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Isolation of Diarrheagenic *Escherichia coli* and their Specific Phages from Rabbits

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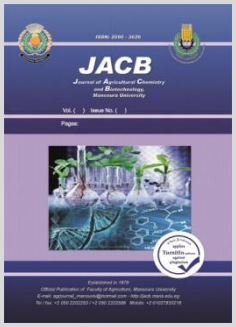

ABSTRACT

This study designed to isolation virulence *E. coli*, specific phages and identify in rabbits biochemically, serologically, detect virulence genes using PCR and susceptibility to antimicrobial range. For propose fifty-fecal-swab samples collected from diarrheic and freshly-dead-young-rabbits were presented to bacteriological examination. Data showed twenty-seven samples (54%) gave positive results of *Escherichia coli*. Data *in-vitro* Pathogenicity-test using Congo-red (CR) binding assay showed eight *E. coli* isolates (29.63%) were Congo-red positive. Antimicrobial susceptibility to several antibiotics was studied. *E. coli* isolates showed different susceptibility degrees to antibiotics, and isolates 3,4 and 8 categorized as multidrug resistant isolates. Three selected *E. coli* isolates were serologically identified classified as O169, O125 and O158 serotypes for isolates 4,8,3 respectively. Conventional polymerase chain reaction for detection *eaeA* and *Stx1* virulence genes revealed *E. coli* serotypes O158 expressed *eaeA* gene, but others, O169 and O125 don't expressed. While, none serotypes expressed *Stx1* gene. Phages may be used effectively to control of pathogenic *E. coli*, colonizing farm rabbit's intestines. However, harsh acidic-conditions and digestive enzymes activities influence phage infectivity, and decrease efficiency in application-trails. Natural-defensive-barrier development was being suitable for oral administration to farm poultry presented acid-stability. Encapsulated pahge beads in gelatin chitosan -matrix showed partial phage titer reductions. Phage beads titers were constant for storage in water, but complete release achieved after 6hr in simulated intestinal solution at 37°C. Finding multidrug resistant enterohaemorrhagic, *E. coli* serotypes created severe health hazard for rabbits and contact human. Encapsulated phages beads are promising and cost-effective method for bacteriophage targeting intestinal bacteria of farm-rabbits.

Keywords: Diarrheagenic *E. coli*, Pathogeniity test *in vitro*, Serotyping, Bacteriophage, Alginate, Chitosan and Phage encapsulation

INTRODUCTION

In Egypt, rabbits breeding were exposed to serious problems and great awareness was directed to this breeding due to the diseases it faces that cause very large economic losses to this industry (Saif-Eldin *et al.*, 1994). Diarrhea is a pattern of digestive disorders in immature rabbits and causes high mortality in baby rabbits. The severity of the disease is due to lead to secondary post infections follow-on reduced immunity (Yang *et al.*, 2017). The enteric diseases related to pathogenic *Escherichia coli* (*E. coli*) that colonized rabbit's intestine (Blanco *et al.*, 1996). *Escherichia coli* is a normal citizen of rabbit digestive flora and it does not affect pathogenic activity in rabbits, but when exposed to other pathogens or any stress they may activate its growth in the intestine causing the death of rabbits (Milon, 1996). The bacterial isolates are more frequent at early weaning period rather than suckling period and may be establish in adults (Shahin *et al.*, 2011). Enteropathogenic *E. coli* (EPEC) is an imperative reason of diarrhea in both animals and it is the only known class of *E. coli* in young rabbits, which encourages acute intestinal disease noticeable by inflammatory lesions of the gut where this *E. coli* is severely colonized (Licois, 2004 and García *et al.*, 2010). EPEC belongs to 12 dissimilar serotypes, the most common types in rabbits are O44 and O158 (Shahin *et al.*, 2011). Antimicrobials play a vital part in human and animal health care and used to treat and prevent bacterial infections in animal breeding (Ben Said *et al.*, 2015 and Reuland *et al.*, 2014). The unnecessary use of antibiotics led to the appearance of antibiotic-resistant bacteria such as *E. coli* in poultry farms (Yeh *et al.*, 2018; Ievy *et al.*, 2020) .The cost-effective fatalities in production of rabbits are difficult to control, because of several antimicrobial agents may not be used in rabbits and all at once numerous EPEC isolates become challenging to the drugs that regularly used (Moyenuddin *et al.*, 1989; Camarda *et al.*, 2004). The implications of the antibiotic resistance for public health require attention from both clinical and economic authorities (Tirumalai *et al.*, 2019). Using bacteriophages has been approved as a prospective biocontrol strategy for infections caused by multi drug resistant bacteria (MDRBs). It represented an alternative method for controlling bacterial infections owing to their capability to aim the specific cells of the bacterial host (Jassim & Limoges 2014 and Taha *et al.*, 2018). Previous studies also recommend using phage therapy to reduce the mortalities rate in infected rabbits farms (Xie *et al.*, 2005). The orally application of phage in human assessments was not describing any unfavorable properties (McCallin *et al.*, 2013). Though, the oral application of phage is not with no complexity because of, disclosure to gastric juice (GJ) in stomach that maybe has an effect on the infectivity of phages (Tothova *et al.*, 2012). Depending on that mentioned

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above, encapsulation techniques of phage have introduced defensive delivery system for bactriophage beside the acidic conditions of stomach by least loss of bacteriophage titer (Choińska-Pulit *et al*., 2015). Previous investigations mentioned the possibility of use some substances such as alginate and chitosan for the encapsulation of phages (Ma *et al*. 2008, 2012; Tang *et al*., 2013; Kim *et al*., 2015 and Colom *et al*., 2017). This study was planned to isolate of *E. coli* from rabbits and detect its susceptibility to their specific phages. Also develop phage beads defensive system for controlling of phages release and increasing its ability to survive pH degree in the animal stomach.

MATERIALS AND METHODS

Samples collection and preparation

By using sterile cotton swabs, a total of 50 fecal swabs were collected from 35 diarrheic and 15 freshly recently dead rabbits aged between 1-10 weeks old from different breeds localities in Cairo, Giza and Qalubia Governorates in Egypt. The breeds had a history of high mortality rates and severe diarrhea in young rabbits. Samples collection and preparation were done according to OIE, (2015).

Isolation of Diarrheagenic *E. coli* isolates

Fecal samples were inoculated on nutrient broth and incubated for 18-24 hrs at 37°C, then subcultured on Eosin methylene blue agar (EMB) and MacConkey's agar media. Cultural and morphological properties of *E. coli* suspected colonies were examined then suspected colonies were picked up and streaked into nutrient agar slants for further studies (Cheesbrough, 1985). Biochemical identification was carried out according to Edward and Ewing, (1972) by some biochemical tests such as, Oxidase, Catalase, Indole, Citrate, Urea utilization, Methyl red, Voges Proskauer , Haemolysis on blood agar and reaction on triple sugar iron agar.

Pathogenicity Testing *In Vitro*:

The pathogenicity of the obtained isolates were tested by Congo red dye binding test (CRDPT) as the method mentioned by Berkhoff and Vinal, (1986). Each isolate was cultured on Trypticase soy agar media complemented with 0.003% Congo red dye and 0.15% bile salts (Sigma). The positive result was recorded as appearance of red colonies after incubation for 24 hrs at 37°C.

Antibiotic sensitivity test for Diarrheagenic *E. coli* isolates.

Using the disc diffusion method mentioned by Bauer *et al*., (1966), the susceptibility of the *E. coli* isolates to antimicrobials was determined. Discs of Amoxicillin (10 µg), Ampicillin (10 µg), Amoxicillin-clavulanate (30 µg), Ciprofloxacin (5 µg), Doxycycline (30 µg), Gentamycin (10 µg), Neomycin (30 µg), Norfloxacin (10 µg), Streptomycin (10 µg), Sulfamethoxazole-Trimethoprim (25 µg) and Tetracycline (30 µg) (Oxoid Laboratory, Oxoid) were used for this purpose. Then plates were incubated for 24 hours at 37°C. Interpretation of the inhibition zone given by manufacturer of Clinical and Laboratory Standards Institute (CLSI) instructions (CLSI, 2015) was used to interpret isolates into sensitive or intermediate or resistant groups.

Serotyping of Diarrheagenic *E. coli* isolates.

Serological identification was carried out for the resistant isolates using slide agglutination test by *E. coli* specific antisera at: Animal Health Research Institute (AHRI); Dokki, and Giza; Egypt according to methods of Edwards and Ewing, (1972).

Detection of Virulence genes of Diarrheagenic *E. coli* isolates

In this study RT-PCR technique, was carried out at: the Animal health research institute; Dokki; Giza, Egypt for three *E. coli* isolates were chosen for detection of virulence genes. DNA extraction was carried out according to, QIAamp DNA mini kit instructions. Preparation of PCR Master Mix was presented according to Emerald Amp GT PCR master mix kit (Takara), code no. RR310A. Visualization of PCR products was accomplished by gel electrophoresis in 1.5% agarose in Tris–acetate EDTA (TAE) buffer at: 100 V. (Sambrook *et al*., 1989). The following primers were used for characterization of pathogenic *E. coli* targeted virulence *eaeA* (248 bp) (Blanco *et al*., 1996) and Stx1gene (614bp) (Brian et al., 1992), respectively:

***eaeA* gene: forward F: ACGTTGCAGCATGGGTAACTC**
R: GATCGGCAACAGTTTCACCTG

***Stx1*gene: forward F: AAATCGCCATTCGTTGACTACTTCT**
R: TGCCATTCTGGCAACTCGCGATGCA

phage isolation and detection

Phages were isolated from different sewage water samples. 50 mL of tryptic soy broth (TSB; Oxoid, England) medium was inoculated with 5.0 mL of sewage water samples and equal volume of overnight liquid culture of bacterial host. The inoculated flasks were incubated overnight at 37^0C under shaking conditions (250 rpm/min). Then, samples were centrifuged at 6000 rpm for 15 min at 4 °C. Chloroform was added at a rate of 1:10 (v/v) to the supernatants followed by shaking for 5.0 min, finally the crude phages lysates were transferred into a sterilized tube (Adams, 1959).Detection of bacteriophages was carried out qualitatively by spot test technique according to the method described by Borrego *et al*. (1987). Obtained Phages were assayed quantitatively by the plaque assay method according to method of Adams, (1959). Propagation of the isolated phages was done as reported by Goodridge *et al*. (2001). Isolated phages were concentrated by the differential centrifugation method of Figrski and Christensen (1974). Phage pellets were resuspended in SM buffer: (100 mM MgSO4·7H2O,10 mM NaCl, 50 mM Tris-HCl pH 7.5) and then filtered by 0.22 µm syringe filters. Bacteriophages were stockpiled in SM buffer, at 4 °C until used (Kropinski *et al*., 2009; Marco *et al*., 2012),

Encapsulation of bacteriophages on chitosan–alginate beads

Encapsulated bacteriophages in chitosan–alginate coating shell were primed by suspending phages in commercial honey (3%), gelatin (2.5%); NaCl0 (15 M) and MgSO4·7H2O (10 mM) according to method mentioned by Farzaneh *et al*.(2017) with some modifications. Then mixed among sodium alginate (1.5%) after that, CaCl2 solution (100 mM) was added by a syringe before washing using distilled water after 30 min. The phage beads were covered by chitosan (0.4% chitosan ; 100 mM acetate buffer solution (pH 4.2)) for 30 min. The phage beads were washed by distilled water and stored at 4 °C.

Stability of encapsulated phages on an artificial intestinal juice

The stability of phage beeds in an artificial intestinal juice was studied using Simulated Gastric Fluid'' (SGF): bile (0.1%) salt (0.4%) and pancreatin (Sigma-Aldrich, MO, USA) in 50 mM KH2PO4; pH 7.5. The beads of encapsulated bacteriophages with titer of 3×10^5 PFU mL−1 were incubated with SGF for 6 hr at 37 °C with shakeup. The free phage titer was determined using plaque assays

method (United States Pharmacopeial Convention, 2004; Kim *et al.,* 2015).

Diffusion properties of bacteriophages beads

30 g from Bacteriophages beads were stored in 1000 mL of distilled water at 4 °C. Samples were collected at a range of times to determine the released phages titers (Kim *et al.,* 2015).

The ‘‘phage-loading efficiency’’ (PLE) can calculate from the following equation:

$$\text{PLE (\%)} = \frac{\text{Amount of phage released after destruction}}{\text{Amount of phages initially used}}$$

RESULTS AND DISCUSSION

Rabbits breeding are considered one of the most important animal industries in Egypt. Great consideration is directed to the diseases causing economic losses, mainly enteric diseases, which lead to high mortality rates, especially in young rabbits (Saif-Edlin *et al.,* 1994). Previous studies reported by Hong *et al.*(2017) showed that high mortality rates of 24% in a rabbit farm and 75% of these deaths were caused by Diarrheagenic *E. coli* isolates. The current study designed to isolate some Diarrheagenic *E. coli f* from rabbits. A total number of 50 fecal samples were assembled from freshly dead and diarrheic rabbits in different localities at Cairo, Giza and Qalubia Governorates in Egypt. *E. coli* was recovered from 27 out of 50 fecal samples with prevalence rate of 54%. These data were virtually comparable to results recorded by Entssar *et al.,* (2000); Alton *et al.* (2013); Sawsan, (2012) and Saif-Eldin *et al.,* (1994).

Gram staining was carried out and gram-negative rods were detected. The obtained isolates produced distinctive green metallic sheen with a black center colonies and pink colonies on Eosin Methylene blue (EMB) and MacConkey’s agar media, respectively. The biochemical tests for the suspected *E-coli* isolates showed negative results with Vogus-proskauer test, Urease test, Citrat utilization test, and Oxidase reduction test. While gaving positive results with Indole test, Catalase production test and Methyle red test. The suspected *E-coli* isolates showed yellow slant and yellow butt with gas production in the T.S.I agar test. As agreement with Edward and Ewing, (1972)

Singh and Gupta, (1996) indicated that isolates of virulent Diarrheagenic *E-coli* can be recognized by its capability to combine to Congo red. As a result of *in vitro* pathogenicity test, 8 from 27 isolates (29.63 %) gave positive results and showed small sized dark brick red colonies. These results were in conformity by Berkhoff and Vinal (1986), which also mentioned a strong link among expression of CR phenotype and virulence in poultry *E. coli*. He also suggested that, it was associated with the presence of p-D-glucan in the bacterial cell wall. On the other hand, Yoder, (1989) has revealed that Congo red binding results did not associated with pathogenicity.

Table no.1 is shown the antimicrobial sensitivity of the obtained isolates to different antibiotics. Data revealed that the isolated *E. coli* showed the highest sensitivity to both Norfloxacin and Gentamycin. The 3 from 8 isolates (no. 3, 4 and 7) were resistant to Ampicillin and Amocxicillin and classified as multidrug resistant isolates. While isolates 5 and 8 were sensitive to many antibiotics. Antimicrobial susceptibility pattern of isolated *E. coli* showed variably susceptibility to other used antibiotics. The noticeable data agrees with Ibrahim, (1977) Moharam *et al.,* (1993) and Abd-El Rahman *et al.,*(2005) who mentioned that most *E. coli* isolates were susceptible to Gentamycin, Norfloxacin and Enerofloxacin and were resistant to penicillin.

Table 1. Antimicrobial pattern of the isolated *E. coli* from rabbits

Antibiotics*	Isolates No.							
	1	2	3	4	5	6	7	8
AM	R	S	R	R	R	S	R	I
AX	I	R	R	R	R	I	R	S
AMC	R	R	R	R	I	R	R	I
CIP	I	S	S	R	S	S	R	S
DO	I	I	I	I	S	S	S	S
CN	S	I	R	S	S	S	I	S
N	I	I	I	R	S	S	S	S
NOR	S	S	R	I	S	S	I	S
S	S	S	R	S	S	R	I	S
T	R	S	R	R	I	S	R	S
SXT	S	S	S	I	R	R	R	S

AM=Amoxicillin (10 µg), AX=Ampicillin (10 µg), AMC=Amoxicillin-clavulanate (30 µg), CIP=Ciprofloxacin (5 µg), DO= Doxycycline (30 µg), CN=Gentamycin (10 µg), N=Neomycin (30 µg), NOR=Norfloxacin (10 µg), S=Streptomycin (10 µg), T=Tetracycline (30 µg) and SXT=Sulfamethoxazole-Trimethoprim (25 µg) ; R; resistant I; intermediate and S; susceptible.

Three multidrug resistant *E. coli* isolates no. 3, 4 and 8 were represented for serotyping by specific antisera. Data revealed that E. *coli* isolates no.4 and 8 were categorized as O169 and O125 serotypes respectively, but isolate no.3 categorized as O158. Different serotypes were recorded as O111 and O114 by Scaletsky *et al.* (1984). El-bakry, (2009) identified *E. coli* O44 as the most common in rabbits. Similar data previously mentioned by Shahin *et al.* (2011) found that O44 and O158 as the most common *E. coli* serotypes in rabbits. Although, dissimilar results reported by Walaa *et al.,* (2016) that identified different serotypes of *E. coli* as O109, O15, O103 and O8.

PCR detection of *eae*A and *Stx*1 virulence genes was carried out for three isolates of *E. coli*. One *of E. coli* serotypes (O158) expressed *eae*A gene at suspected size of 248bp. On the other hand, all tested serotypes did not express *Stx*1genes as shown in fig.1. Camarda *et al.* (2003) reported that the *eae*A gene was found in 28.57% of the isolated *E. coli*. Despite the fact that reported by Pohl *et al.,*(1993) and Blanco *et al.,* (2006) that showed many serotypes isolated from diarrheic rabbits and all were did not contain genes encoding enterotoxins. Hassan and Al-Azeem, (2009) revealed that 31% of tested *E. coli* isolates possessed *eae*A gene, one shows *Stx*1 gene and all isolates did not express *LT* gene. The *eae*A gene is an adhesion factor that facilitates the attachment of bacteria to intestinal epithelial cells, producing attaching and effacing lesions that most important to enteropathogenic and enterohaemorrhagic diarrhea.

For bacteriophages isolation, different five samples of sewage water were collected from different locations. Spot test was effectively used to detect qualitatively the presence of *E. coli* phages in the collected samples. 4 out of 5 samples gave positive results and confirmed the presence of coliphages. Quantitatively assaying was carried out for the positive samples using the plaque assay technique. Concentrations of isolated phages were 3.5×10^4, 4×10^5, 3×10^4 and 2×10^5 for bacteriophage isolates no.1,3,4 and 5 , respectively, as shown in Table 2 and fig 3.

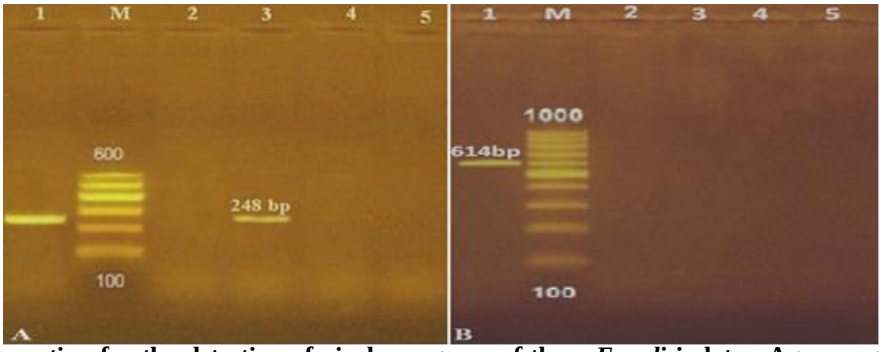

Fig. 1. RT-PCR reaction for the detection of virulence genes *of* three *E. coli* isolates. Agarose gel electrophoresis presenting PCR amplification of (A): *eae*A virulence gene; (B): *Stx*1 virulence gene. M; 100 bp ladder as molecular size DNA marker; 1 and2: Positive and negative control samples respectively., 3-5: *E. coli* isolates no. 3 ,4 and 8 respectively.

Table 2. Qualitative and quantitative assaying of coliphages:

Sources of Sewage water	Qualitative assay by Spot test	Quantitative assay (pfu/ml)
1.El-Gabl Al-Asfar	Positive	3.5×10^4
2.Giza	Negative	*ND
3.Shoubra Elkhema	Positive	4×10^5
4. El-Sharkaya	Positive	3×10^4
5.El-Mariotya	Positive	2×10^5

***ND= Not Determined.**

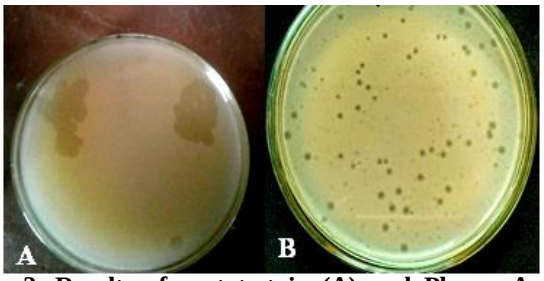

Fig. 2. Results of spot test in (A) and Plaque Assay technique in (B).

Making sure that the stability of phages is a explanation to the success of therapy and biocontrol. Encapsulation of Phage is a talented method that provides work for feed attuned supplies that have no unfavorable effects on phage activity. Phage encapsulated beads demonstrated that, it can organize the delivery of phage in simulated intestinal fluids and keep it from difficult conditions in the stomach to assist therapeutic delivery to poultry. The used technique created a success encapsulation and improved acid stability parallel to earlier information of phage encapsulation mentioned by Tang et al. 2013; Colom et al. 2017). Alginate is considered a very good system for encapsulation of bacteriophages causing their ability to resist acidity, and to control and preserve the release of live products to the gut such as phages and probiotic bacteria (Gbassi *et al.* 2009; Lee and Heo 2000). Chitosan is a natural polymer. It is unsuitable for use as a core solution (Sudarshan *et al.* 1992), other than can be used as a cover coating material because of its solubility in acid conditions, as well as its brilliant biodegradable and biocompatible properties (Allan *et al.* 1984). Prepared beads were stocked up in water at 4 °C. Samples were collected for a variety of times of storage to determine the maintenance and stability of the encapsulated phage beads. Along of the experiment; phage release was not observed in over the storage conditions. The alginate-based microbeads are shown in fig 3.

Determination of the release rate of encapsulated phages under artificial intestinal conditions was done after incubation in SGF. The phages beads produced titers in the range of 3.5 to 4.1 log6 PFU mL−1 later than 1 hr after incubation and reached to 6 to 7.3 log7 PFU mL−1 after 6 hr. Data approved that, full release occurred after 6 hrs of incubation in gateric fluid. Phage cocktail that administered to poultry farms must be tolerating the acidic condition of the bird's intestine. Phages beads represented greater stability rate than the non-encapsulated phages under simulated acidic conditions, produced. These explanations are reliable with those reported previously by Koo *et al*. (2000). The assimilation of gelatin and honey in the preparation of beads increased their aptitude to keep the diffusion rate by increasing the beads viscosity (Ma *et al*., 2012).

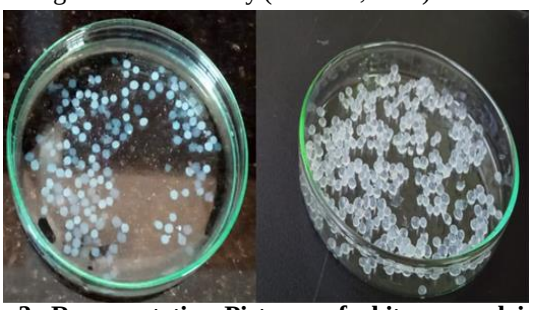

Fig. 3. Representative Pictures of chitosan - alginate phages beads

CONCLUSION

High level of isolated virulence *E. coli* from the examined rabbits pointed to a challenge to the rabbit farming industry that requires stricter hygienic and preventive trials. Further, serotyping by specific antisera and PCR are considered fast and trustworthy diagnostic tools in the detection of *E. coli* isolates. In conclusion, this investigation presented the proficient defensive effects of encapsulated phage beads alongside inactivation by acidic condition, and to maintain phage lysis and release activity for a long period on farm applications.

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عزل ميكروب الايشيريشيا كولاى المسبب للإسهال والفاجات المتخصصة لها من الأرانب

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صُممت هذه الدراسة لعزل سلالات من ميكروب الإيشريشيا كولاى وكذلك البكتريوفاجات الخاصة بها. وأيضا للتعرف على الإيشريشيا كولاى المعزولة من الأرانب كيميائيا ، مصليا ، الكشف عن جينات الضراوة باستخدام تكنيك تفاعل البلمرة المتسلسل والكشف عن مدى قابليتها لمجموعة من المضادات الميكروبية. ولهذا الغرض ، تم التحصل على خمسين عينة مسحة برازية تم جمعها من الأرانب الصغيرة الناقفة المصابة بالإسهال وكذلك الميتة حديثًا وتم عمل الفحص البكتريولوجي. أظهرت النتائج أن 27 عينة بنسبة 54٪ أعطت نتائج إيجابية لعزل الميكروب هدف الدراسة. أشارت النتائج التي تم الحصول عليها من اختبار القدرة الامراضية باستخدام تكنيك أحمر الكونجو،إلى أن ثمانية بنسبة 29.63٪ كانت موجبة لاختبار أحمر الكونجو. تمت دراسة مدى حساسية الميكروب للعديد من المضادات الحيوية. أظهرت عزلات الميكروب درجات مختلفة من الحساسية للمضادات الحيوية المستخدمة. تم تصنيف العزلات رقم 3 و 4 و 8 على أنها عزلات مقاومة للعديد من المضادات الحيوية. وتم التعرف على عزلات الميكروب الثلاثة التى تم اختيارها وتصنيفها مصليا على أنها تتبع O169 و O125 و O158 للمعزولات 4 و 8 و 3 على التوالي. أظهر تفاعل البلمرة المتسلسل التقليدي للكشف عن جينات الضراوة eaeA و Stx1 أن النمط المصلي O158 عبرت عن جين eaeA لكن الأنماط المصلية الأخرى O169 و O125 لم تعبر عنه. من ناحية أخرى ، لم يعبر أي من الأنماط المصلية عن جين Stx1. ويمكن استخدام البكتريوفاجات بشكل فعال لعلاج ميكروب الايكولاى الممرض الذى يستعمر أمعاء الأرانب. ومع ذلك ، فإن الظروف الحمضية الغير ملائمة بالإضافة إلى نشاط الإنزيمات الهضمية جميع هذه العوامل تؤثر على حيوية البكتريوفاجات وتقلل من كفاءتها عند التطبيق. تم تطوير حاجز دفاعي طبيعي مناسب للإعطاء عن طريق الفم لمزارع الدواجن والذي يوفر ثباتًا عند التعرض للظروف الحمضية فى معدة الحيوان. أدت حبيبات الشيتوزان والألجينات المغلفة مع العسل والجيلاتين إلى تقليل هذا التأثير. وكانت حبيبات البكتريوفاجات المغلفة محتفظة بثباتها عند التخزين في الماء ولكنها حققت تحرر شبه كامل من داخل الحبيبات بعد 6 ساعات في المحلول المعوي المجهز عند 37 درجة مئوية. في هذه الدراسة ، تم البحث عن طريقة لعزل وتعريف العزلات القولونية فى محاولة للسيطرة على المرض بإستخدام البكتريوفاجات. وتعد حبيبات الفاجات المغلفة طريقة واعدة وفعالة من حيث التكلفة لاستهداف البكتيريا المعوية فى ارانب المزرعة .