Hemolysin and Bacteriocin Production of *E. Coli* Isolated from Urinary Tract Infection

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**Abstract**

A sixty isolates of *E. coli* isolated from urine of patients with urinary tract infection were included in the study.

The hemolysin and bacteriocin produced by *E. coli* was studied, and it was found that 12 (40%) out of the 30 isolates of *E. coli* were able to produce hemolysin, whereas 18 (60%) were found no ability to produce hemolysin.

In (30) isolates of *E. coli*, bacteriocin production was seen in 17 (56.7%), whereas 13 (43.3%) were found no ability to produce bacteriocin. We conclusion that *E. coli* isolates are considered good producer for bacteriocin that acts as factor.

**Introduction**

The urinary tract infection (UTI) is an infection caused by pathogenic organisms (for example, bacteria, fungi, or parasites) in any of the structures that comprise the urinary tract (Davis, 2011). Urinary tract infection caused by bacteria (most often *E. coli*) that travel up the urethra to the bladder. A bladder infection is called cystitis. If bacteria spreads to the kidney and ureters, the condition is called pyelonephritis. Cystitis is considered a lower urinary tract infection. Pyelonephritis is an upper urinary tract infection and is much more serious (Foster, 2008).

Uropathogenic strains of *E. coli* (UPEC) from a subgroup of extra-intestinal pathogenic *E. coli* (EXPEC) strains and caused human urinary tract infection (UTIS).

Previous studies showed that there are several virulence factors associated with UPEC strains including adhesions, hemolysin and aerobactin production, cytotoxic necrotizing factor, and microcin V (Smajs, et al., 2010).

Hemolysin are pro-forming proteins, they caused destruction of red blood cells, with subsequent release of hemoglobin that can occur by any one of the numerous substances such as a bacterial hemolysin that appears to aid the invasive power of bacteria (Mosby, 2009).

Hemolysins are produced by quite divergent species of bacteria, including *Escherichia coli*, *Clostridium perfringins*, *Bacillus cereus*, *Vibrio cholera*, *Listeria monocytogenes*, *Streptococcus pneumonia*, *Staphylococcus aureus* and *Streptococcus pyogenes* (Chesters, et al., 2000).

*E. coli* produce many types of hemolysin, that most common type of which alpha-hemolysin. It non-specifically adheres to the cell membrane (Martin, et al., 2000).

Bacterocin production is an important characteristic of *E. coli* and several related species in the Enterobacteriaceae family. Within the genus *Escherichia,*
bacteriocin production is exclusively associated with strains of *E. coli* (Smarda, *et al*., 2002).

Moreover, there is increasing evidence indicating that bacteriocins are important elements in bacterial ecology and are linked to their possible probiotic effects (Crusinol, *et al*., 2006).

Bacteriocins proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strains. They are typically considered to be narrow spectrum antibiotics, though this has been debated (Farkas-Himsley, 1980).

Bacteriocins were first discovered by A. Gordon, 2006. He was involved in the process of searching for ways to kill bacteria, which also resulted in the development of antibiotics and the discovery of bacteriophage, all within a span of a few years. He called his first discovery a colicin (Gordon, 2006).

**Materials and Methods**

Sixty *E. coli* strains were isolated from urine samples from both sexes who suffering from urinary tract infection who were admitted to Hilla Teaching Hospital between October 2010 and January 2011.

**Bacteriocin production**

1. Stab inoculate multiple strains on separate multiple brain-heart infusion agar petri-dishes.
2. Incubate at 37°C for 24 hours.
3. Remove the cell growth with a sterile glass slide.
4. Kill the residual cells on the agar surface by exposure the chloroform vapor for 30 minutes.
5. Incubate all the plates again at 30°C for 24 hours.

The presence of bacteriocin can be detected by zones of growth inhibition around stabs (Chung, 2003).

**Hemolysin production**

Hemolysin production was carried out by inoculating a blood agar medium with bacterial isolates at 37°C for 24 hours.

The appearance of a clear zone around the colonies referred to a complete hemolysis (*β*-hemolysis). The appearance of greenish zone around the colonies referred to a partial hemolysis (*α*-hemolysis), whereas no change of colour referred to non hemolysis (*γ*-hemolysis). (DeBoy *et al*., 1980).

**Results**

In this study, 60 urine samples were obtained from patients (of both sexes) suffering from urinary tract infections.

Hemolysin production by *E. coli* was studied, and it was found that 12 (40%) out of 30 isolates of *E. coli* were able to produce hemolysin, on human blood agar. Other isolates of *E. coli* 18 (60%) had no ability to produce hemolysin. The results are shown in table (1).

**Table (1): Hemolysin production of *E. coli* isolated from patients UTI**

<table>
<thead>
<tr>
<th>Hemolysin production</th>
<th>No. and %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>12 (40)</td>
</tr>
<tr>
<td>Negative</td>
<td>18 (60)</td>
</tr>
<tr>
<td>Total</td>
<td>30 (100)</td>
</tr>
</tbody>
</table>

The results of the present study were indicated that out of (30) urinary tract infection *E. coli* isolates, 17 (56.7%) produced bacteriocin, table (2).
Table (2): Bacteriocin production of *E. coli* isolated from patients UTI

<table>
<thead>
<tr>
<th>Bacteriocin production</th>
<th>No. and %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Produced</td>
<td>17 (56.7)</td>
</tr>
<tr>
<td>Non-produced</td>
<td>13 (43.3)</td>
</tr>
<tr>
<td>Total</td>
<td>30 (100)</td>
</tr>
</tbody>
</table>

**Discussion**

Virulence factors enable *E. coli* to colonise selectively that mucosal uro epithelium, evoke an inflammatory reaction and eventually produced from lower urinary tract to renal cavities and tissue invasions. The capacity of *E. coli* to produce many virulence factors contributes to its pathogenicity.

These virulence factors enable some members of the normal flora to elicit an infection by over coming the host defence mechanisms (Embody, *et al.*, 2003).

Hemolysin production as a virulence factor by urinary isolates of *E. coli* has been shown by previous workers (Raksha, *et al.*, 2003).

Hemolysin production is associated with pathogenicity of *E. coli*, especially the more severe forms of infection (Sharma, *et al.*, 2007).

Hemolysin production by *E. coli* was studied and it was found that 12 (40%) out of (30) isolates of *E. coli* were able to produce hemolysin on human blood agar. Other isolates of *E. coli* 18 (60%) had no ability to produce hemolysin.

These results agreed with the results obtained by Bhakdi *et al.*, (1988) who found that many strains of *E. coli* elaborate α-hemolysin, responsible for the zone of β-hemolysis surrounding bacterial colonies on blood agar. The hemolysin was usually synthesized as precursor proteins, it was covalently modified to yield an active hemolysin, and it was secreted via specific export systems that differ for various types of hemolysins (Focareta, 1991).

The results of this study resemble the results obtained by Raksha *et al.*, (2003) who found that among the 220 cases, 91 (41.36%) were hemolytic, but do not resemble the results obtained by Sharma *et al.*, (2007) who found that out of the total 152 isolates of *E. coli*, only 36 (23.7%) were hemolytic on blood agar plate.

Some studies have reported that hemolysin production is positive among *E. coli* strains in the range of 16.6% – 41% (Yasmeen *et al.*, 2009).

Bacteriocin are the interest in medicine because they are made by non pathogenic bacteria that normally colonize the human body. Loss of these harmless bacteria following antibodies use may allow opportunistic pathogenic bacteria to invade the human body (Farkas-Himsley, 1985).

Investigation of (568) clinical isolates of uropathogenetic strains of *E. coli* collected in New Zealand revealed lower incidence of bacteriocin producers (42.6%) (O’Brien *et al.*, 1996). An even lower incidence (32.3%) was found among 440 *E. coli* UTI strains tested in 2001 in the Czech Republic (Smarda and Obdrzalek, 2001).

Gordon and O’Brien found 102 (38%) bacteriocin production strains among 266 human *E. coli* strains (Gordon and O’Brien, 2006).

The result of present study disagreed with these studies.

The present study agree with Smajs, (2010) who reported that 195 (54%) bacteriocin producing of UTI *E. coli* strains, were identified among 361 tested.
References


