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Outstanding Thesis Awards

Biological Science Section

Dr. David Yembilla Yamik

Doctoral Degree Program

Major in Biotechnology

Prevalence of Antibiotic-Resistant *Escherichia coli* in Farm Animals, Food, and the Environment and Evaluation of Phage Treatment as an Alternative to Antibiotics

FACULTY OF AGRO-INDUSTRY
DEPARTMENT OF BIOTECHNOLOGY

Advisory committee
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Introduction

The management of infections has been complicated by the rapid emergence of antibiotic resistance among bacterial pathogens, particularly *Escherichia coli* [1,2]. Livestock is the primary reservoir of resistant *E. coli*. Cross contamination can occur from farm to food and into the environment, posing significant public health concerns [3]. This research characterized *E. coli* phages, including their genomic features, to evaluate their safety and evaluated their potential as biocontrol agents for controlling antibiotic-resistant *E. coli* isolated from farm animals, food, and the environment in Thailand.

Materials and methods

This study isolated and characterized (phenotypically and genotypically) *E. coli*, from farm animals, meat-processing facilities, and the surrounding environment (wastewater) as well as isolated and and characterized (phenotypically and genotypically) phages, and evaluated an *E. coli* phage cocktail against biofilm formation resistant pathogenic *E. coli* in meat.

Results and Discussion

Prevalence of *E. coli* and virulence factors

Prevalence of *E. coli* across various sources varied significantly ($p = 0.000$). Out of the 383 samples, 230 samples (60.1%) were positive for *E. coli* (0.551–0.649, 95% CI): 72.3% (180/249) from animal sources, 33.7% (28/83) from food sources, and 43.1% (22/51) from environmental samples. The *sheA* gene was the most frequently detected virulence marker among the *E. coli* isolates (92.0%), followed by *eae* (4.0%), while the *stx1* gene was the least detected (0.9%) (Figure 1). Thirteen isolates harbored multiple virulence genes, with the following combinations observed: *eae-sheA, stx1-stx2-sheA-ehxA*, *sheA-ehxA*, and *stx2-sheA-ehxA*. However, suposedly nonpathogenic *E.coli* (NPE) recorded 9.6%

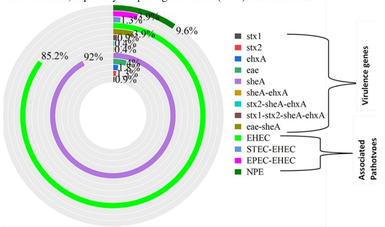


Figure 1. Virulence genes and pathotype of *E. coli*

Antibiotic resistance profile

All *E. coli* isolates showed resistance to at least one of the tested antibiotics. Overall, the antibiotic resistance profile indicated 28.4% resistance, 22.9% intermediate susceptibility, and 48.8% full susceptibility. The resistance exceeded 50.0% for streptomycin, ampicillin, tetracycline, and ciprofloxacin. In contrast, the lowest resistance was observed for amoxicillin-clavulanic acid (1.7%) (Figure 2).

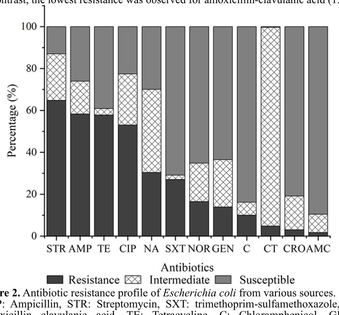


Figure 2. Antibiotic resistance profile of *Escherichia coli* from various sources.
AMP: Ampicillin, STR: Streptomycin, SXT: trimethoprim-sulfamethoxazole, NA: Nalidixic, AMC: Amoxicillin clavulanic acid, TE: Tetracycline, C: Chloramphenicol, GEN: Gentamicin, CIP: Ciprofloxacin, CT: Colistin, CRO: Ceftriaxone, NOR: Norfloxacin.

Multidrug resistance

Multidrug resistance (MDR) was observed among isolates from all sources, though the differences were not statistically significant ($p = 0.850$). Multidrug resistance (MDR) was detected in 61.1% of animal samples, 57.1% of food samples, and 50.0% of the environmental isolates (Figure 3).

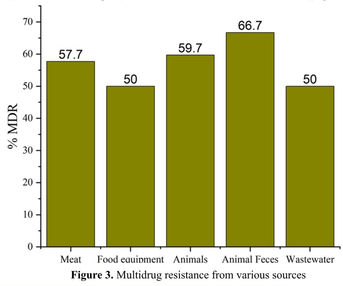


Figure 3. Multidrug resistance from various sources

Pathotype and multidrug resistance based on network associations

Resistance profiles also varied across *E. coli* pathotypes. Based on network associations, EHEC pathotypes predominantly exhibited MDR, with some isolates reaching pan-drug resistance (PDR) levels. EPEC-EHEC pathotypes were primarily associated with XDR, while NPE strains showed mainly MDR profiles (Figure 4). In total, 31 resistance gene patterns were observed, with *tetA-gyrA-strA-blaTEM* (22.1%) showing the most common in all the sources investigated.

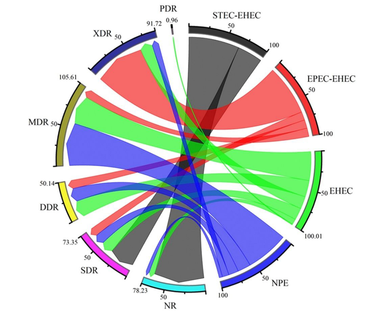


Figure 4. Network associations of resistance of levels and pathotype
NPE: non-pathogenic *E. coli*; EHEC: Enterohemorrhagic *E. coli*; EPEC-HEC: hybrid enteropathogenic/enterohemorrhagic *E. coli*; STEC-EHEC: hybrid Shiga toxin/enterohemorrhagic *E. coli*; NR: non-resistance; SDR: single drug-resistance; DDR: double drug-resistance

Phage characterization

All the phages have icosahedral heads, long contractile tails, base plates, and short tail fibers, consistent with the family *Myoviridae* (Figure 5). The phages vary in length, width, and total size (Figure 5)

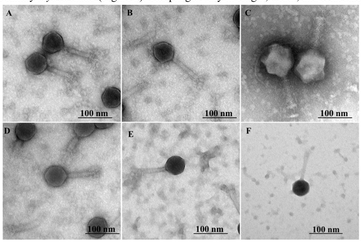


Figure 5. Morphology *E. coli* phages WPEC1 (A), WPEC2 (B), WPEC3 (C), WPEC4 (D), WPEC5 (E), and WPEC6 (F) under 100,000x magnifications under TEM analysis, and the scale bar is 100nm.

Whole genome sequencing of *Escherichia coli* phages

Whole-genome sequencing showed variation in genome size, ORF/tRNA, and DTRs among the six *E. coli* phages. Three phages contained high-confidence depolymerases, with no antibiotic resistance, virulence, or lysogeny-associated genes detected. Phylogenetic analysis classified WPEC1, WPEC2, WPEC4, and WPEC6 as *Phapecoctavirus* and WPEC3 and WPEC5 as *Asteriusvirus* (Figure 6).

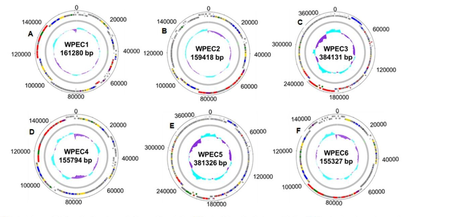


Figure 6. Circular genome maps and genomic annotation of *Escherichia* phages WPEC1, WPEC2, WPEC3, WPEC4, WPEC5, and WPEC6

Host-range determination and one-step growth

A total of 231 antibiotic-resistant *E. coli* strains, both pathogenic and non-pathogenic, originating from animals, sources, food, and the environment, were used to determine the host range of the six phages. From the results, the phage isolates exhibited varying lytic abilities (48.1 to 56.7%) with an average lytic ability of over 50% against all the *E. coli* strains tested. They also exhibited a similar latent period (10 min) and burst sizes ranging 16-192 particles/cell (Figure 7)

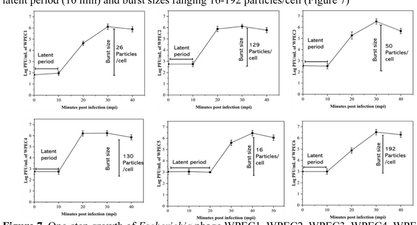


Figure 7. One-step growth of *Escherichia* phage WPEC1, WPEC2, WPEC3, WPEC4, WPEC5, and WPEC6 following infection with their natural host *Escherichia coli* EC3.18 at an MOI of 0.01 in LB broth at 37 °C. Values represent the means of three replications, with error bars indicating the standard deviation.

Phage susceptibility

The phage cocktail was stable in preservatives, across pH 2–11, temperature from 4–45℃, in 0.1 1.0% (v/v) chlorine, and different concentrations of sodium chloride (Salinity).

Phage application

The co-culture method at MOI 10000 was the most effective against biofilm formation (Figure 8). The 10% (v/v) phage cocktail was the most effective against *E. coli* in meat, including plant-based meat, eradicating *E. coli* chicken and beef within 0 h and 24 h (Figure 9; Figure 10), respectively, and significantly reducing *E. coli* in plant-based meat.

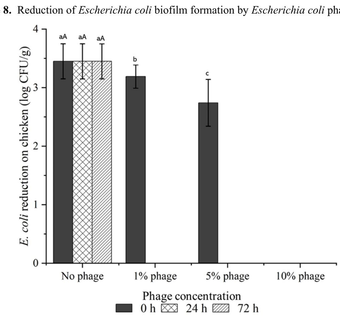


Figure 8. Reduction of *Escherichia coli* biofilm formation by *Escherichia coli* phage cocktail

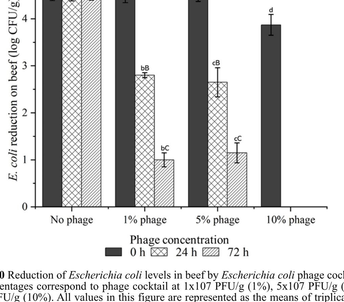


Figure 9. Reduction of *Escherichia coli* in chicken meat by *Escherichia coli* phage cocktail

Figure 10 Reduction of *Escherichia coli* levels in beef by *Escherichia coli* phage cocktail. The percentages correspond to phage cocktail at 1x107 PFU/g (1%), 5x107 PFU/g (5%), and 1x108 PFU/g (10%). All values in this figure are represented as the means of triplicate (n =3) treatments, with error bars indicating standard deviations. Different lowercase letters (a, b, c, d) indicate significant differences ($p < 0.05$) among phage cocktail concentrations and the control, whereas different uppercase letters (A, B, C) indicate significant differences ($p < 0.05$) among treatment times.

Conclusions

In conclusion, these findings underscore the need for enhanced antimicrobial stewardship, improved surveillance programs, and alternative control measures to mitigate resistance and its transmission from farms to food. The phage cocktail demonstrated strong potential for reducing resistant *E. coli* and biofilm formation.

References

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Thesis Book File