR-TET-TMP/SMX
60	2021	11-Jun	110–2708	Pingtung	5	AMO-AMP-APR-DOX-FFC-KAN-OTC-STR
61	2019	8-Jan	108-043	Pingtung	15	AMO-AMP-APR-DOX-GEN-KAN-OTC-STR
62	2015	4-Nov	104–1337	Pingtung	15	AMO-AMP-DOX-ERY-KAN-OTC-SP-STR-TET-TMP/SMX
63	2019	3-Jun	108–910	Pingtung	5	APR-KAN-OTC-SP-STR
64	2019	2-Oct	108–1885 ^a	Hualien	15	AMO-AMP-APR-DOX-KAN-OTC-SP-STR-TIL

^a Whole genome sequences reported in the Ke et al. [19]^b Farms are presented in geographic sequence—beginning with the northernmost farms on Taiwan's west coast (Miaoli) and progressing southward to Pingtung, before turning eastward to Hualien^c Antimicrobial abbreviations: AMO Amoxicillin, AMP Ampicillin, APR Apramycin*, CEF Ceftiofur, CFX Cephalexin, CTN Cephalothin, DOX Doxycycline, ENR Enrofloxacin, ERY Erythromycin*, FFC Florfenicol, GEN Gentamicin, KAN Kanamycin, LS Lincospectin* (lincomycin + spectinomycin), OTC Oxytetracycline, SP Spiramycin*, STR Streptomycin, TET Tetracycline*, TIL Tilmicosin, TMP/SMX Trimethoprim/Sulfamethoxazole

Antibiotics marked with an asterisk were not included in the susceptibility panel of the Ke et al., 2024 but were tested here to reflect local therapeutic usage

between serovars using serological techniques can give misleading results [4, 8]. A recent study by the Ke et al. [19], using next-generation sequencing (NGS), investigated *A. pleuropneumoniae* isolates ($n = 38$) and found that serovar 15 predominated (47.4%, 18/38), followed by serovar 7 (18.4%, 7/38), serovar 5 (15.8%, 6/38), serovar 1 (13.2%, 5/38), and serovar 2 (5.3%, 2/38). In the present study, a molecular assay differentiating 19 *A. pleuropneumoniae* serovars was used [6]. Among the *A. pleuropneumoniae* isolates from clinical outbreak cases, serovar 15 (40.4%) was the predominant isolate, followed by serovar 5 (29.3%), serovar 1 (15.2%), serovar 7 (11.1%) and serovar 2 (4.0%). This distribution contrasts markedly with an earlier (2002–2007) Taiwanese slaughter-pig survey [7], which found serovar 1 to be most frequent (65.4%). Compared with the more recent (2017–2022) limited the Ke et al. study [19], which analyzed only 38 isolates—16 of which overlapped with the present study—our findings from 99 determinations show a slightly lower proportion of serovar 15 (40.4% vs. 47.4%) and a considerably higher frequency of serovar 5 (29.3% vs. 15.8%). With a larger sample size, our study provides a more accurate and comprehensive picture of the current epidemiological situation of *A. pleuropneumoniae* in Taiwan. Our serovar data revealed that both serovars 15, 1 and 2 were prevalent during 2015–2016 in Taiwan. Surprisingly, after 2019, only two serovar 1 isolates were identified (both in 2020) (Fig. 1B). Four farms with more than one serovar in clinical-case pigs were identified in the present study. Multiple serovars identified in the same farm has been reported in Portugal, it was due to introduction of multiple sources of replacement gilts [12]. Herein we could not rule out the possibility of received gilts carrying and introducing new different serovars, because the presence of *A. pleuropneumoniae* in clinically healthy carrier animals is difficult to determine [4]. Three isolates, 109-520, 110-450, and 107-2618 showed dual serotype banding patterns (“15 + 7”, “5 + 7”, and “1 + 5”, respectively) with the multiplex PCR panel validated at Imperial College London. Each had already been re-streaked in the National Pingtung University of Science and Technology diagnostic laboratory to obtain a single colony, yet mixed growth can occasionally elude visual selection. Given the well-documented specificity for the mPCR assay, a technical artefact is unlikely; instead, the data indicate the presence of two serovars persisting even after subculture. The presence of more than one serotype in a single pig has been described previously [20, 21]; these findings underscore the need for additional purification steps (e.g., re-streaking and re-serotyping) when further characterization of individual isolates is required.

To date, Taiwan and Australia are among the few countries where *A. pleuropneumoniae* serovar 15 has been reported as the most prevalent [18, 19]. This raises the intuitive speculation that Taiwan may have imported pigs from Australia, where serovar 15 dominates. However, according to government data dating back to 1971, Taiwan has not imported pigs from Australia. Over the past 20 years, the countries of origin for breeding pigs imported into Taiwan have been [22]: (1) United States: 46.2%; (2) Denmark: 22.0%; (3) Canada: 14.6%; (4) France: 5.1%; (5) United Kingdom: 4.0%; (6) Sweden: 3.8%; (7) Norway: 2.5%; (8) Finland: 2.0%. Given this data, the origin and potential transmission route of serovar 15 in Taiwan remain unclear and warrant further investigation.

Current Taiwanese *A. pleuropneumoniae* isolates showed the lowest resistance to cephalosporin drugs such as ceftiofur, cephalexin and cephalothin, with only 3.2%, 5.2% and 6.3% of isolates, respectively, classified as resistant. These results are similar to those in previous studies in 2002 [10], 2011 [7] and 2024 [19]. Only 9.3% of isolates were resistant to enrofloxacin, a fluoroquinolone antibiotic commonly used in swine. Gentamicin is a commonly used antimicrobial agent in Taiwan; the resistance of *A. pleuropneumoniae* isolates was 28.4% in this study, considerably lower than that reported in 2011 [7].

A similar phenomenon was found for the older antimicrobial agent lincospectin: resistance was 97.6% in a previous study [7] compared with 11.8% in this study. Florfenicol is a broad-spectrum antibiotic used exclusively in farm animals to replace chloramphenicol. Our results showed that 45.1% of isolates were resistant to florfenicol, which is comparable to resistance rates reported in Korea (36%) [23] and China (47.4%) [24]. The data contrast with many non-Asian countries where florfenicol resistance is rare [9]. A high level of resistance (96.7%) to florfenicol was also observed in *Salmonella enterica* serovar Choleraesuis in Taiwan [25]. These findings imply that AMR remains a severe clinical problem in Asian countries. In addition, our data suggest that certain traditional antimicrobial agents, e.g. gentamicin and lincospectin, are still relatively active against current *A. pleuropneumoniae* isolates owing to their comparatively low resistance rates.

Building on our findings regarding AMR, we propose practical stewardship measures to mitigate antimicrobial use and safeguard therapeutic efficacy in Taiwanese pig production. These measures include: (i) establishing a tiered antimicrobial formulary classifying first-, second-, and third-line antibiotics according to their impact on human and animal health; (ii) transitioning from routine group medication in feed to targeted, case-based parenteral treatment guided by rapid diagnostic testing and local antimicrobial susceptibility profiles; (iii) strengthening evidence-based vaccination programs to reduce

overall disease prevalence; (iv) investing in improved husbandry and biosecurity, specifically optimizing climate control, stocking density, and quarantine practices to limit pathogen transmission; and (v) introducing a national benchmarking system to transparently monitor and report farm-level antimicrobial usage, incentivizing sustained reductions. Collectively, these strategies support an "injectable-only-when-needed" approach, aligning Taiwanese practices with international principles for AMR management.

Conclusions

This study determined the serovar prevalence and AMR patterns of clinical Taiwanese *A. pleuropneumoniae* isolates. Clinically relevant *A. pleuropneumoniae* serovars present in Taiwanese farms included, in descending order of prevalence, 15, 5, 1, 7, and 2. Local *A. pleuropneumoniae* isolates exhibited high resistance to antimicrobials currently used in the Taiwanese pig production, such as amoxicillin, ampicillin, and florfenicol. In addition, our study indicates that *A. pleuropneumoniae* serovar distribution and resistance patterns can be diverse and change over time. Therefore, alternative strategies for the control and treatment of *A. pleuropneumoniae* are essential, including the design of suitable vaccination programs and increased attention to ventilation (climatic conditions and air quality), management practices, stress factors, and biosecurity [2].

Methods

Specimen collection and bacterial isolates

From 2015 to 2022, 96 isolates of *A. pleuropneumoniae* from 64 different pig farms were isolated from diseased pigs. The clinical specimens—including lung, pleura, and pericardial swabs—were primarily obtained from necropsied pigs that had succumbed to clinical respiratory disease. Bacterial isolation was performed at the Animal Disease Diagnostic Center of National Pingtung University of Science and Technology (NPUST), Taiwan. Only samples associated with clearly documented outbreak events were included, ensuring that the isolates represented confirmed respiratory disease cases. Moreover, the farms in this study spanned all major swine-producing regions in Taiwan. *A. pleuropneumoniae* isolates were isolated overnight on chocolate agar plates supplemented with PolyViteX (bioMérieux, Marcy l'Etoile, France) at 37 °C with 5% CO₂. Isolates were purified and then characterized by standard biochemical and morphological tests to confirm their identities as described previously [7].

Antimicrobial susceptibility testing (AST)

All bacterial strains were immediately subcultured on supplemented chocolate agar plates used for the primary isolation (see above) and submitted for AST by

the disk diffusion test as described previously [7]. The test employed commercially available antimicrobial-impregnated disks, including a disk labeled "Lincospectin," which contains approximately 33.3% lincomycin and 66.6% spectinomycin (aligning with its common field formulation). Isolates were categorized as susceptible, intermediate or resistant using zone diameter standards as described previously [10], and the NCCLS standard used is equivalent to the current CLSI standard. In total 19 antimicrobial agents were tested. Fourteen of these (AMO, AMP, CEF, CFX, CTN, DOX, ENR, FFC, GEN, KAN, OTC, STR, TIL, TMP/SMX) follow the panel and three-letter codes reported by the Ke et al., 2024 [19].

To provide a broader overview of current on-farm practice in Taiwan we additionally included apramycin, spiramycin, erythromycin, tetracycline (base molecule) and the combination lincospectin (lincomycin + spectinomycin). Their in-house codes (APR, SP, ERY, TET and LS, respectively) are flagged in the legend beneath Table 2 and clearly distinguished from the Ke et al., 2024 panel [19].

A. pleuropneumoniae serotyping

Serotyping was done at Imperial College London by using capsule-loci-based multiplex PCRs (mPCRs) designed to detect all 19 known serovars of *A. pleuropneumoniae* [6, 26]. In brief, based on methods previously reported [27], an aliquot of *A. pleuropneumoniae* cultured in the laboratory was added to FTA cards and sent from Taiwan to London where DNA was eluted by a simple water wash, and used as template in the serotyping mPCRs. Serovars were confirmed using DNA obtained from *A. pleuropneumoniae* serovar reference strains as template. The reference strains used were: (4074) serovar 1, S1536 (serovar 2), L20 (serovar 5), WF83 (serovar 7), HS143 (serovar 15) as described in Stringer et al. [6].

Acknowledgements

We thank all members of the Animal Disease Diagnostic Center of National Pingtung University of Science and Technology for assisting with the diagnostic work.

Authors' contributions

YL, JB, and PL were responsible for the serotyping; HJC, MTC provided materials and clinical samples; CNL, WFK, PL, and PM drafted the manuscript. All authors read and approved the submitted version.

Funding

This study was partly funded by Ceva, and The United Kingdom Biotechnology and Biological Research Council (BB/S005897/1 and BB/S002103/1).

Data availability

Data is provided within the manuscript.

Declarations

Ethics approval and consent to participate

This study did not involve any animal experiments, the Institutional Animal Care and Use Committee (IACUC) of the National Pingtung University of

Science and Technology (NPUST) did not deem it necessary for this research group to obtain formal approval to conduct this study. All clinical samples were submitted to the Animal Disease Diagnostic Center in NPUST for diagnostic purpose.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Ceva Animal Health, Taipei, Taiwan. ²Department of Infectious Disease, Imperial College London, London W2 1PG, UK. ³Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan. ⁴Sustainable Swine Research Center, National Pingtung University of Science and Technology, Pingtung, Taiwan. ⁵Animal Disease Diagnostic Center, College of Veterinary Medicine, National Pingtung University of Science and Technology, Pingtung, Taiwan. ⁶Ceva Corporate Swine, Libourne, France.

Received: 27 February 2025 Accepted: 13 June 2025

Published online: 04 August 2025

References

- Shope RE. Porcine contagious pleuropneumonia: I. Experimental transmission, etiology, and pathology. *J Exp Med*. 1964;119(3):357–68.
- Sassu EL, Bossé JT, Tobias TJ, Gottschalk M, Langford PR, Hennig-Pauka I. Update on *Actinobacillus pleuropneumoniae*—knowledge, gaps and challenges. *Transbound Emerg Dis*. 2018;65:72–90.
- Martin PR, Shea RJ, Mulks MH. Identification of a plasmid-encoded gene from *Haemophilus ducreyi* which confers NAD independence. *J Bacteriol*. 2001;183(4):1168–74.
- Gottschalk M. The challenge of detecting herds sub-clinically infected with *Actinobacillus pleuropneumoniae*. *Vet J*. 2015;206(1):30–8.
- Mortensen P, Toft N, Kiss I, Palya V, Smits H, Tenk M. Comparative efficacy in challenge dose models of a toxin expressing whole-cell vaccine against eight serovars of *Actinobacillus pleuropneumoniae* in pigs. *Animals*. 2022;12(23):3244.
- Stringer OW, Bossé JT, Lacouture S, Gottschalk M, Fodor L, Angen Ø, Velazquez E, Penny P, Lei L, Langford PR. Proposal of *Actinobacillus pleuropneumoniae* serovar 19, and reformulation of previous multiplex PCRs for capsule-specific typing of all known serovars. *Vet Microbiol*. 2021;255:109021.
- Yang CY, Lin CN, Lin CF, Chang TC, Chiou MT. Serotypes, antimicrobial susceptibility, and minimal inhibitory concentrations of *Actinobacillus pleuropneumoniae* isolated from slaughter pigs in Taiwan (2002–2007). *J Vet Med Sci*. 2011;73(2):205–8.
- O'Neill C, Jones SCP, Bossé JT, Watson CM, Williamson SM, Rycroft AN, Kroll JS, Hartley HM, Langford PR. Prevalence of *Actinobacillus pleuropneumoniae* serovars in England and Wales. *Vet Rec*. 2010;167(17):661.
- Michael GB, Bossé JT, Schwarz S. Antimicrobial resistance in *Pasteurella* of veterinary origin. *Microbiol Spectr*. 2018;6(3). <https://doi.org/10.1128/microbiolspecarba-0022-2017>.
- Chang CF, Yeh TM, Chou CC, Chang YF, Chiang TS. Antimicrobial susceptibility and plasmid analysis of *Actinobacillus pleuropneumoniae* isolated in Taiwan. *Vet Microbiol*. 2002;84(1–2):169–77.
- Li YC, Chiou MT, Lin CN. Serodynamic analysis of the piglets born from sows vaccinated with modified live vaccine or E2 subunit vaccine for classical swine fever. *Pathogens*. 2020;9(6):427.
- Nunes T, Costa F, Li Y, Bossé JT, Langford PR, Mortensen P. Identification of *Actinobacillus pleuropneumoniae* serotypes 4, 5, and 17 in swine pleuropneumonia outbreak in Portuguese swine herds. In: 12th European Symposium of Porcine Health Management. 2021;2021:343.
- Kardos G, Sárközi R, Laczkó L, Marton S, Makrai L, Bányai K, Fodor L. Genetic diversity of *Actinobacillus pleuropneumoniae* serovars in Hungary. *Vet Sci*. 2022;9(10):511.
- Li Y, Bossé JT, Williamson SM, Maskell DJ, Tucker AW, Wren BW, Rycroft AN, Langford PR. *Actinobacillus pleuropneumoniae* serovar 8 predominates in England and Wales. *Vet Rec*. 2016;179(11):276.
- Cohen LM, Bossé JT, Stegger M, Li Y, Langford PR, Kielland C, Klem TB, Gulliksen SM, Ranheim B, Grøntvedt CA. Comparative genome sequence analysis of *Actinobacillus pleuropneumoniae* Serovar 8 isolates from Norway, Denmark, and the United Kingdom indicates distinct phylogenetic lineages and differences in distribution of antimicrobial resistance genes. *Front Microbiol*. 2021;12:729637.
- Schuwerk L, Hoeltig D, Waldmann K-H, Valentin-Weigand P, Rohde J. Sero- and *apx*-typing of German *Actinobacillus pleuropneumoniae* field isolates from 2010 to 2019 reveals a predominance of serovar 2 with regular *apx*-profile. *Vet Res*. 2021;52:1–10.
- Ozawa M, Kawano M, Abo H, Issiki Y, Kumakawa M, Kawanishi M, Kojima A, Iwamoto S. Characterization of *Actinobacillus pleuropneumoniae* isolated from pigs in Japan using whole genome sequencing. *Comp Immunol Microbiol Infect Dis*. 2023;102:102062.
- Yee S, Blackall P, Turni C. Genetic diversity and toxin gene distribution among serovars of *Actinobacillus pleuropneumoniae* from Australian pigs. *Australian Vet J*. 2018;96(1–2):17–23.
- Ke CH, Lai PY, Hsu FY, Hsueh PR, Chiou MT, Lin CN. Antimicrobial susceptibility and resistome of *Actinobacillus pleuropneumoniae* in Taiwan: a next-generation sequencing analysis. *Vet Q*. 2024;44(1):1–13.
- Arnal Bernal JL, Gottschalk M, Lacouture S, Galofré-Milán M, Lacasta D, Domínguez L, de la Fuente L, Pastor-García A, Maldonado J, Magallón-Botaya R. Serotype diversity of *Actinobacillus pleuropneumoniae* detected by real-time PCR in clinical and subclinical samples from Spanish pig farms during 2017–2022. *Vet Res*. 2024;55:165.
- Cuccato M, Divari S, Ciaramita S, Sereno A, Campelli D, Biolatti PG, Biolatti B, Meliotta F, Bollo E, Cannizzo FT. *Actinobacillus pleuropneumoniae* serotypes by multiplex PCR identification and evaluation of lung lesions in pigs from Piedmont (Italy) farms. *Animals*. 2024;14(15):2255.
- Council of Agriculture, Executive Yuan. Pig Breeding Data from 1971 to 2023. https://pigbase.angrin.tlri.gov.tw/pigfarm/pigreg_import3.asp?rsn=DM. Accessed on 2025/2/19.
- Kim B, Hur J, Lee JY, Choi Y, Lee JH. Molecular serotyping and antimicrobial resistance profiles of *Actinobacillus pleuropneumoniae* isolated from pigs in South Korea. *Vet Q*. 2016;36(3):137–44.
- Yao X, Song Q, Zhu W, Wei J, Shao D, Liu K, Li Z, Qiu Y, Ma Z, Xia L. Characterization of small plasmids carrying florfenicol resistance gene *florR* in *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* isolates from swine in China. *Front Vet Sci*. 2023;10:1084491.
- Le AX, Hsu FY, Hsueh PR, Lin WH, Chen HH, Chiou MT, Lin CN. Antimicrobial resistance profile in *Salmonella enterica* serovar Choleraesuis isolates from diseased pigs in Taiwan. *J Microbiol Immunol Infect*. 2024;57:660–4.
- Li Y, Bossé JT, Stringer OW, Hennig-Pauka I, Mortensen P, Langford PR. Detection of Novel *Actinobacillus pleuropneumoniae* Serovars by Multiplex PCR: a Cautionary Tale. *Microbiol Spectr*. 2023;11(2):e04461–e4422.
- Stringer OW, Bossé JT, Lacouture S, Gottschalk M, Fodor L, Angen Ø, Velazquez E, Penny P, Lei L, Langford PR. Rapid detection and typing of *Actinobacillus pleuropneumoniae* serovars directly from clinical samples: Combining FTA® card technology with multiplex PCR. *Front Vet Sci*. 2021;8:728660.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.