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# TRIAL FOR DETECTION OF COLICIN-PRODUCING *ESCHERICHIA COLI*

BY

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## ABSTRACT

Avian colibacillosis,a critical disease in poultry,is caused byGram-negative *Enterobacteriaceae* avian pathogenic *E. coli* APEC. APEC is responsible for many infections, such as septicemia, chronic respiratory disease, swollen-head syndrome, enteritis, cellulitis, salpingitis, omphalitis, synovitis, pericarditis, and peritonitis. Antibiotic-resistant pathogens are an emerging threat, so an alternative to antibiotics is necessary. Colicins are high-molecular-weight proteins of antimicrobial effect that affect phylogenetically relevant bacteria. They do not sacrifice for the microbiota and are not harmful to human cells but only to bacteria carrying the protein receptors BtuB, Cir, FhuA, and OmpF. The study investigates the in vitro inhibitory effect of colicin-producing *E. coli* from different sources of animals and humans on APEC. They use various mechanisms of action and distinct receptor or translocation systems to avoid mutation and resistance in receptor or translocation systems. Eighteen isolates were detected from 35 chicken organ samples. Four isolates were retrieved from 10 fecal sheep samples. Five isolates were obtained from 10 fecal cat samples. Fifteen isolates were revealed from 18 fecal cow samples. Eleven isolates were from 17 human urine samples. Fifty-three isolates were isolated from 90 samples (58.88%). Non-avian isolates did not have a colicin inhibitory effect on avian pathogenic *Escherichia coli* APEC. APEC antibiotic resistance for almost all antibiotics is partly responsible for therapeutic failure. Moreover, antibiotic-resistant APEC may possess a zoonotic potential. Consequently, antibiotic alternatives such as colicins may prove useful tools in antibiotics-resistant APEC control without antibiotics drawbacks.

**Keywords:**

*E. coli*, Avian pathogenic *E. coli,* colibacillosis, poultry, human uropathogenic *E. coli*, sheep, cow, cat, colicins, Egypt.

## INTRODUCTION

Avian colibacillosis is a disease with significant influence on poultry caused by Gram-negative *Enterobacteriaceae* avian pathogenic *E. coli* (APEC) **(Paixão *et al.*, 2016; Mahmud *et al.*, 2018).** APEC is chargeable for many infections, such as septicemia, chronic respiratory disease, swollen-head syndrome, enteritis, cellulitis, salpingitis, omphalitis, synovitis, pericarditis, and peritonitis **(Ibrahim *et al.*, 2019; Nolan *et al.*, 2020)**. Colibacillosis is a top cause of economic losses that affect mortality, performance, condemnation of carcasses, and preventive and control costs **(Messai *et al.*, 2015; Lounis *et al.*, 2017; Nolan *et al.*, 2020)**. Antibiotic-resistant pathogens are a significant menace, so antibiotic alternatives are necessary. Colicins are high molecular weight proteins of antibacterial effect that affect phylogenetically relevant bacteria with no collateral damage to microbiota and are not toxic to human cells but only to bacteria with protein receptors BtuB, Cir, FhuA and OmpF **(Cascales *et al.*, 2007; Bano *et al.*, 2024).** Colicins are a class of bacteriocin Selective, potent, biodegradable, non-replicative alternative antimicrobial to multidrug-resistant pathogens **(Ghequire and De Mot 2018)**. Mechanism of action through receptor binding to target strains **(Ghequire and De Mot 2018)**. Transportation by porins Tol dependent type A or TonB dependent type B pathways. Toxic activity by pore formation (colicins, N, U, E1, A, S4, B, Ia, Ib,5,10) or enzymatic by peptidoglycan degradation (colicin M) or blockage of protein synthesis (colicinsE3, E4, E6 ribosomal and E5, D tRNA) or nucleic acid degradation DNAse (colicinsE2, E7, E8, E9). Many detected the antimicrobial efficacy of colicin from different hosts (**Chad *et al.*, 2004; Schamberger and Diez-Gonzalez 2004; Stahl *et al.*, 2004; Trautner *et al.*, 2005; Rijavec *et al.*, 2007; Cutler *et al.*, 2008).** This study tries to investigate colicins from different animals and human sources in vitro inhibitory MIC (Minimum Inhibitory Concentration) effect on avian pathogenic *E.coli* with various modes of action and distinct receptor /translocation systems to avoid mutation in receptor or translocation systems and hence resistance **(Budic *et al.*, 2011)**.

## MATERIAL AND METHODS

**1. Sample collection:**

Livers, lung, hert, and spleen of 35 broiler carcasses were selected from 14 flocks with symptoms and lesions suggesting colibacillosis were taken (mortalities, respiratory signs, diarrhea, colisepticemia, airsacculitis, perihepatitis, and pericarditis) in (Table 1). Seventeen human urine samples with pus and symptoms of urinary tract infection were collected. Ten sheep fecal samples, 18 cow fecal samples, and ten cat fecal samples were obtained. All samples were retrieved from farms and laboratories in greater Cairo governorates. Different host systems were used to obtain different types of colicins **(Schamberger and Diez-Gonzalez 2004).** The samples were obtained in sterile containers in an aseptic condition and sent instantly for further analysis.

**2. Bacteriological isolation:**

*Escherichia coli* isolates were obtained from the organs of chickens and human urine and feces of sheep, cows, and cats pre-enriched in buffered peptone water aerobically at 37°C for 24 hours. Moreover, they were Subcultured on MacConkey agar and aerobically incubated at 37°C for 18-24 hours in Fig. (1). Gram-negative, oxidase-negative, and catalase-positive typical colonies were detected using the biochemical tests (oxidase strips and triple sugar iron agar were from Oxoid, UK; urea, Simmons' citrate agar, and peptone water were from Lab M; and Kovacs reagent was from HiMedia, India) **(Nolan *et al.* 2020).**

**Fig. (1):** *E.coli* isolation on MacConkey agar.

**3. Screening for colicin production:**

The method described by **(Gordon and O'Brien 2006)** to detect colicin production from non-avian samples is used. In Brief, each non-avian strain was grown in Luria Bertani broth overnight. 1 ml of culture was introduced to 10 ml Luria broth and incubated for one hour at 37 °C with shaking at 150 r.p.m. Afterward, mitomycin C 0.1-0.2 ug/ml (for colicin induction SOS agent) was inserted and the culture was incubated for four hours at 37 °C with shaking at 150 r.p.m. A 1.5 ml aliquot of the overall culture was transported to a microfuge tube, and subsequently centrifuged for 5 min at 10,000 g. Next, the supernatant was taken to another microfuge tube holding 50 ml chloroform. The sterile extract was refrigerated at 5 °C. The crude extract was spotted on the Luria soft agar of the avian pathogenic *E. coli* strains.

## RESULTS

Eighteen isolates were revealed from 35 chicen organ samples. Four isolates were obtained from 10 fecal sheep samples. Five isolates were retrieved from 10 fecal cat samples. Fifteen isolates were detected from 18 fecal cattle samples.Eleven isolates were recovered from 17 human urine samples. Fifty-three total isolates were retrieved from 90 samples (58.88%) in (Table 1). Non-avian isolates did not possess colicin inhibitory effect on avian pathogenic *Escherichia coli* in Fig. (2).

**Table (1):** Frequency of isolate recovery.

| Species | Number of isolates | Number of samples | Percentage |
|---|---|---|---|
| **Chicken** | **18** | **35** | **51.4%** |
| **Cattle** | **15** | **18** | **83.3%** |
| **Cat** | **5** | **10** | **50%** |
| **Sheep** | **4** | **10** | **40%** |
| **Human** | **11** | **17** | **64.7%** |
| **Total** | **53** | **90** | **58.88%** |

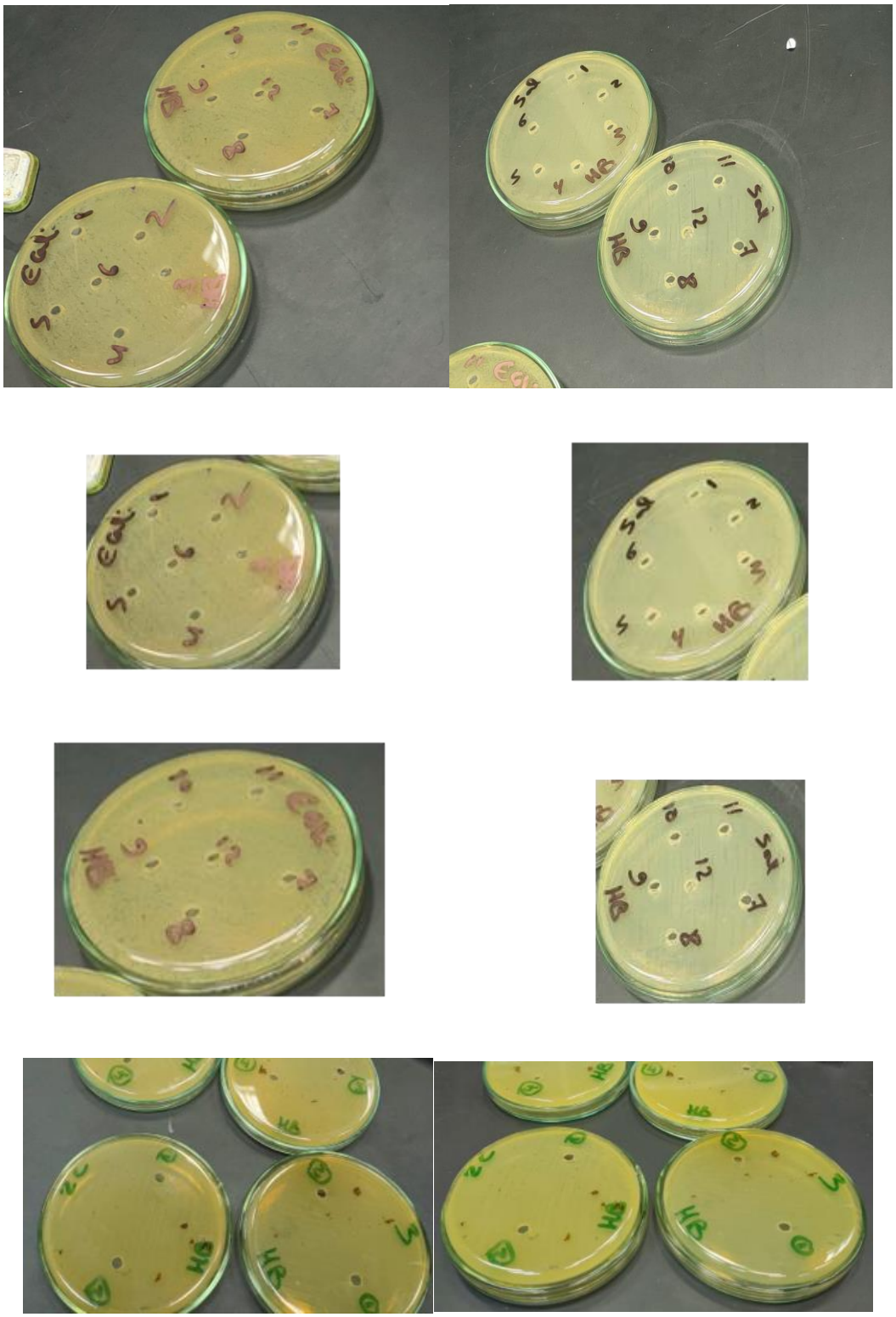

**Fig. (2):** shows the absence of APEC inhibition by colicin from different *E.coli* isolates.

## DISCUSSION

The most significant disease colibacillosis is blamed for huge economic losses in the poultry industry. It affects public health due to *Escherichia coli* transmission of drug-resistance genes between poultry and humans in addition to its zoonotic potential. Thus, implementing antibiotic regulations, biosecurity and hygienic measures, and human food hygiene is essential. Consequently, an alternative to antibiotics is necessary. Colicins are macromolecular proteins with antibacterial effects for phylogenetically relevant bacteria with no damage to microbiota and human cells but only to bacteria carrying protein receptors BtuB, Cir, FhuA and OmpF **(Cascales *et al.*,2007).**Colicins are Selective, potent, biodegradable, non-replicative bacteriocin alternative antimicrobials **(Ghequire and De Mot 2018).** It acts through receptor binding, transportation by porins (Tol and TonB), and toxic activity **(Ghequire and De Mot 2018).** Types of colicins are pore-forming colicins (Colicins N, U, E1, A, S4, B, Ia, Ib,5,10) or enzymatic by peptidoglycan degradation (colicin M) or blockage of protein synthesis (colicins E3, E4, E6 ribosomal and E5, D tRNA) or nucleic acid degradation DNAse (colicins E2, E7, E8, E9).(**Schambergerand Diez-Gonzalez 2004)**and others detected the antimicrobial efficacy of colicin from different hosts and its relation to virulence factors. Fifty-three isolates were isolated from 90 samples (58.88%). APEC isolation rate was 18/35 farms (51.4%). However higher isolation results were recovered by **(Liu *et al.*, 2016)** 88%, **(Khalaf *et al.*, 2020)** 70%, and **(Bhattarai *et al.*, 2024)** 90.4%. In contrast, lower isolation percentages were obtained by **(Shecho *et al.*, 2017)** 11%, **(Moawad *et al.*, 2018)** 13.4%, **(Ibrahim *et al.* 2019*)*** 34%, and (**Radwan *et al*., 2020)** 26.7%. Different isolation rates attributed to different localities and time. Four isolates were obtained from 10 fecal sheep samples. Five isolates were retrieved from 10 fecal cat samples. Fifteen isolates were detected from 18 fecal cattle samples. Eleven isolates were recovered from 17 human samples. APEC antibiotic resistance has soared in the last decades at an alarming rate globally **(Jarlier *et al.*, 1988; Gregova and Kmet 2020).**Hence, antibiotic-resistant APEC exchanges genetic material (conjugative plasmids or transposons), which impacts public health and the poultry industry due to mortalities and costs of control. Accordingly, alternatives for antibiotics and new legislation for antibiotic misuse and antimicrobial susceptibility testing before use must be implemented.

There are many causes for the absence of colicin activity such as the mutation in the receptor, translocation, colicin activity gene *cxi*, lysis gene for release, and the transmission of immunity gene (With the colicin activity gene *cxi* by a plasmid), and proteases **(Cascales *et al.*, 2007).** Although there was no colicin inhibitory activity from non-avian *Escherichia coli* on APEC, further studies are needed such as in vivo colicin study and the relation between colicin, antibiogram, phylogroup, and virulence factors.

The success of the poultry business and general public health are seriously threatened by avian colibacillosis. The misuse of antibiotics, inadequate sanitation practices, and poor human food safety are the main causes of *Escherichia coli*'s growing virulence in poultry and people. Therefore, a diversified approach to colibacillosis is crucial like bacteriocins (Colicins), probiotics, organic acids, pre-biotics, essential oils, bacteriophages, innate immune stimulants, virulence inhibitors and growth inhibitors, antimicrobial peptides, and vaccination.

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