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Original article

# *E. coli* O124 K72 alters the intestinal barrier and the tight junctions proteins of guinea pig intestine

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ABSTRACT

Our research group previously isolated and identified a strain of pathogenic Escherichia coli from clinical samples called *E. coli* O124 K72. The present study was aimed at determining the potential effects of *E. coli* O124 K72 on intestinal barrier functions and structural proteins integrity in guinea pig.

Guinea pigs were grouped into three groups; control (CG); *E. coli* O124 K72 (*E. coli*); and probiotics *Lactobacillus rhamnosus* (LGG). Initially, we create intestinal dysbiosis by giving all animals Levofloxacin for 10 days, but the control group (CG) received the same volume of saline. Then, the animals received either *E. coli* O124 K72 (*E. coli*) or Lactobacillus rhamnosus (LGG) according to their assigned group. *E. coli* O124 K72 treatment significantly affected colon morphology and distorted intestinal barrier function by up-regulating Claudin2 and down-regulating Occludin. In addition, *E. coli* upregulated the mRNA expression of MUC1, MUC2, MUC13 and MUC15. Furthermore, suspected tumor was found in the *E. coli* treated animals. Our results suggested that *E. coli* O124 K72 strain has adverse effects on intestinal barrier functions and is capable of altering integrity of structural proteins in guinea pig model while at same time it may have a role in colon carcinogenesis.



## 1. Introduction

In modern life, antibiotics abuse of and unhealthy lifestyle pose a serious danger to the balance of the intestinal microbiome, which eventually could be effect the intestinal barrier function [1], and may cause a bacterial invasion through intestinal barrier into blood stream and infect body organs [2].

Our previous work revealed that bacteria exist in bile of acute cholecystitis patients [3]. Most specifically, we identified and isolated a pathogenic strain of Escherichia coli called *E. coli* O124 K72 from acute cholecystitis patients. Moreover, recent Studies pointed that intestinal pathogenic bacteria can damage the structural barrier by changing the intestinal tight junction proteins [4]. This eventually cause and creates a favorable atmosphere for bacteria to invade and infect the host organs [5]. Since CLDN2 and Occludin have been identified as major integral membrane proteins at tight junctions [6] they were explored in regards to this study. Notably, CLDN2 and Occludin are antagonistic to each other in relation to intestinal barrier function. Such that when one is upregulated, the other is automatically downregulated.

Mucins play a vital role in intestinal barrier function [7,8]. In the current study we searched whether the pathogenic bacteria consume the intestinal mucus as carbon source, then break through the intestinal barrier into blood.

Mucins were usually divided into two types: secreted mucins and membrane-bound mucins [9]. The secreted mucins such as MUC2 are produced by goblet cells [10,11]. MUC2 secrete a protective mucosa lubricant. while the membrane-bound mucins such as MUC1, MUC13 and MUC15 commonly acted as a protective barrier to the epithelium. More importantly, they can transmit signals from external environment into cells as sensors. [12]. Furthermore, the membrane bound mucins MUC1; MUC13; and MUC15 have been evidenced to be linked to inflammatory bowel diseases and colon cancer [8,13,14].

This work focused on the effects of administration of the pathogenic *E. coli* O124 K72 strain on the intestinal barrier functions and the structural protein integrity in guinea pigs, and The effect of *E. coli* O124 K72 on the formation and secretion of

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MUC2 as well as the expression level of membrane-bound mucins MUC1, MUC13 and MUC15.

## 2. Materials and methods

### 2.1. Animal model for intestinal dysbiosis

Five weeks old pigmented guinea pigs were obtained from Dalian Medical University Laboratory Animal Center (Liaoning, China). Briefly, fifteen male guinea pigs were divided into three groups of five animals each.

Following seven days's acclimatization, two groups received 200 mg/kg/day of Levofloxacin by oral gavage for 10 days [15], while the control group received an equal volume of saline. At the end of antibiotic administration, the animals were either given *E. coli* O124 K72 ($1 \times 10^8$ CFU), or *Lactobacillus rhamnosus* (LGG) depending on their assigned group (Ecoli; LGG) respectively.

By the end of the 4th week, the guinea pigs were sacrificed by decapitation. Serum was separated from blood by centrifuge. The animals' colon, heart, liver, kidneys, and spleen were collected and placed in 10% Neutral formaldehyde Buffer.

### 2.2. Bacterial strains

*E. coli* O124 K72 used in this study was isolated from the bile of patients with acute cholecystitis [3]. *Lactobacillus rhamnosus*(LGG) was obtained from Chinese General Microbiological Culture Collection (CGMCC 1.2134), while *E. coli* NovaBlue was bought from Novagen company with pMD18-T cloning vector being supplied by TaKaRa Biotech (Dalian. China).

### 2.3. Faecal total DNA extraction and PCR amplification

The faecal total DNA obtained from QIAamp DNA stool kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol, and the V3 region of 16sRNA was amplified using the universal bacterial primers: F: 5′- GC clamp (CGCCCGGGGCGCGCCCCGGGCGGGGCG GGGGCAC GGGGGG) −CCTACGGGAGGCAGCAG-3′; the reverse primer R: 5′-ATTACCGCGGCTGCTGG-3′.

### 2.4. DGGE and sequence analysis

Denaturation Gradient Gel Electrophorsis (DGGE) was performed using Universal Mutation Detection System (Bio-Rad, USA); the method was similar to the work previously reported [3]. Differently we use 40–50% of denaturing gradient in DGGE electrophoresis. Selected DGGE bands were excised and sent to the TaKaRa Biotech (Dalian, China) for sequencing.

### 2.5. Immunohistochemistry

3 mm thin sections of paraffin-embedded sections of guinea pig colon were prepared and mounted into adhesive microscopic glass slides at the Histopathology department of Dalian Medical University, Immunohistochemistry was done according to Eugene's method [16]. And Image J was used to analyze the differences between the tested samples.

### 2.6. cDNA synthesis and qRT PCR

cDNA was synthesized from total RNA as per the manufacturer's manual (Promega RT-PCR kit, USA). The primers were designed and synthesized from TaKaRa (Japan) as presented in Table 2. The reaction conditions were done according Gamallat et al. [17].

### 2.7. Statistical analysis

The statistical analysis was performed using SPSS 16.0 and Graphpad Prism5 software. All data are presented in the text as the mean ± SD. The Statistical significance between the groups was presented if *p value* of $<0.05$.

## 3. Results

### 3.1. Intestinal microbiota dysbiosis in guinea pig model

Antibiotic usage significantly altered the composition of intestinal microbiota as evidenced by reduced microbiota diversity (Fig. 1). We evaluated the alteration of diversity composition of the microbiota with levofloxacin treated animals using PCR-DGGE, a technique which can analyze rapidly the DNA fingerprint of the microbial community [18]. Similar DGGE bands profiles indicated similar patterns of microbial community structure and diversity as in Fig. 1B, the three dominant bands (A, B, C), we cut them down for cloning and sequencing. The gene sequencing results of the bands in the DGGE profiles (Table 1) indicated that a significant reduction in the *Bifidobacterium magnum*, and *Streptococcus constellatus* was increased after antibiotics treated. Then we used the software Phoretix 1D to analyze these samples. The 12 fecal samples were divided into two clusters using the UPGMA analysis (Fig. 1B).

### 3.2. E. coli *O124 K72 treatment altered colon morphology*

Hematoxylin and eosin (HE) staining of colon tissues in Fig. 2, CG group revealed a neat and delicate arrangement of the intestinal villi. In addition, a normal intestinal lamina propria thickness and thin longitudinal muscle layer were observed. While *E. coli* group colon tissues revealed incomplete or damaged intestinal wall thickening and intestinal villi. The excess number of inflammatory cell infiltration in the intestinal tissue and appearance of a large number of vacuoles signifies hyperplasia of the goblet cells. C group of intestinal villus tissue structural is integrity and intestinal lamina propria is normal thickness, longitudinal muscle layer is normal. In contrast to the *E. coli* O124 K72 treated group, LGG have no adverse effect on the intestinal wall structure.

### 3.3. E. coli *O124 K72 alter the intestinal barrier proteins CLDN2 and occludin*

In order to test whether *E. coli* O124 K72 have influence on the intestinal barrier function protiens. We checked the expression of

**Table 1**
Band A–E sequencing results with similarity matching using NCBI data.

| Selected band | Blast result | Similarity |
|---|---|---|
| A | *Desulfotomaculum thermocisternum* | 100% |
| B | *Prevotella oralis* | 100% |
| C | *Bifidobacterium magnum* | 100% |
| D | *Streptococcus constellatus* | 99% |
| E | *DesulFovibrio* | 100% |

**Table 2**
List of primer sequences used for qRT PCR.

| Gene | Forward Primer (5′–3′) | Reverse Primer (5′–3′) |
|---|---|---|
| Muc1 | *GCTGGTGCTGGTCTGTGTTC* | *GGTGGTAGGTGTCCCGTGTT* |
| Muc13 | *TCGATGAAATTAAGTGCTCACACG* | *CCAGCCAAACCCAGACAAA* |
| Muc15 | *GCCTGCCTAAAAGTGCAAACA* | *CACCCAGAAACCAGACAGACC* |
| β-actin | *GGCACCAGGGAGTCATGGTA* | *TGGGGTATTTCAGGGTCAGG* |