VIROLOGICA SINICA 2015, 30 (1): 73-75
DOI 10.1007/s12250-014-3541-8

LETTER

T4-like coliphage ΦKAZ14 virulent to pathogenic and extended spectrum β-lactamase-producing *Escherichia coli* of poultry origin

Dear Editor,

Bacteriophages (otherwise called phages) are a type of virus that infect bacteria. This viral type has found useful applications in the control of bacterial pathogens in foods and food processing environments. In addition, phages may be useful to prevent colonization and shedding of bacteria into the surrounding environment. Bacteriophages have been applied in the biocontrol of colibacillosis caused by avian pathogenic *Escherichia coli* (APEC) 078 serotype (Lau et al., 2010). There is a scarcity of literature on the application of bacteriophages in the biocontrol of APEC 01 serotype and extended-spectrum β-lactamase (ESBL)-producing *E. coli* in poultry. APEC 01 causes mortality in chickens and is a probable cause of urinary tract infection and newborn meningitisin in humans; thus, APEC 01 is a threat to public health. The aim of the present study was to isolate bacteriophages for the pre-harvest biocontrol of APEC 01 and ESBL-producing *E. coli* in chicken, in order to mitigate the risk of these pathogens to the food chain. Isolation and characterization of the T4-like coliphage ΦKAZ14, lytic to APEC 01 and ESBL-producing *E. coli,* is reported and discussed.

Avian pathogenic *E. coli* (ATCC 11775: Serovar O1:K1:H7) was purchased from ATCC, and ESBL-producing *E. coli* was isolated by plating cloacal swabs onto Chromocult® coliform agar (Chromocult, Merck, UK). Blue or violet colonies observed on the media were sub-cultured onto CHROMagar-ESBL (CHROMagar, Paris, France) and incubated aerobically overnight at 37 ºC. Colonies that appeared dark pink to reddish in color were presumptively identified as ESBL-producing *E. coli* (Coudron et al., 2000; Lagacé-Wiens et al., 2010; Turner et al., 2000). Isolate identities were confirmed by polymerase chain reaction (PCR) using specific primers for the detection of the blaTEM gene, which confers resistance to cephalosporins in Enterobacteriaceae. The primers used were 5'-AAAATTCTTGAAGACG-3' (forward) and 5'-TTACCAATGCTTAATCA-3' (reverse). PCR was performed as previously described (Bora et al., 2014; Sharma et al., 2010). Bacteriophages were isolated based on an established method (Oliveira et al., 2009). Host range and susceptibility of host bacteria to the isolated bacteriophage were determined by the modified double-layer technique (Carey-Smith et al., 2006). The isolated bacteriophage was purified and its morphology examined using a transmission electron microscope (LEO 912AB EFTEM) at 100,000–260,000×magnification (Carey-Smith et al., 2006). The identity of the phage was confirmed based on PCR to detect the g23 capsid gene (Filée et al., 2005) using the following primers: MZIA1bis (5′-GATATTTGIGGIGTTCAGCCIATGA-3′, forward), MZIA6 (5′-CGCGGTTGATTTCCAGCATGATTTC-3′, reverse).

E. coli was isolated from chicken cloaca and characterized based on presumptive phenotypic identification, using Chromocult coliform agar and CHROMagar as selective media for isolation of ESBL-producing *E. coli*. CHROMagar has been demonstrated as a suitable medium for specific isolation of ESBL-producing bacteria (Lagacé-Wiens et al., 2010). These isolates were resistant to some cephalosporins (cefotaxime, cefpodotaxime, ceftoxime and cetriaxone) based on disk sensitivity test. Molecular characterization was performed to confirm that *E. coli* isolates produced ESBL. PCR was performed with primers specific to the blaTEM gene, and the gene was detected in all five wild-type *E. coli* isolates. The blaTEM gene was identified 1080 base pairs (bp) in length on the gel (Figure 1A). The blaTEM gene is prevalent and confers resistance to cephalosporins in *E. coli*, *Kleibsella pneumonaea,* and other Enterobacteriaceae (Bora et al., 2014; Sharma et al., 2010; Manoharan et al., 2011). These investigations confirmed that *E. coli* isolated from the chicken cloaca were ESBL-producing.

Interest in the application of phages as biocontrol agents against bacterial infections and antibiotic resistance has steeply risen and is continuing to grow (Huff et al., 2004; Sulakvelidze et al., 2001; Chanishvili et al., 2001; Yosef et al., 2014). In the present study, we isolated a bacteriophage lytic to APEC 01 and ESBL-producing *E. coli*. The phage infected and lysed APEC 01 and three out of five ESBL-producing *E. coli* strains (used as host range indicators) (Table 1). The fact that

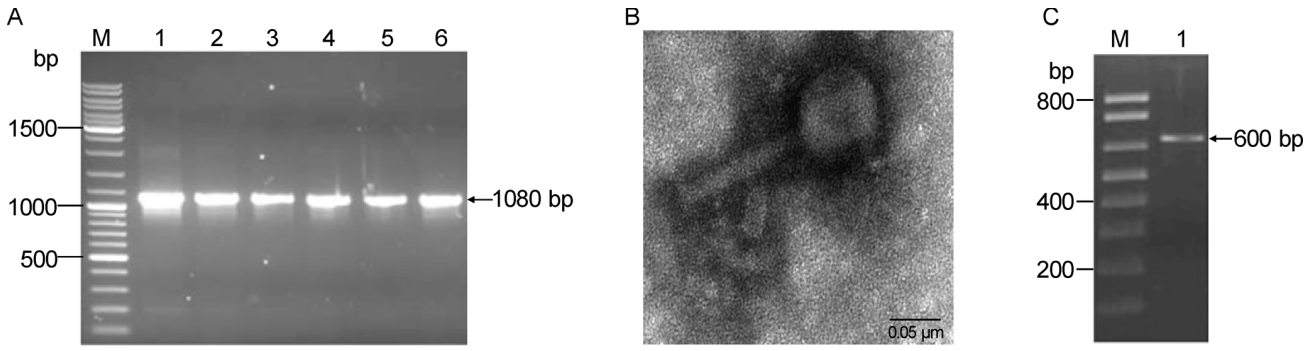

Figure 1. (A) Detection of blaTEM genes in *E. coli* isolated from chicken. M: Marker (100 bp); Lane 1: *Klebsiella pneumoniae* (ATCC 700603); Lane 2-6: *E. coli* isolated from chicken. The blaTEM gene was detected at 1080 bp. (B) Micrograph of field transmission electron microscopy of coliphage ΦKAZ14 isolated from chicken faeces capable of infecting avian pathogenic and extended spectrum *β*-lactamase producing *E. coli* from Chicken. (C) Gel electrophoresis amplified g23 gene fragments from coliphage ΦKAZ14 isolated from faeces of Chicken. M: 100 bp marker; 1: detected g23 gene fragment.

only three strains were infected and lysed indicates that the other two strains were not susceptible or lacked receptors for the initial attachment of the phage necessary for infection. The ability of the isolated phage to infect both APEC 01 and ESBL-producing *E. coli* demonstrates its polyvalent nature. This may provide a dual advantage compared with monovalent-type phages in biocontrol applications. Morphological observations indicated that the phage had an icosahedral head measuring 50 nm by 45 nm, with a long contractile tail measuring 78 nm by 10 nm (Figure 1B). Phages with similar morphological characteristics have been identified in previous investigations based on transmission electron microscopy (Ackermann and Nguyen, 1983). Previously, molecular characterization using PCR led to the detection of a 600 bp g23 capsid gene, consistent with other isolated bacteriophages classified as T4-like coliphages (Filée et al., 2005). The phage identified in the present study belongs to the family *Myoviridae* (Figure 1C). The detected g23 capsid gene is widespread in T4-type myoviruses, further confirming the classification of the identified phage as a T4-type myovirus.

Although T4-like phages infectious to avian pathogenic *E. coli* strains have been previously reported (Oliveira et al., 2009), there is a scarcity of published studies on T4-like bacteriophages virulent to ESBL-producing *E. coli* of poultry origin. We report for the first time identification of a phage designated as ΦKAZ14, which was lytic to cephalosporins resistant and ESBL-producing *E. coli* strains isolated from chicken. These results may be useful in the biocontrol of susceptible APEC 01, cephalosporins resistant and wild-type ESBL-producing *E. coli* in chicken. Further studies on the applications of ΦKAZ14 may help in developing better strategies to improve food safety and security.

Table 1. Host range spectrum of the isolated bacteriophage ΦKAZ14

No.	Species of Host	Strain	Plaque formation and size* (mm)
1	*Escherichia coli*	APEC (O1:K1:H7)	+
2	*Escherichia coli*	ESBL 1	-
3	*Escherichia coli*	ESBL 2	-
4	*Escherichia coli*	ESBL 3	+
5	*Escherichia coli*	ESBL 4	+
6	*Escherichia coli*	ESBL 5	-

* Approximately 2mm; ESBL: Extended spectrum beta lactamase *E. coli* strains; APEC (O1:K1:H7): Avian pathogenic E. coli; "+": positive lytic zones; "-": negative.

FOOTNOTES

Research University Grant Scheme (RUGS; grant number 9329400), University Putra Malaysia, partly funded the research. We acknowledge Drs. Abdulrasheed Aliyu Bello, Faruku Bande, and Ibrahim Mohammed Jalo for assistance with some reagents during the course of the study. All the authors declare that they have no competing interest. This article does not contain any studies with human or animal subjects performed by any of the authors.

Kaikabo Adamu Ahmad[1,3], Abdulkarim Sabo Mohanmmed[1✉], Faridah Abas[1], Sieo Chin Chin[2]

1. Department of Food Science, Faculty of Food Science and Technology, Universitiy Putra Malaysia, Selangor 43300, Malaysia
2. Department of Microbiology, Faculty of Biotechnology and Biomolecular Sciences, Universitiy Putra Malaysia, Selangor

43300, Malaysia
3. Bacterial Research Department, National Veterinary Research Institute, Plateau State 930010, Nigeria
✉Correspondence:
Phone: +60-03-8946-8537, Email: karimsabo@upm.edu.my
ORCID: 0000-0001-6233-1006

Published online: 3 February 2015

REFERENCES

Ackermann HW, Nguyen TM. 1983.Appl Environ Microbiol, 45: 1049–1059.
Bora A, Hazarika NK, Shukla SK,et al. 2014. Indian J Pathol Micr, 57:249–254.
Carey-Smith GV, Billington C, Cornelius AJ, et al. 2006. FEMS Microbiol Lett, 258: 182–186.
Coudron PE, Moland ES, Thomson KS. 2000. J Clin Microbiol, 38: 1791–1796.
Chanishvili N, Chanishvili T, Tediashvili M, et al. 2001. J Chem Technol Biot, 76: 689–699.
Filée J, Tétart F, Suttle CA, et al. 2005. Proc Natl Acad Sci U S A, 102: 12471–12476.
Huff WE, Huff GR, Rath NC, et al. 2004. Poult Sci, 83:1944–1947.
Manoharan A, Premalatha K, Chatterjee S, et al.2011. Indian J Med Microbi, 29:161–164.
Oliveira A, Sillankorva S, Quinta R, et al. 2009. J Appl Microbiol, 106: 1919–1927.
Lagacé-Wiens P, Tailor F, Simner P, et al. 2010. European Congress on Clinical Microbiology and Infectious Diseases, Vienna Austria.
Lau GL, Sieo CC, Tan WS, et al. Poult Sci, 89: 2589-2596.
Sharma J, Sharma M, Ray P. 2010. Indian J Med Res, 132:332–336.
Sulakvelidze A, Alavidze Z, Morris JG Jr. 2001. Antimicrob Agents Ch, 45: 649–659.
Turner KM, Restaino L, Frampton EW. 2000. J Food Protect, 63:539–541.
Yosef I, Kiro R, Molshanski-Mor S, et al. 2014. Bacteriophage, 4: e28491.