Molecular Characterization of *Escherichia Coli* Isolated from Poultry Meat and its Products

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**ABSTRACT**

This study was conducted on chicken breast, chicken thigh, Shish Taouk, chicken shawarma, chicken nuggets and chicken luncheon (35 of each) collected from different supermarkets at Menoufia Governorates for isolation and identification of *E. coli* and using PCR for detection of virulence gene. The obtained results indicated that the incidence of *E. coli* was 14.3%, 20%, 14.3%, 17.1%, 14.3% and 20% of examined samples of chicken breast, chicken thigh, Shish Taouk, chicken shawarma, chicken nuggets and chicken luncheon, respectively. Moreover, the incidence of serologically identified *E. coli* O78, O26: H11, O2: H6, O41: H21; O42: H18, O128: H2; O13: H7, O103: H2, O127: H4, O114: H4 and O158: PCR results showed shiga toxin 2 gene (stx2) detected in (O1), (O2), (O114) & (O127), while shiga toxin 1 gene (stx1) detected in (O42), (O128) & (O158), also (O78) & (O91) Positive *E. coli* strains for stx1 and stx2 genes, and (O26) & (O103) Positive *E. coli* strains for stx1, stx2 and eaeA genes.

**Key words:** *E. coli*, poultry products, PCR, virulence Gene

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1. **INTRODUCTION**

The production of poultry meat products has increased throughout the world due to its specific sensory attributes and the consumer's belief that white meat is healthier than red meat. Poultry products are highly perishable foods. Therefore, the industry is focused on methods to increase the overall safety and quality of poultry products (Colak et al., 2011) poultry meat and its products are very popular food throughout the world and no wonder since They are delicious, nutritious and considered as a good and cheap source of protein characterized by good flavor and easily digested on the other hand, they rank first or second in foods associated with food borne disease as *E. coli* in most of the countries all over the world where USA ranked third of the reported food-borne disease outbreaks. Many researchers have reported that poultry meat and its products were contaminated by several pathogenic bacteria (Basaran Khraman and Ak 2012 and Urumova et. al., 2014).

*E. coli* is a commensal inhabitant of the gastrointestinal tract of mammals and birds, is also the causative agent of several diseases in animals and human worldwide. Pathogenic *E. coli* strains have been divided into intestinal pathogenic *E. coli* and extra intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection they are causing. EPEC strains are responsible for a variety of infections, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (Russo and Johnson, 2000 and Kaperet al., 2004). At least six different categories of pathogenic *E. coli* causing enteric infections have been identified and further characterized (Alfredo et al., 2010). These pathotypes are: Verocytotoxigenic *E. coli* (VTEC), Enterotoxigenic *E. coli* (ETEC), Enter-invasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAggEC), Diffusely adherent *E. coli* (DAEC) Also, certain strains of *E. coli* known as verocytotoxin-producing *E. coli* (VTEC) produce a potent poison, or toxin, which causes illnesses ranging from mild diarrhea through to very
severe inflammation of the gut. Occasionally this can cause complications such as kidney failure, and anemia. The most important toxin-producing strain associated with human illness is known as E. coli O157 (Nagwa et al., 2012). Most E. coli strains do not cause disease, but virulent strains can cause gastroenteritis, urinary tract infections, and neonatal meningitis. It can also be characterized by severe abdominal cramps, diarrhea that typically turns bloody within 24 hours, and sometimes fever. In rarer cases, virulent strains are also responsible for bowel necrosis (tissue death) and perforation without progressing to hemolytic-uremic syndrome, peritonitis, mastitis, septicemia, and Gram-negative pneumonia (Todar, 2007). STEC represent a hazardous public health problem worldwide causing various human gastrointestinal tract diseases, including watery or bloody diarrhea and might develop a life-threatening disease, such as hemorrhagic colitis (HC), Thrombotic Thrombocytopenic Purpura (TTP) and Hemolytic Uremic Syndrome (HUS). The latter is characterized by thrombocytopenia, microangiopathic hemolytic anemia and acute renal failure (Pennington, 2010).

Polymerase Chain Reaction (PCR) based methods have been identified as a powerful diagnostic tool for the detection of pathogenic microorganisms (Malorny et al., 2003). Compared to other methods of detection, these methods are rapid, highly specific and sensitive in the identification of target organisms (Wang et al., 2007).

The aim of the present study was to determine the occurrence, serovars and virulence gene profile of E. coli isolated poultry meat (chicken thigh and chicken breast) and some of its product (chicken shawarma, Shish Taouk, chicken nuggets and chicken luncheon).

2. MATERIAL AND METHODS

Collection of Samples: A total 210 random samples of fresh raw chicken cuts (chicken breast and chicken thigh), some half-cooked chicken products (chickenshawarma, chennuggets and Shish Taouk) and Cooked Products(chickenluncheon)(35 of each) collected from different supermarkets at Menoufia governorate.

2.1. Preparation of Samples according to (APHA, 1992)

2.2 Isolation and identification of Escherichia coli according to (ICMSF, 1996)

2.3 Polymerase Chain Reaction (PCR) of E. coli

1. Materials used for PCR:
   1.1. Reagents used for agarose gel electrophoresis:
   1.1.1. Agarose powder, Biotechnology grade (Bioshop®, Candainc.lot No: OE16323).
   1.1.2. Tris acetate EDTA (TAE) electrophoresis buffer (50×liquid concentration) (Bioshop®, Candainc. lot No: 9E11854).
   1.1.3. Ethedium bromide solution (stock solution) biotechnology grade (Bioshop® Candainc, Lot No: 0A14667):
   1.2. Gel loading buffer (6×stock solution) (Fermentas, lot No: ooo56239).
   1.3. DNA ladder (molecular marker):
   1.4. 5X Taq master (Fermentas): 100 bp (Fermentas, lot No: 00052518).

2.3.2. Amplification reaction of E. coli (Fagan et al., 1999): The amplification was performed on a Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany). PCR assays were carried out in 1 ml of nucleic acid template prepared by using reference EHEC isolates (approximately 30 ng of DNA), 10 mM Tris-HCl (pH 8.4), 10 mM KCl, 3 mM MgCl2; 2 mM concentrations of each primer, 0.2 mM concentrations of each 29-deoxynucleoside 59-triphosphate, and 4 U of Ampli Taq DNA polymerase (Perkin-Elmer). Amplification conditions consisted of an initial 95°C denaturation step for 3 min followed by 35 cycles of 95°C for 20 secs, 58°C for 40 s, and 72°C for 90 sec. The final cycle was followed by 72°C incubation for 5 min. The reference strains were E. coli O157:H7 Sakai (positive for stx1, stx2 and eaeA) and E. coli K12DH5a (a nonpathogenic negative control strain) that does not possess any virulence gene. Amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (AppliChem, Germany, GmbH) in 1x TBE buffer stained with ethidium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment size.
Table (1) Primer sequences of E. coli used for PCR identification system

<table>
<thead>
<tr>
<th>Primer</th>
<th>Oligonucleotide sequence (5′ → 3′)</th>
<th>Product size (bp)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1 (F)</td>
<td>5′ ACACTGGATGATCTCAGTGG ’3</td>
<td>614</td>
<td>Dhanashree and Mallya (2008)</td>
</tr>
<tr>
<td>Stx1 (R)</td>
<td>5′ CTGAATCCCCCCTCCATATTG ’3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stx2 (F)</td>
<td>5′ CCAATGACACGACACGACGATG ’3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stx2 (R)</td>
<td>5′ CCTGTCAACTGAGCAGCACTTTG ’3</td>
<td>779</td>
<td></td>
</tr>
<tr>
<td>eaeA (F)</td>
<td>5′ GTGGCGAATACTGGCGAGACT</td>
<td>890</td>
<td>Mazaheri et al, (2014)</td>
</tr>
<tr>
<td>eaeA (R)</td>
<td>5′ CCCCAGTCTTTTTCACCGTCG ’3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (2): Cycling conditions of the different primers during PCR.

<table>
<thead>
<tr>
<th>Target gene</th>
<th>Primary denaturation</th>
<th>Secondary denaturation</th>
<th>Annealing</th>
<th>Extension</th>
<th>Final extension</th>
</tr>
</thead>
<tbody>
<tr>
<td>stx1</td>
<td>95°C 3 min.</td>
<td>95°C 20 sec.</td>
<td>58°C 20 sec.</td>
<td>72°C 1.5 min.</td>
<td>72°C 5 min.</td>
</tr>
<tr>
<td>stx2</td>
<td>95°C 3 min.</td>
<td>95°C 20 sec.</td>
<td>58°C 20 sec.</td>
<td>72°C 1.5 min.</td>
<td>72°C 5 min.</td>
</tr>
<tr>
<td>eaeA</td>
<td>95°C 3 min.</td>
<td>95°C 20 sec.</td>
<td>58°C 20 sec.</td>
<td>72°C 1.5 min.</td>
<td></td>
</tr>
</tbody>
</table>

Table (3) Incidence of identified E. coli serotypes isolated from the examined samples of chicken meat products (n=35).

<table>
<thead>
<tr>
<th>Isolated Bacteria</th>
<th>Raw Products</th>
<th>Half Cooked</th>
<th>Cooked Products</th>
<th>Types</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breast</td>
<td>Thigh</td>
<td>Nuggets</td>
<td>Shish Taouk</td>
</tr>
<tr>
<td>O2: H6</td>
<td>No</td>
<td>%</td>
<td>No</td>
<td>%</td>
</tr>
<tr>
<td>O14: H4</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>O4: H8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O1: H7</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O98</td>
<td>3</td>
<td>8.6</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>O158</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O26: H11</td>
<td>2</td>
<td>5.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O31: H12</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>O10: H2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>O128: H3</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>O127: H4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>14.3%</td>
<td>7</td>
<td>20%</td>
</tr>
</tbody>
</table>

3. RESULTS

3.1 Prevalence of E. coli isolated from poultry meat and some of its products:

A total of 210 chicken cuts and some of chicken by product were examined bacteriologically, incidence of E. coli was 14.3%, 20%, 14.3%, 17.1%, 14.3% and 20% of examined sample of chicken breast, chicken thigh, Shish Taouk, chicken shawarma, chicken nuggets and chicken luncheon, respectively.

3.2 Results of PCR amplification of the stx1, stx2 and eae A genes of Enteropathogenic E. coli serogroups:

The genomic DNA of Enteropathogenic E. coli serogroups were tested using 3 sets of primers for detection of 3 virulence genes that play a role in virulence of Enteropathogenic E. coli. The genes were shiga toxin 1 gene(stx1), shiga toxin 2 gene(stx2) and intimin gene (eaeA). It was applied on random isolated Enteropathogenic E. coli serogroups (O1, O127 and O103 from Shish Taouk; O2 and O91 from thigh;
O114 and O158 from luncheon; O44 and O128 from nuggets; O78 from breast and O26 from Shawarma. PCR results showed shiga toxin 2 gene (stx2) detected in (O1), (O2), (O114) & (O128), while shiga toxin 1 gene (stx1) detected in (O44), (O127) & (O158), also (O78) & (O91). Positive E. coli strains for stx1 and stx2 genes, and (O26) & (O103) Positive E. coli strains for stx1, stx2 and eae A genes. The genomic DNA of Enteropathogenic E. coli serogroups were tested using specific primer for the stx1 gene. The stx1 gene was amplified in (O44), (O127), (O158), (O78), (O91), (O26) and (O103) which giving product at (614 bp) as showing in Photograph (1).

While genomic DNA of Enteropathogenic E. coli serogroups were tested using specific primer for the stx2 gene. The stx2 gene was amplified in (O1), (O2), (O114), (O128), (O78), (O91), (O26) and (O103) which giving product at (779 bp) as showing in Photograph (1).

And genomic DNA of Enteropathogenic E. coli serogroups were tested using specific primer for the eae A gene. The eae A gene was amplified in (O26) & (O103) which giving product at (890 bp) as showing in Photograph (1).

Photograph (1): Agarose gel electrophoresis of multiplex PCR of stx1 (614 bp), stx2 (779 bp) and eaeA (890 bp) genes characterization of Enteropathogenic E. coli. Lane M: 100 bp ladder as molecular size DNA marker. Lane C+: Control positive E. coli for stx1, stx2 and eaeA genes. Lane C-: Control negative. Lanes 1 (O1), 2 (O2), 8 (O114) & 11 (O128): Positive E. coli strains for stx2 gene. Lanes 4 (O44), 9 (O127) & 10 (O158): Positive E. coli strains for stx1 gene. Lanes 5 (O78) & 6 (O91): Positive E. coli strains for stx1 and stx2 genes. Lanes 3 (O26) & 7 (O103): Positive E. coli strains for stx1, stx2 and eaeA genes.

4. DISCUSSION

The incidence of E. coli in poultry meat and some of its products revealed that incidence of isolation of E. coli in the examined samples of (chicken breast, chicken thigh, Shish Taouk, chicken Shawarma, chicken Nuggets and chicken Luncheon), respectively. Was (14.3 % (5), 20% (7), 14.3% (5), 17.1% (6), 14.3% (5) and 20% (7), respectively. Incidence of isolation of E. coli in the examined samples of thigh and breast 20% (7) and 14.3 % (5), respectively. These results nearly agreed with other authors Vaidya et al., (2005) isolated E. coli from examined breast with percentage rate 14.57 % on the other hand, lower incidence from thigh and breast reported by Mohamed (2004) isolated E. coli from the examined samples of chicken breast with percentage of 7.5% and in chicken thigh with percentage of 2.5%, Marionette et al., (2009) isolated E. coli from the examined samples of chicken breast and thigh with percentage of 6.67%, 10%, respectively. Edris - Shimaa et al., (2011) isolated E. coli from the examined samples of chicken breast with percentage of 12% and in chicken thigh with percentage of 16%, Hassanin et al., (2014) isolated E. coli from examined breast with percentage rate 10%. Khaled et al., (2015) isolated E. coli from examined breast with percentage rate 10%. and Riyad (2011) isolated E. coli from the examined samples of chicken breast and thigh with percentage of 8.7% in both samples.

On the other side higher incidence from thigh and breast was documented by several authors Al-Dughaym and Altabari (2010) isolated E. coli from the examined samples of chicken thigh with percentage of 60%, Ruban and fairoze (2011) isolated E. coli from examined thigh and breast in the range
of 42 to 88%, Cook et al., (2012) isolated E. coli from the skin-off chicken breasts, 33 (33%) and from the skin-on chicken breasts, 77 (41%), James Andrews (2013) isolated E. coli from examined breast with percentage of 65%, Robert Roos (2013) isolated E. coli from examined breast with percentage of 65.2%, E. coli was isolated from the examined thigh with percentages of 33.33%. Edris et al., (2015) isolated E.coli from examined thigh and breast with percentages of 88%, 70%, respectively. Khalafalla et al., (2015) isolated E. coli from both examined thigh and breast with percentages of 100% and Khaled et al., (2015) isolated E. coli from examined thigh with percentages of 30%.


Incidence of isolation of E. coli in the examined samples of chicken luncheon, nuggets and shawarma, were 20%, 14.3% and 17.1%, respectively. Some authors nearly agreed with our results in luncheon as Rady et al., (2011) who isolated E. coli with percentage of 20%.

Lower incidence of E. coli from chicken luncheon reported by Fawzy (2004), Naglaa et al., (2009)and Samaha et al., (2012)isolated E. coli with percentage of 8%,10% and 8%, respectively. Also, higher incidence of E. coli in chicken luncheon reported by El Sabagh-Rasha (2010), Hashim (2003)and Sharaf- Eman and Sabra-Sherifa (2012) isolated E. coli with percentage of 35%, 19 (22.35) % and 25%, respectively.


Higher incidence of E. coli in chicken shawarma, chicken nuggets and Shish Taouk reported by Mahmoud (2006), Eglezos et al., (2008) and Naglaa. et al., (2009) E. coli percentage was 35.6%, 47% and 20%, respectively. Also, higher incidence of E. coli in chicken shawarma reported by Hassanin et al., (2014), Mohamed -Walaa (2014), Sharaf- Eman and Sabra -Sherifa (2012) and Nimri –Laila et al., (2014) 33.3%, 33.3%, 20% and 29.0%, respectively.

On the other side lower incidence of E. coli in chicken shawarma documented by Saad et al., (2015) isolated E. coli with percentage of 10%. Hamid and Majid (2008), Abd El-Rahman et al., (2010) reported low incidence of E. coli in Shish Taouk and nuggets with percentage of 5.5%, 10.6%, respectively.


On the other side higher incidence from E. coli in chicken nuggets and Shish Taouk reported by AL-Dughaym and Altabari (2010), Hassanin et al., (2014) isolated E. coli with percentage of 60% and 26.7%, respectively.

Also, higher incidence reported by Mohamed -Walaa (2014), Saad et al., (2015) isolated E. coli with percentage of 26.7%, 25% of examined shish and nuggets, respectively.


The variation of the results between different authors may be due to the differences in manufacture practices, handling from producers to consumers, storage and the effectiveness of hygienic measures applied during production. The presence of E. coli in food of animal origin is considered as indicator of faults during preparation, handling, storage or services (Tebbutt, 1999). The incidence of identified E. coli serotypes in poultry meat and some of its product. The results reported in table (3) revealed that the isolated serotypes of E. coli in chicken luncheon were O2: Hs, O144:Hs, O155, O26:H11 and O93:H21 with incidence of 2(5.71%), 12.9%, 1(2.9%), 1(2.9%), 1(2.9%) and 1(2.9%), respectively. this result agrees with El Sbagh-Rasha (2010) who isolated O26:K50, O55:K59, O111:K58 and O124:K72 on the other hand this result not agree with Fawzy (2004) who cannot detect any serotypes that present in luncheon and isolated O55:K59 and O124:K72.

The results reported in table (5) revealed that the isolated serotypes of E. coli in thigh were O2: Hs, O78 and O93:H21 with incidence of 2(5.71%), 2(5.71%) and 2(5.71%), respectively and serotypes in breast were O78 and O26:H11 with incidence of 3(8.6) and 2(5.71%), respectively. this result agrees with Edris et al., (2015) who isolated O78 from thigh and O26, O78 from breast, Marionette et al., (2009) O78:K40 from breast and thigh, Mostafa-
Hemmat et al., (2014) isolated O78: k80 from thigh and Hassannin et al., (2014) isolated O26: k60 from breast.

on the other hand, this result not agree with Khalafalla et al., (2015) who cannot detect any serotypes that present in thigh and breast and isolated O157 and O158. Edris-Shimaat et al., (2011) cannot detect any serotypes that present in thigh and breast but isolated O55: K59 and O119: K69 (B10) and Riyad (2011) cannot detect any serotypes that present in thigh and breast but isolated O124: K72, O119: K69, O128: K67, respectively.

The results reported in table (3) revealed that the isolated serotypes of E. coli in chicken shawarma were O26:H11 and O78 with incidence of 2(5.71%) and 3(8.6%), respectively. this result agrees with Saad et al., (2015) isolated O26(5%). on the other hand, this result not agree with Mahmoud (2006), Nimri–Laila et al., (2014) who cannot detect any serotypes that present in chicken shawarma but isolated E. coli O157: H7 with incidence of 11.1 %, Rawash-Rabab (2014) and Fatin, et al., (2015) also not agree but isolated serotype O119:H4.

The results reported in table (3) revealed that the isolated serotypes of E. coli in chicken nuggets were O2; H6, O44:H18, O78 and O123:H2 with incidence of 1(2.9%), 1(2.9%), 2(5.71%) and 2(5.71%), respectively. this result not agree with Saad et al., (2015) who isolated O119:H4(5%), O86(5%), O125:H21(5%), O124(5%) and O26(5%). Also, this result not agree with Abd El-Rahman et al., (2010) who isolated O103: k3(12%) and E. coli O119: K69 (8%).

The results obtained showed that the examined thigh samples are more contaminated than other samples and this may have attributed to exposure of thigh samples to fecal contamination by worker’s hands during evisceration. The presence of E.coli in high numbers indicates the presence of organisms originating from fecal pollution. This is due to improper slaughtering techniques, contaminated surfaces and/or handling of the meat by infected food handlers (Neletal., 2004). Also, the presence of these pathogens can be due to contamination taking place during the meat processing at slaughter house or due to the poor handling of the retailers of meat(Kagambéga et al., 2011). The presence of E.coli in the examined chicken products considered as indicator for improper handling or unhygienic conditions (Hashim, 2003).

The present study was directed to recognize some virulence genes that may play a role in virulence of Enteropathogenic E.coli by using one of the recent development molecular biological techniques (PCR), the genes were shiga toxin 1 gene(stx1), shiga toxin 2 gene(stx2) and intimin gene(eaeA). it was applied on random isolated Enteropathogenic E. coli serogroups (O1, O127 and O103 from Shish Taouk; O2 and O93 from chicken thigh; O114 and O158 from chicken luncheon;O44 and O128 from chicken nuggets;O78 from breast and O26 from chicken shawarma(Photograph1). PCR results showed shiga toxin 2 gene(stx2) detected in (O1), (O2), (O114) & (O128), while shiga toxin 1 gene(stx1) detected in (O44), (O127) & (O158), also (O78) & (O93) Positive E. colistrains for stx1 and stx2 genes, and (O26) & (O103) Positive E. colistrains for stx1, stx2 and eaeA genes(Photograph1).

Shiga toxins are central to the pathogenesis of bloody diarrhea and hemolytic uremic syndrome through cytopathic effect on vascular endothelial cells of kidney, intestine, central nervous system and other organs (Broden et al., 2000). Ethelberg (2004). Although Stx1 and Stx2 have similar structures and modes of action their toxicities appear to be distinct. Stx2 was 1000 times more cytotoxic than Stx1 towards human renal microvascular endothelial cells, the putative target of Shiga toxins in the development of HUS (Louise and Obrig 1995).

Although fimbiae and OMPs have been identified which may be associated with STEC adhesion, most studies have concentrated on intimin as a potential adhesin. There is a precedent for this as intimin was shown to be involved in both initial bacterial attachment (Hicks et al., 1998) and in intimate attachment (Frankel et al., 1998) of EPEC. Intimate attachment is necessary for full expression of the effects of EPEC on the host cell cytoskeleton. Mutants at the eaeA locus remain capable, however, of subtiler cytoskeletal alterations and of inducing host-cell tyrosine kinase activity (Rosenshine et al., 1992). We speculate that this signal transduction event results in elevated intracellular calcium concentrations and fluid secretion (Baldwin et al., 1991) Loss of microvilli caused by EPEC attaching and effacing lesions is an alternative mechanism for EPEC diarrhea. While malabsorption may be a factor in protracted EPEC infection, the acute onset of diarrhea in volunteers (in as little as 2.9 h) indicates that secretory mechanisms are also operative. Interestingly, the eaeA mutant was not completely avirulent; diarrhea developed in 4 of 11 volunteers. Since diarrhea does not develop in volunteers who ingest nonpathogenic E. coli. This suggests that
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