Veterinary Integrative Sciences 2026; 24(2): e2026037-1-14. DOI: 10.12982/VIS.2026.037

Veterinary Integrative Sciences
ISSN; 2629-9968 (online)

Research article

Lytic activity of novel bacteriophages recovered from pig farm sewage against multidrug-resistant *Escherichia coli*

Vilakone Luangmanyvongkhao[1], Nattha Vigad[1], Wattana Pelyuntha[2,3], Kitiya Vongkamjan[4] and Kridda Chukiatsiri[1,*]

[1] *Faculty of Animal Science and Technology, Maejo University, Chiang Mai 50290, Thailand.*
[2] *Futuristic Science Research Center, School of Science, Walailak University, Nakhon Si Thammarat 80160, Thailand.*
[3] *Research Center for Theoretical Simulation and Applied Research in Bioscience and Sensing, Walailak University, Thasala, Nakhon Si Thammarat 80160, Thailand.*
[4] *Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok 10900, Thailand.*

Abstract

Antimicrobial resistance (AMR) presents a critical challenge to both human and animal health, with multidrug-resistant (MDR) *Escherichia coli* (*E. coli*) posing serious risks in livestock systems, particularly in pig farming. This study aimed to isolate and characterize bacteriophages (phages) from pig farm sewage and evaluate their lytic efficacy against MDR *E. coli* recovered from diarrheal pig feces. Forty *E. coli* isolates (n = 40) from pigs at different production stages exhibited high resistance to amoxicillin (100%) and oxytetracycline (95%), while maintaining susceptibility to enrofloxacin (98%) and gentamicin (85%). Thirteen phages (ECVL1–ECVL13) were isolated from pig farm sewage samples, among which ECVL1, ECVL2, and ECVL6 showed strong lytic activity, lysing over 90% of the tested isolates. A phage cocktail composed of these three phages was tested against representative *E. coli* isolates at various multiplicity of infections (MOIs). At MOI 10^5, bacterial reductions of up to 71% were observed within 6 h, particularly for isolate VL19, indicating a dose-dependent and time-sensitive response. The findings support the feasibility of using highly lytic phage cocktails to reduce MDR *E. coli* in pig production environments. These results highlight phage therapy as a viable biocontrol strategy for AMR mitigation, especially in agricultural systems burdened by high antibiotic use.

Keywords: Antibiotic resistance, Biocontrol, Colibacillosis, *Escherichia coli*, Phage cocktail.

*Corresponding author: Kridda Chukiatsiri, Faculty of Animal Science and Technology, Maejo University, Chiang Mai 50290, Thailand. E-mail: kridda003@hotmail.com.

Article history; *received manuscript: 30 April 2025, revised manuscript: 23 May 2025, accepted manuscript: 12 July 2025, published online: 18 August 2025,*
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

Antimicrobial resistance (AMR) continues to be a major global health concern, posing significant threats to both human and animal populations (World Health Organization, 2020). Among drug-resistant pathogens, *E. coli* is especially worrisome due to its remarkable ability to acquire and disseminate resistance genes, resulting in infections that can be increasingly challenging to treat (McEwen and Collignon, 2018). Multidrug-resistant (MDR) *E. coli* strains, those resistant to multiple classes of antibiotics, commonly arise in clinical and agricultural contexts, leading to prolonged illnesses, increased healthcare expenses, and elevated mortality rates (Rasheed et al., 2014).

The swift expansion of MDR *E. coli* is closely linked to the overuse and misuse of antibiotics in human healthcare and animal husbandry (Van Boeckel et al., 2017). Within livestock production, antibiotics are often utilized for therapeutic, prophylactic, and growth-promoting purposes, generating selective pressure that hastens the evolution and spread of resistant bacterial populations (World Health Organization, 2020). Pig farming, in particular, has been pinpointed as a hotspot for MDR *E. coli* due to high stocking densities, elevated antibiotic usage, and frequent interactions among animals, humans, and the surrounding environment (O'Neill, 2016). The emergence of MDR *E. coli* poses a significant threat to human health, as infections caused by these strains are more difficult to treat, often requiring last-resort antibiotics and leading to higher morbidity, mortality, and healthcare costs. Moreover, the potential for zoonotic transmission further amplifies the public health risk. Consequently, there is an urgent need for new, more sustainable approaches to control MDR bacterial infections.

Bacteriophages (phages), viruses that specifically infect and lyse bacterial cells, offer a compelling alternative to conventional antibiotics (Altamirano and Barr, 2019). Unlike broad-spectrum antibiotics, phages typically exhibit narrow host ranges, thus limiting adverse effects on beneficial microbiota (Pires et al., 2016). Their self-propagating nature allows them to multiply as long as susceptible bacterial targets remain, potentially reducing the risk of incomplete treatments (Principi et al., 2025). Indeed, phage therapy has shown promise for tackling MDR *E. coli* in numerous applications, including wastewater treatment, food safety, and clinical care (Abedon, 2018). Nevertheless, successful phage therapy depends on several critical factors, such as isolating highly lytic phages, ensuring phage stability across diverse environments, and mitigating the emergence of phage-resistant bacterial mutants.

Recognizing the urgent need for innovative strategies, this study aimed to isolate and characterize phages from pig farm sewage, evaluate their lytic activity against multidrug-resistant *E. coli*, and select effective phage candidates for potential cocktail development. The outcomes of this research could contribute to the advancement of phage-based biocontrol methods, with important implications for agricultural sustainability, food safety, and public health efforts to combat antibiotic resistance.

MATERIALS AND METHODS

Isolation and identification of *Escherichia coli* recovered from diarrheal pig feces

This study was approved by the Animal Care and Use Committee for Science and Technology at Maejo University (approval no. MACUC013A/2566), and all procedures involving animals were conducted in accordance with relevant ethical guidelines and regulations.

Fresh diarrheal fecal samples were collected from pigs exhibiting clinical signs of diarrhea at commercial pig farms, with farm owner consent obtained prior

to sampling. Samples were collected aseptically using sterile cotton swabs directly from the rectum of individual pigs to minimize contamination. The swabs were then placed in sterile, labeled containers. Each sample was labeled with relevant metadata, including farm location, pig age, and date of collection. The samples were transported on ice to the laboratory and processed immediately upon arrival.

In the laboratory, one gram of each fecal sample was suspended in 9 mL of sterile phosphate-buffered saline (PBS) and vortexed thoroughly to obtain a homogenous suspension. A 100-µL aliquot of the suspension was then spread onto eosin methylene blue (EMB) agar plates using a sterile loop. The plates were incubated aerobically at 37 °C for 18–24 hours. Following incubation, colonies displaying typical *E. coli* morphology and metallic green sheen colonies on EMB were selected for further identification. Single colonies were picked and re-streaked on Luria-Bertani (LB) agar to obtain pure isolates. Presumptive *E. coli* isolates were subjected to a series of standard biochemical tests to confirm their identity: Gram staining, oxidase test, catalase test, IMViC series test (to differentiate among members of the Enterobacteriaceae family based on their metabolic characteristics, helping to identify *E. coli* and related bacteria), and triple sugar iron (TSI) test (to assess an organism's ability to ferment sugars (glucose, lactose, sucrose) and to produce hydrogen sulfide and gas, aiding in the identification and characterization of enteric bacteria).

Antibiotic susceptibility test of *Escherichia coli*

The antibiotic resistance (ABR) test was performed using the standard agar disk diffusion method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2024). Bacterial isolates from culture broth were adjusted to an optical density (OD625) of 0.08–0.1 using Luria-Bertani (LB) broth. The diluted bacterial suspension was evenly spread onto Mueller-Hinton agar (MHA) using a sterile cotton swab. Antibiotic disks, including cephalexin (CL), neomycin (NEO), enrofloxacin (ENR), sulfamethoxazole-trimethoprim (SXT), oxytetracycline (OTC), gentamicin (GEN), amoxicillin (AMX), and colistin (CT), were placed on the surface of the MHA plates. The plates were then incubated at 37 °C for 18 hours, after which the inhibitory zones were measured. Based on the CLSI standard guidelines, the results were interpreted and classified as sensitive, intermediate, or resistant. All experiments were performed in two independent replicates.

Isolation and characterization of phages from pig farm sewages

A total of 11 sewage samples were collected from a pig farm at the Faculty of Animal Science and Technology, Maejo University, Chiang Mai, Thailand. Samples were collected in sterile 1 L bottles and kept on ice during transport to the laboratory. All samples were stored at 4 °C until they were processed for phage isolation. *E. coli* phages were isolated using an enrichment technique with a mixture of three *E. coli* host strains (VL2, VL5, and VL12), following the procedure described by Pelyuntha et al. (2021).

Briefly, 100 mL of each water sample was mixed with 2.5 g of LB powder and 3 mL of the host mixture, then incubated at 37 °C for 18 h. After centrifugation, the supernatant was filtered through 0.20 µm syringe filters. The resulting filtrate was used in a double-layer agar method prepared with LB, followed by incubation at 37 °C for 18–24 h. A distinct plaque was picked and suspended in 300 µL of Salt-Magnesium (SM) buffer for purification. An appropriate dilution of this plaque suspension underwent three consecutive passages using the double-layer agar technique with the specific host that had produced a positive result (Pelyuntha and Vongkamjan, 2023). A plaque from the third purification passage was subsequently used to prepare 10-fold serial dilutions in SM buffer for another overlay and for phage lysate preparation. The phage titer was then determined by counting plaques on each plate at a given dilution.

Host-range determination

A spot assay was performed to determine each phage's lytic ability. A 10 µL aliquot of phage lysate, containing approximately 8 log PFU/mL, was carefully spotted onto a bacterial lawn of a specific *E. coli* strain. The bacterial lawn was prepared by mixing an overnight culture of the strain (adjusted to an appropriate optical density, typically OD600 = 0.1–0.2) with 0.7% soft agar, which was then overlaid onto a solid agar plate. After the phage suspension was applied, the plates were left undisturbed for 10–15 minutes at room temperature to allow absorption. The plates were then incubated at 37°C for 24 hours. Following incubation, the plates were examined for clear zones (plaques), which indicate successful bacterial lysis due to phage infection (Chanthavong et al., 2025; Pelyuntha et al., 2025b). All experiments were performed in two independent replicates.

Lytic capability of phage cocktail on multidrug-resistant *Escherichia coli*

To prepare a phage cocktail, three phages were selected for cocktail preparation based on their highest lytic activity from host-range determination test against the target bacterial strains. Each phage (9 log PFU/mL), including ECVL1, ECVL2, and ECVL6, was mixed in an equivalent quantity (1: 1: 1) to obtain a working cocktail stock. SM buffer or PBS was used as a diluent to obtain the desired phage titer (Pelyuntha et al., 2024; Pelyuntha et al., 2025a). Phage cocktail titers were also determined by observing the plaques in each dilution plate.

A suspension of phage cocktail (9 log PFU/mL, 20 mL) was mixed with a suspension of each host (VL19, VL28, and VL40), presenting ~2 to 3 log CFU/mL (20 mL) in a flask (MOI 10^7). Three *E. coli* isolates were chosen to represent each pig age group. The culture of each *E. coli* without the phage cocktail was used as a control. All mixtures were incubated at 37 °C for 24 h. The number of *E. coli* cells in the control and phage treatment conditions was evaluated at every 6 h interval for 24 h using a spread plate technique on eosin methylene blue (EMB) agar (Chanthavong et al., 2025). All treatments and controls were run in triplicate.

Statistical analysis

Statistical analysis was performed using SPSS (Version 22.0) of Windows statistics software (SPSS Inc., Chicago, IL, USA). Data on *E. coli* count during the incubation time of the lytic capability experiment were subjected to variance analysis followed by Tukey's range test. A significant difference between the control and treatments was calculated using the independent-sample T-test. A difference was also considered statistically significant at a p-value of less than 0.05.

RESULTS

Antibiotic resistance profiles of *Escherichia coli* isolated from diarrheal pig feces

Forty *E. coli* were isolated from pig fecal samples of sucking piglets (n =20), weaning piglets (n =10), and fattened pigs (n = 10). The antibiotic sensitivity test of *E. coli* isolated from pigs at various growth stages showed that isolates were highly sensitive to enrofloxacin (98%) and gentamicin (85%). Intermediate sensitivity was observed for sulfamethoxazole-trimethoprim (60%), neomycin (45%), and cephalexin (50%). Colistin showed predominantly intermediate sensitivity (92%), as shown in Figure 1. Conversely, significant resistance was detected toward amoxicillin (100%) and oxytetracycline (95%), suggesting the limited effectiveness of these antibiotics against *E. coli* in pigs.

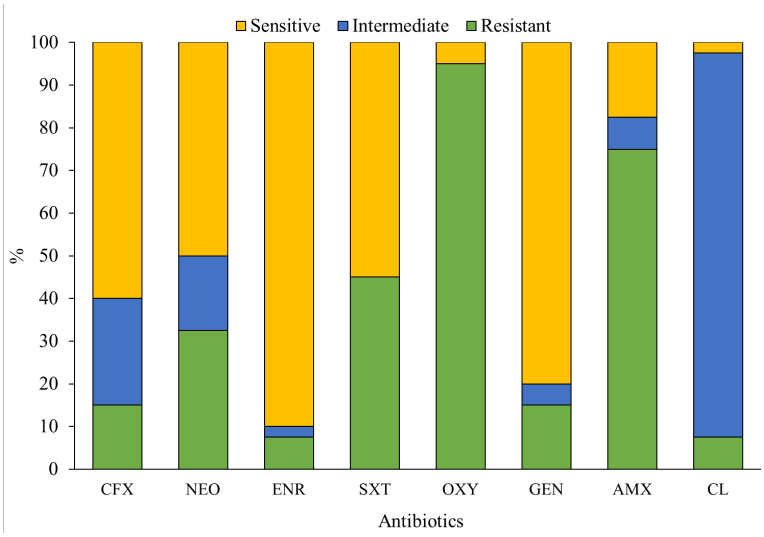

Figure 1 Antibiotic resistance profiles of *Escherichia coli* isolated from diarrheal pig feces.

The results indicated varying levels of antibiotic resistance, intermediate susceptibility, and sensitivity of *E. coli* across different growth stages of pigs (Figure 2). For suckling piglets (N=20), high resistance was observed against oxytetracycline (90%), amoxicillin (90%), and neomycin (65%), with intermediate resistance to colistin (85%) and intermediate sensitivity to enrofloxacin (80%) and gentamicin (60%). In weaning piglets (N=10), resistance notably shifted to oxytetracycline (100%) and amoxicillin (50%), with complete sensitivity (100%) to enrofloxacin, gentamicin, and colistin. Finally, in fattened pigs (N=10), there was full resistance (100%) to oxytetracycline and high resistance to sulfa-trimethoprim (60%) and amoxicillin (70%), while strong sensitivity (100%) was found for cephalexin, enrofloxacin, and gentamicin, and intermediate resistance to colistin (90%).

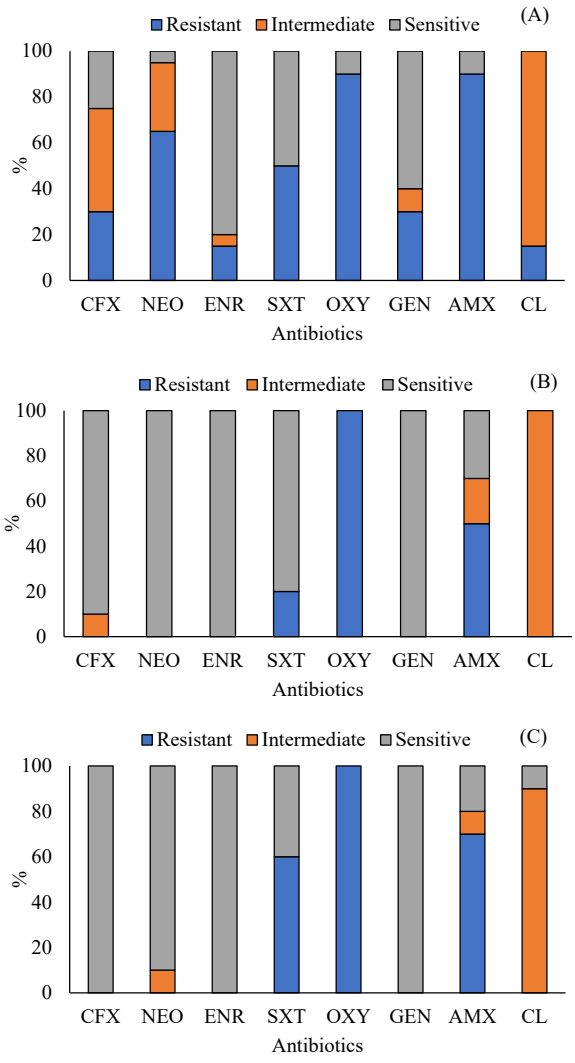

Figure 2 Antibiotic resistance profiles of *Escherichia coli* isolated from diarrheal pig feces with different growth stages. (A) suckling stage, (B) weaning stage, (C) fattened stage.

Phage lytic profile on *Escherichia coli* isolates

The heatmap illustrates the lytic activity of 13 phages (ECVL1-ECVL13) against 40 *E. coli* isolates (VL1-VL40), as shown in Figure 3. Phages ECVL1, ECVL2, and ECVL6 exhibited the highest lytic activity, with effectiveness percentages of 95%, 95%, and 92.5%, respectively, lysing most isolates (particularly VL1-VL40). Other phages displayed minimal effectiveness, with lytic percentages ranging from 2.5-7.5%.

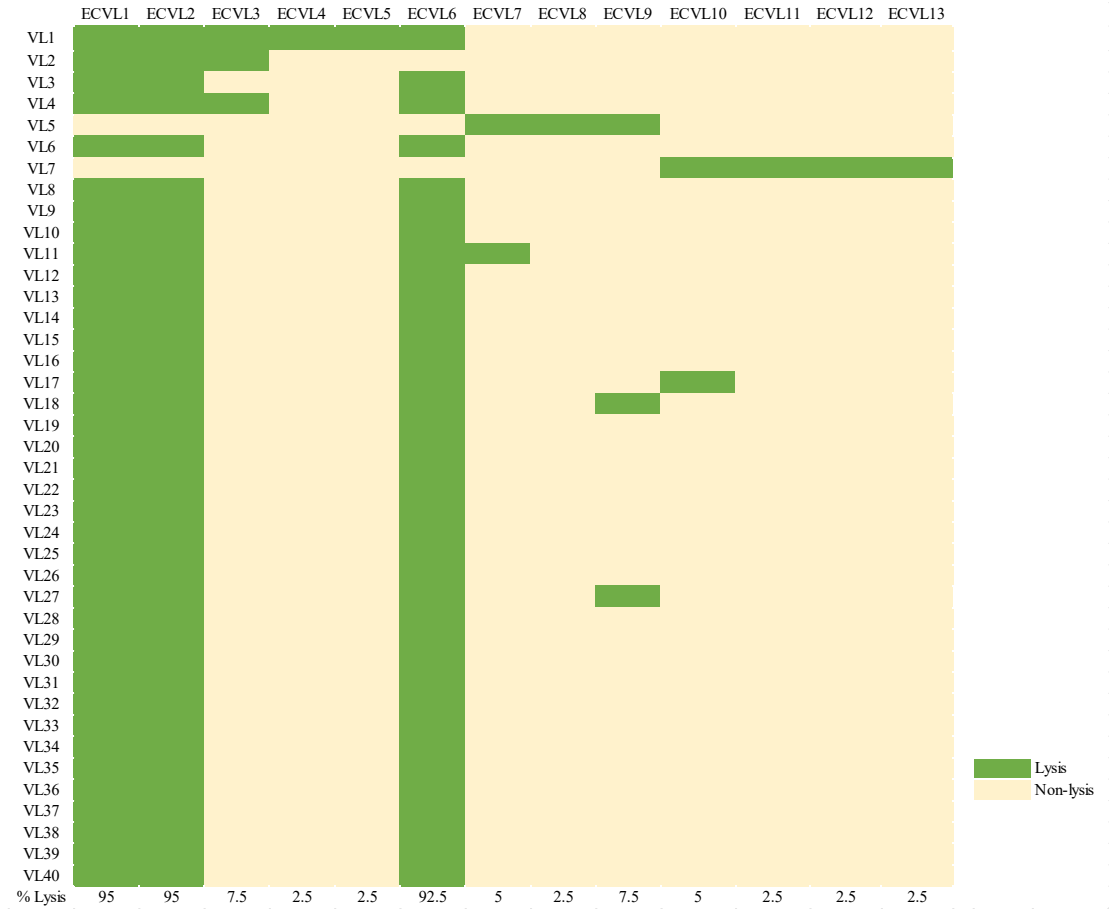

Figure 3 Heatmap illustrates the lytic activity of 13 phages (ECVL1-ECVL13) against 40 *E. coli* isolates (VL1-VL40). The green areas indicate lysis, and the gold areas indicate non-lysis.

Reduction of *Escherichia coli* by a phage cocktail

The study investigated the efficacy of the phage cocktail against three *E. coli* isolates (VL19, VL28, and VL40) at different concentrations (MOI 10^3, 10^4, and 10^5) over 24 h (Table 1). For isolate VL19, the highest reduction was observed at 6 h with MOI 10^5 (71.35% reduction from 8.69 to 2.49 log CFU/mL), followed by MOI 10^4 (64.56%) and MOI 10^3 (64.33%). The reductions were substantial at 12 h, with 65.31%, 50.86%, and 44.00% for MOI 10^5, 10^4, and 10^3, respectively. For VL28, the maximum reduction occurred at 6 h with MOI 10^5 (60.90% reduction), while MOI 10^4 achieved a 47.26% reduction. At 12 h, MOI 10^5 maintained effectiveness with a 43.52% reduction. For VL40, the highest reductions were at 6 h, with MOI 10^5 (34.47%) and MOI 10^4 (33.48%). All isolates showed a pattern where phage effectiveness generally decreased over time, with the 24-hour reductions being lower than those at 6 and 12 h across all MOI levels. Notably, MOI 10^5 consistently demonstrated the most significant reduction for all three isolates throughout the experiment, with VL19 showing the highest susceptibility to phage treatment. These results suggest that higher phage concentrations (MOI 10^5) provide more effective control of *E. coli* populations, particularly during the first 12 h of treatment, with varying effectiveness among different bacterial isolates.

Table 1 Effect of *Escherichia coli* phage cocktail at different multiplicities of infection (MOI) on the reduction of representative *Escherichia coli* isolates

Isolates	Times (h)	*E. coli* count (log CFU/mL)			
		Control	Phage cocktail		
			MOI 10^3	MOI 10^4	MOI 10^5
VL19	0	3.11 ± 0.10^{aA}	3.05 ± 0.07^{aA}	3.08 ± 0.05^{aA}	3.05 ± 0.01^{abA}
	6	8.69 ± 0.04^{bC}	3.10 ± 0.10^{bB}	3.08 ± 0.04^{aB}	2.49 ± 0.00^{aA}
	12	9.34 ± 0.01^{cD}	5.23 ± 0.31^{cC}	4.59 ± 0.02^{bB}	3.24 ± 0.05^{bA}
	18	9.56 ± 0.21^{cD}	8.34 ± 0.03^{dC}	7.24 ± 0.14^{cB}	5.65 ± 0.04^{cA}
	24	9.75 ± 0.33^{cC}	9.52 ± 0.37^{eC}	8.20 ± 0.23^{dB}	6.11 ± 0.07^{dA}
VL28	0	3.11 ± 0.05^{aA}	3.04 ± 0.08^{aA}	3.15 ± 0.02^{aA}	3.13 ± 0.21^{aA}
	6	8.21 ± 0.03^{bD}	6.92 ± 0.05^{aC}	4.33 ± 0.03^{bB}	3.21 ± 0.03^{aA}
	12	8.52 ± 0.33^{bC}	8.62 ± 0.06^{bC}	6.54 ± 0.03^{cB}	4.84 ± 0.17^{bA}
	18	8.94 ± 0.02^{bD}	8.18 ± 0.03^{cC}	7.64 ± 0.22^{dB}	5.74 ± 0.02^{cA}
	24	9.52 ± 0.12^{cB}	9.30 ± 0.11^{cB}	9.37 ± 0.00^{eB}	6.56 ± 0.09^{dA}
VL40	0	3.05 ± 0.00^{aA}	3.08 ± 0.02^{aA}	3.00 ± 0.19^{aA}	3.08 ± 0.06^{aA}
	6	7.05 ± 0.14^{bC}	5.80 ± 0.09^{bB}	4.69 ± 0.33^{bA}	4.62 ± 0.18^{bA}
	12	8.70 ± 0.11^{cB}	6.71 ± 0.09^{cA}	6.32 ± 0.14^{cA}	6.24 ± 0.05^{cA}
	18	9.27 ± 0.03^{dD}	8.52 ± 0.01^{dC}	7.95 ± 0.03^{dB}	6.62 ± 0.04^{cA}
	24	9.79 ± 0.10^{dC}	9.52 ± 0.10^{eC}	8.33 ± 0.38^{eB}	7.39 ± 0.24^{dA}

All values were provided as mean ± standard deviations of triplicate (n = 3). The different lowercase letters (a, b, c, d, or e) indicate a significant difference ($p < 0.05$) in the same column (time) of each treatment and control, whereas the different uppercase letters (A, B, C, or D) indicate a significant difference ($p < 0.05$) in the same row of each treatment and control.

DISCUSSION

The antibiotic resistance pattern of *E. coli* isolates from pig sources in our study demonstrates significant variability across different antibiotic classes. Oxytetracycline showed the highest resistance rate (~94%), followed by amoxicillin (~75%) and sulfamethoxazole-trimethoprim (~45%). In contrast, enrofloxacin remained the most effective, with only ~8% resistance observed. This resistance profile aligns with findings from Phayao Province, Thailand, where 92.77% of pig-derived *E. coli* isolates showed resistance to multiple antibiotics (Boonkerd and Chaikhiandee, 2022). Notably, they observed high resistance to ampicillin (80–95.7%), trimethoprim-sulfamethoxazole (84%), tetracycline (62%), and chloramphenicol (61%). The high resistance to tetracyclines, particularly oxytetracycline, aligns with national surveillance data from China, which reported a 96.26% resistance rate among pig isolates (Peng et al., 2022). This widespread resistance is likely linked to the extensive use of tetracyclines in pig farming, where they are often employed not only for therapeutic purposes but also for prophylaxis and growth promotion. The prolonged and sometimes indiscriminate use of these antibiotics contributes to selective pressure that facilitates the development and spread of resistant strains. Similarly, the substantial resistance to amoxicillin (~75%) can be attributed to its common usage in pig production systems in many Southeast Asian countries. Beta-lactam antibiotics like ampicillin and amoxicillin are frequently used due to their affordability and broad-spectrum activity, but this also accelerates the emergence of resistance, as reflected in both our findings and those from other regional studies. The intermediate level of resistance to sulfamethoxazole-trimethoprim (~45%) observed in our study is lower than that reported in healthy pigs raised in deep litter systems in western Thailand (70–80%) (Mitchaothai and Srikijkasemwat, 2021), yet similar to findings from studies along the Thailand-Laos border (Pungpian, 2020). This variation may be influenced by

differences in antibiotic usage practices, farm management systems, and biosecurity levels across regions. The relatively low resistance to enrofloxacin (~8%) suggests limited use or more regulated application of fluoroquinolones in the sampled farms. However, the emergence of resistance, even at low levels, warrants attention due to the critical importance of fluoroquinolones in both veterinary and human medicine. The alarming 90% intermediate resistance to colistin contrasts with Chinese data in 2017, showing only 3.79% of agricultural settings. (Peng et al., 2022), Given that colistin is considered a last-resort antibiotic for treating multidrug-resistant Gram-negative infections, this elevated resistance rate may indicate the emergence and spread of mobile colistin resistance genes (such as *mcr* variants), horizontal gene transfer among bacterial populations, or increased selective pressure due to inappropriate or excessive use of colistin in animal production. These findings highlight an urgent need for surveillance and stewardship to prevent further dissemination of colistin resistance. Growth stage variations are evident across studies, with Thailand's Phayao Province reporting MDR rates of 82.62% in piglets compared to 36.66% in adult swine and 66.66% in sick swine (Boonkerd and Chaikhiandee, 2022), demonstrating how resistance accumulates differently across production phases. This pattern mirrors global trends where piglets and weaning stages exhibit higher resistance rates due to intensive antimicrobial use during these vulnerable phases. Comparing regional data, Thailand's resistance patterns (63% of surveyed farms in Khon Kaen province) (Huber et al., 2021) appear comparable to regional neighbors but lower than China's extensive MDR rates (90.54% nationwide) (Peng et al., 2022), highlighting geographical variations in antimicrobial use practices. Medium-scale intensive farms in Thailand demonstrate significantly higher antimicrobial resistance than small-scale extensive farms (Huber et al., 2021), reflecting how production systems influence resistance development across Southeast Asia and globally.

The heatmap analysis of phage lysis activity against 40 *E. coli* isolates reveals a striking pattern of variable effectiveness among the 13 tested phages. Phages ECVL1, ECVL2, and ECVL6 demonstrated exceptional lytic activity (95%, 95%, and 92.5%, respectively), while the remaining phages showed substantially lower effectiveness. This variability in phage performance aligns with findings from multiple swine-focused studies, though the high efficacy of the top-performing phages in this analysis exceeds typical results reported elsewhere. A comparable study with 17 phages isolated from Polish pig farms showed similar variability in efficacy, but with lower overall effectiveness – their phages lysed between 1.9% and 57.7% of tested ESBL/AmpC *E. coli* strains (Skaradzińska et al., 2017). This contrast suggests that the ECVL phage collection contains particularly potent candidates for phage therapy applications. The Polish study also found that approximately 17.3% of bacterial isolates were completely resistant to all tested phages (Skaradzińska et al., 2017), indicating a common phenomenon of bacterial resistance across different phage collections. The observed pattern of having a few highly effective phages among a larger collection matches findings from other swine studies. For instance, research evaluating five phages (WPEC1-WPEC5) isolated from pig environments showed lytic profiles ranging from 46.0% to 64.0% against antibiotic-resistant *E. coli* isolates (Chanthavong et al., 2025), still notably lower than the top performers in the current heatmap. This validates the exceptional nature of phages ECVL1, ECVL2, and ECVL6 within the broader context of swine phage research. Such variable host-phage interactions have been documented in other studies and highlight the complex dynamics of phage-bacterial relationships (de Souza and Stefani, 2024). This variability underscores the importance of thorough host range testing when developing phage therapies. Regarding in vivo applications, several swine studies have demonstrated the efficacy of phage therapy against *E. coli* infections. A single dose of phage cocktail (9 log PFU/mL) reduced *E. coli* counts in piglet gastrointestinal tracts by 1.33 log units after 7 days and improved fecal scores compared to control groups (Chanthavong et al.,

2025). Other research has shown that phages can significantly decrease *E. coli* concentrations within hours of application (Songphasuk et al., 2022), though bacterial regrowth was consistently observed in all phage treatments, suggesting the need for repeated applications or carefully designed cocktails. The time dynamics of phage therapy merit consideration – studies show greater bacterial reduction at 48-96 h post-treatment compared to measurements taken within 24 h (Songphasuk et al., 2022). This delayed peak effectiveness might explain the mixed results observed in short-duration trials and suggests that extended monitoring periods may be necessary to fully evaluate phage efficacy.

The cocktail approach, combining multiple phages with complementary host ranges, has proven particularly effective in swine studies. In the Polish pig farm research, a three-phage cocktail achieved 80.8% coverage of tested strains (Skaradzińska et al., 2017), comparable to what might be achieved by combining the most effective phages (ECVL1, ECVL2, and ECVL6) from the current heatmap. Infection and cell lysis assays with swine-derived phages have demonstrated approximately 75% reduction in bacterial growth after just 2 h (de Souza and Stefani, 2024), highlighting the rapid action potential of well-selected phages. Beyond direct antibacterial effects, phage supplementation has shown broader benefits in pig production. Studies report improved nitrogen digestibility in pigs fed phage-supplemented diets compared to negative controls (Yan et al., 2012), suggesting nutritional and growth advantages beyond pathogen control. This collective evidence confirms the exceptional nature of the highly effective phages identified in the current heatmap and supports the potential development of a targeted phage cocktail using ECVL1, ECVL2, and ECVL6 for controlling *E. coli* infections in swine production systems. Such an approach aligns with the growing interest in phage therapy as an alternative to antibiotics in animal agriculture, particularly valuable in addressing the rising concern of antimicrobial resistance.

The observed reduction in *E. coli* counts with higher MOI values (particularly 10^5) aligns with findings from Kudva et al. (1999), who demonstrated that phage efficacy depends significantly on MOI, with superior results occurring at higher concentrations (MOI of 10^3) (Niu et al., 2021). Similarly, the dose-dependent effect observed in our study corresponds with findings where "higher phage concentrations were more effective" at reducing pathogenic *E. coli* counts (Dissanayake et al., 2019). The variable effectiveness against different *E. coli* isolates in our study (with VL19 showing greater susceptibility than VL28 and VL40) parallels observations by Cornelissen et al. (2011), who noted that phage cocktail effectiveness varies significantly depending on the targeted *E. coli* strains (Niu et al., 2021). Additionally, our finding that effectiveness generally decreased over time for all isolates corresponds with observations in biofilm studies where some *E. coli* strains (like DS515) showed the ability to recover after initial phage suppression (Sanchez et al., 2022). The greater efficacy of higher MOI (10^5) treatment in our study, particularly in the first 12 h, reflects similar findings in catheter biofilm studies where higher doses of phage cocktails (10^9 PFU/mL) resulted in a greater reduction (~4 log CFU/mL) in bacterial burden compared to medium doses (Sanchez et al., 2022). This emphasizes the importance of appropriate phage concentration for successful treatment. Interestingly, while our study showed consistent effectiveness at higher MOI values, O'Flynn et al. (2004) reported optimal MOI values as low as 0.1 to 0.01 for their phage CE1 (Tang et al., 2023), highlighting how phage efficacy parameters can vary significantly between different phage preparations and target strains. Our observation of a significant reduction in all three *E. coli* isolates at higher phage concentrations is consistent with findings from engineered phage cocktails that demonstrated better reduction of *E. coli* load than their components (Gencey et al., 2024). However, it is important to note that in vivo studies have shown that promising in vitro results do not always translate to similar outcomes in living systems, as demonstrated by studies where phage cocktails that reduced *E. coli* by 1.5 log CFU/mL in fermentation systems failed to achieve a significant reduction in murine models (Buttimer et al., 2022). These comparisons

highlight that phage effectiveness depends on multiple factors, including phage concentration, target strain susceptibility, treatment duration, and testing environment. Despite the promising potential of phages as alternatives to antibiotics, several limitations affect their practical application in real-life settings, especially in livestock. One challenge is the need for a high multiplicity of infection (MOI) to ensure efficacy, which can be difficult to achieve and maintain in the gastrointestinal environment of animals (Abedon, 2011). The host immune system may also neutralize phages upon repeated exposure, reducing their effectiveness (Dąbrowska, 2019). Additionally, harsh conditions in the gut, including low pH, enzymes, and bile salts, can degrade phages before they reach their target bacteria (Jończyk et al., 2011). The complex gut microbiota and bacterial biofilms may further hinder phage access and reduce lytic efficiency (Salmond and Fineran, 2015). Therefore, future studies should focus on improving phage delivery systems and testing efficacy under realistic in vivo conditions. The significant reduction in *E. coli* counts achieved with higher MOI values in our study supports the potential of phage cocktails as an alternative to antibiotics, though optimization of treatment parameters remains critical for successful application.

CONCLUSIONS

This study demonstrates the promising potential of using highly lytic phage cocktails, particularly ECVL1, ECVL2, and ECVL6, to target MDR *E. coli* in pig farming systems. The dose- and strain-dependent reductions observed validate the therapeutic promise of phages as sustainable alternatives to antibiotics. For practical on-farm applications, future work should focus on in vivo trials to assess safety, efficacy, and optimal dosing strategies under real production conditions. Additionally, studies on phage stability under varying environmental and gastrointestinal conditions will be vital. Expanding phage isolation to other farm environments and targeting different resistant pathogens will further strengthen phage-based solutions in animal agriculture. The holistic integration of phage therapy into farm biosecurity and antibiotic stewardship programs is key to transforming AMR management across livestock systems.

ACKNOWLEDGEMENTS

The authors acknowledge the Faculty of Animal Science and Technology, Maejo University, Chiang Mai, Thailand, and the Department of Biotechnology, Faculty of Agro-Industry, Kasetsart University, Bangkok, Thailand, for instruments and facilities to conduct the research work.

AUTHOR CONTRIBUTIONS

Vilakone Luangmanyvongkhao: Conceptualization, Data Curation, Investigation, Methodology, Visualization, and Writing – Original Draft Preparation. **Nattha Vigad**: Conceptualization, Data Curation, Investigation, Methodology, Project Administration, Visualization. **Wattana Pelyuntha**: Conceptualization, Data Curation, Investigation, Methodology, Supervision, Visualization, Writing – Original Draft Preparation, and Writing - Review & Editing. **Kitiya Vongkamjan**: Conceptualization, Resources, Supervision. **Kridda Chukiatsiri**: Conceptualization, Funding Acquisition, Resources, Supervision, and Writing-Review & Editing. All authors approved the final draft of the manuscript.

CONFLICT OF INTEREST

The authors declare that this research study was conducted without the influence of any commercial or financial relationships that could possibly be construed as a potential conflict of interest.

REFERENCES

Abedon, S.T., 2011. Lysis from without. Bacteriophage. 1(1), 46-49.

Abedon, S.T., 2018. Phage therapy: various perspectives on how to improve the art. In: Medina, C., López-Baena, F. (Eds.), Host-pathogen interactions. Methods in molecular biology. Humana, New York, pp. 113-127.

Altamirano, F.L.G., Barr, J.J., 2019. Phage therapy in the post antibiotic era. Clin. Microbiol. Rev. 32(2), e00066-18.

Boonkerd, N., Chaikhiandee, S., 2022. Antibiotic resistance of *Escherichia coli* isolated from swine feces in Phayao Province. Burapha Sci. J. 1530-1543.

Buttimer, C., Sutton, T., Colom, J., Murray, E., Bettio, P.H., Smith, L., Bolocan, A.S., Shkoporov, A., Oka, A., Liu, B., Herzog, J.W., Sartor, R.B., Draper, L.A., Ross, R.P., Hill, C., 2022. Impact of a phage cocktail targeting *Escherichia coli* and *Enterococcus faecalis* as members of a gut bacterial consortium in vitro and in vivo. Front. Microbiol. 13, 936083.

Chanthavong, V., Vigad, N., Pelyuntha, W., Yamik, D.Y., Vongkamjan, K., Yingkajorn, M., Chaisowwong, W., Tippaya, K., Tadee, P., Chukiatsiri, K., 2025. Effectiveness of a single-dose phage cocktail on the reduction of multidrug-resistant *Escherichia coli* in suckling piglets. Vet. Microbiol. 302, 110395.

Clinical and Laboratory Standards Institute, 2024. Performance standards for antimicrobial susceptibility testing, 34th edition. Wayne, PA, USA, CLSI.

Cornelissen, A., Ceyssens, P.J., T'Syen, J., Van Praet, H., Noben, J.P., Shaburova, O.V., Krylov, V.N., Volckaert, G., Lavigne, R., 2011. The T7-related *Pseudomonas putida* phage ϕ15 displays virion-associated biofilm degradation properties. PLoS ONE, 6(4), e18597.

de Souza, A.L.F., Stefani, L.D.C.M., 2024. Isolation of lytic bacteriophages of *Escherichia coli* from swine. Braz. J. Vet. Res. Anim. Sci. 61, e222458.

Dąbrowska, K., 2019. Phage therapy: What factors shape phage pharmacokinetics and bioavailability? Systematic and critical review. Med. Res. Rev. 39(5), 2000-2025.

Dissanayake, U., Ukhanova, M., Moye, Z.D., Sulakvelidze, A., Mai, V., 2019. Bacteriophages reduce pathogenic *Escherichia coli* counts in mice without distorting gut microbiota. Front. Microbiol. 10, 1984.

Gencay, Y.E., Jasinskytė, D., Robert, C., Semsey, S., Martínez, V., Petersen, A., Brunner, K., de Santiago Torio, A., Salazar, A., Turcu, I.C., Eriksen, M.K., Koval, L., Takos, A., Pascal, R., Schou, T.S., Bayer, L., Bryde, T., Johansen, K.C., Bak, E.G., Smrekar, F., Doyle, T.B., Satlin, M.J., Gram, A., Carvalho, J., Jessen, L., Hallström, B., Hink, J., Damholt, B., Troy, A., Grove, M., Clube, J., Grøndahl, C., Haaber, J.K., van der Helm, E., Zdravkovic, M., Sommer, M.O.A., 2024. Engineered phage with antibacterial CRISPR-Cas selectively reduce *E. coli* burden in mice. Nat. Biotechnol. 42(2), 265-274.

Huber, L., Hallenberg, G.S., Lunha, K., Leangapichart, T., Jiwakanon, J., Hickman, R.A., Magnusson, U., Sunde, M., Järhult, J.D., Van Boeckel, T.P., 2021. Geographic drivers of antimicrobial use and resistance in pigs in Khon Kaen Province, Thailand. Front. Vet. Sci. 8, 659051.

Jończyk, E., Kłak, M., Międzybrodzki, R., Górski, A., 2011. The influence of external factors on bacteriophages. Folia Microbiol. 56, 191-200.

Kudva, I.T., Jelacic, S., Tarr, P.I., Youderian, P., Hovde, C.J., 1999. Biocontrol of *Escherichia coli* O157 with O157-specific bacteriophages. Appl. Environ. Microbiol. 65(9), 3767–3773.

McEwen, S.A., Collignon, P.J., 2018. Antimicrobial resistance: a one health perspective. Microbiol. Spectr. 6(2), 1-26.

Mitchaothai, J., Srikijkasemwat, K., 2021. Antimicrobial resistance in fecal *Escherichia coli* from different pig production systems. Anim. Biosci. 35(1), 138.

Niu, Y.D., Liu, H., Du, H., Meng, R., Sayed Mahmoud, E., Wang, G., McAllister, T.A., Stanford, K., 2021. Efficacy of individual bacteriophages does not predict efficacy of bacteriophage cocktails for control of *Escherichia coli* O157. Front. Microbiol. 12, 616712.

O'Flynn, G., Ross, R.P., Fitzgerald, G.F., Coffey, A., 2004. Evaluation of a cocktail of three bacteriophages for biocontrol of *Escherichia coli* O157:H7. Appl. Environ. Microbiol. 70(6), 3417–3424.

O'Neill, J., 2016. Tackling drug-resistant infections globally: final report and recommendations. The Review on Antimicrobial Resistance. Available online: https://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover. pdf

Pelyuntha, W., Ngasaman, R., Yingkajorn, M., Chukiatsiri, K., Benjakul, S., Vongkamjan, K., 2021. Isolation and characterization of potential *Salmonella* phages targeting multidrug-resistant and major serovars of *Salmonella* derived from broiler production chain in Thailand. Front. Microbiol. 12, 662461.

Pelyuntha, W., Vongkamjan, K., 2023. Control of *Salmonella* in chicken meat by a phage cocktail in combination with propionic acid and modified atmosphere packaging. Foods. 12(22), 4181.

Pelyuntha, W., Ngasaman, R., Yingkajorn, M., Chukiatsiri, K., Guyonnet, V., Vongkamjan, K., 2024. Phage cocktail administration to reduce *Salmonella* load in broilers. Res. Vet. Sci. 169, 105163.

Pelyuntha, W., Narkpao, T., Yamik, D.Y., Kiatwuthinon, P., Sanguankiat, A., Kovitvadhi, A., Vongkamjan, K., 2025a. Efficiency, cytotoxicity, and survivability evaluation of *Salmonella* phage cocktail against *Salmonella* derived from broiler sources. Vet. World. 18(2), 475.

Pelyuntha, W., Yamik, D.Y., Vetboocha, N., Vongkamjan, K., 2025b. Effect of novel phage cocktail on *Salmonella* recovered from broiler sources and its anti-biofilm effect on food contact surface model. Food Control. 169, 111000.

Peng, Z., Hu, Z., Li, Z., Zhang, X., Jia, C., Li, T., Dai, M., Tan, C., Xu, Z., Wu, B., Chen, H., Wang, X., 2022. Antimicrobial resistance and population genomics of multidrug-resistant *Escherichia coli* in pig farms in mainland China. Nat. Commun. 13(1), 1116.

Pires, D.P., Oliveira, H., Melo, L.D., Sillankorva, S., Azeredo, J., 2016. Bacteriophage-encoded depolymerases: their diversity and biotechnological applications. Appl. Microbiol. Biotechnol. 100(5), 2141-2151.

Principi, N., Silvestri, E., Esposito, S., 2019. Advantages and limitations of bacteriophages for the treatment of bacterial infections. Front. Pharmacol. 10, 513.

Pungpian, C., 2020. Genetic characteristics of antimicrobial resistance in *Escherichia coli* isolated from pigs, pork and humans in Thailand and Lao PDR border provinces (Theses and Dissertations). Chulalongkorn University, p. 403.

Rasheed, M.U., Thajuddin, N., Ahamed, P., Teklemariam, Z., Jamil, K., 2014. Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources. Rev. Inst. Med. Trop. Sao. Paulo. 56, 341-346.

Salmond, G.P., Fineran, P.C., 2015. A century of the phage: past, present and future. Nat. Rev. Microbiol. 13(12), 777-786.

Sanchez, B.C., Heckmann, E.R., Green, S.I., Clark, J.R., Kaplan, H.B., Ramig, R.F., Muldrew, K.L., Hines-Munson, C., Skelton, F., Trautner, B.W., Maresso, A.W.,

2022. Development of phage cocktails to treat *E. coli* catheter-associated urinary tract infection and associated biofilms. Front. Microbiol. 13, 796132.

Skaradzińska, A., Śliwka, P., Kuźmińska-Bajor, M., Skaradziński, G., Rząsa, A., Friese, A., Roschanski, N., Murugaiyan, J., Roesler, U.H., 2017. The efficacy of isolated bacteriophages from pig farms against ESBL/AmpC-producing *Escherichia coli* from pig and turkey farms. Front. Microbiol. 8, 530.

Songphasuk, T., Imklin, N., Sriprasong, P., Woonwong, Y., Nasanit, R., Sajapitak, S., 2022. Bacteriophage efficacy in controlling swine enteric colibacillosis pathogens: An in vitro study. Vet. World. 15(12), 2822.

Tang, Z., Tang, N., Wang, X., Ren, H., Zhang, C., Zou, L., Han, L., Guo, L., Liu, W., 2023. Characterization of a lytic *Escherichia coli* phage CE1 and its potential use in therapy against avian pathogenic *Escherichia coli* infections. Front. Microbiol. 14, 1091442.

Van Boeckel, T.P., Glennon, E.E., Chen, D., Gilbert, M., Robinson, T.P., Grenfell, B.T., Levin, S.A., Bonhoeffer, S., Laxminarayan, R., 2017. Reducing antimicrobial use in food animals. Sci. 357(6358), 1350-1352.

World Health Organization, 2020. Global antimicrobial resistance surveillance system (GLASS) report: early implementation *2020*. World Health Organization. Available online: https://iris.who.int/handle/10665/332081.

Yan, L., Hong, S.M., Kim, I.H., 2012. Effect of bacteriophage supplementation on the growth performance, nutrient digestibility, blood characteristics, and fecal microbial shedding in growing pigs. Asian-Australas J. Anim. Sci. 25(10), 1451.

How to cite this article;
Vilakone Luangmanyvongkhao, Nattha Vigad, Wattana Pelyuntha, Kitiya Vongkamjan, and Kridda Chukiatsiri. Lytic activity of novel bacteriophages recovered from pig farm sewage against multidrug-resistant *Escherichia coli.* Veterinary Integrative Sciences. 2026; 24(2): e2026037-1-14.