Serotypes, pathogenic potential and antimicrobial resistance of
Escherichia coli isolated from subclinical bovine mastitis milk samples
in Egypt

Rabee Alhossiny Ombarak, Mahmoud Gamaleldin Zayda, Atsushi Hinenoya, and Shinji
Yamasaki

Received: January 10, 2019. Accepted: April 8, 2019
Published online: April 26, 2019
DOI:10.7883/yoken.JJID.2018.538
Serotypes, pathogenic potential and antimicrobial resistance of *Escherichia coli* isolated from subclinical bovine mastitis milk samples in Egypt

Rabee Alhossiny Ombarak\(^1,2\), Mahmoud Gamaleldin Zayda\(^1\), Atsushi Hinenoya\(^2\), Shinji Yamasaki\(^2\)*

\(^1\) Faculty of Veterinary Medicine, Sadat City University, Sadat City, 32897, Menoufia, Egypt.

\(^2\) Graduate School of Life and Environmental Sciences, Osaka Prefecture University, 1-58, Rinkuourai-kita, Izumisano, Osaka 598-8531, Osaka, Japan.

*Corresponding author: Shinji Yamasaki, Ph.D.
Graduate School of Life and Environmental Sciences, Osaka Prefecture University
1-58, Rinkuourai-Kita, Izumisano, Osaka 598-8531, Japan
Tel/Fax: +81-72-463-5653
E-mail: shinji@vet.osakafu-u.ac.jp

**Running head:** *Escherichia coli* in subclinical mastitis milk

**Keywords:** antimicrobial resistance, *E. coli*, subclinical mastitis milk, virulence factors
Domestic author information

日根野谷 淳 2）、山崎 伸二 2）

2. 大阪府立大学大学院 生命環境科学研究科
〒598-8531 大阪府泉佐野市りんくう往来北 1-58
Summary

Subclinical mastitis (SCM) is regarded as not only a problem to dairy producers worldwide but also a threat to human health due to the potential bacterial contamination of milk and dairy products, particularly those made from raw milk. In this study, *E. coli* were isolated from fourteen (9.3%) SCM milk samples. The isolated *E. coli* (*n*=14) were serotyped, their potential pathogenicity and antimicrobial resistances (AMRs) were investigated. Serotyping results showed that the *E. coli* isolates belonged to the O55:H7 (*n*=2), O111:H4 (*n*=2), O127:H6 (*n*=2), O128:HUN (*n*=2), O26:HUN (*n*=1), O44:H18 (*n*=1), O114:H21 (*n*=1), O86:HUN (*n*=1), O124:HUN (*n*=1) and O127:H7 (*n*=1) serogroups. Potential pathogenicity was detected in 93% (13/14) of the isolates. In particular, thirteen isolates possessed at least one of the examined virulence genes. Ten isolates (71%) exhibited AMR to at least one of the tested antimicrobials, 4 (40%) of them were multidrug-resistant and one isolates showed ESBL production. The obtained results indicate that SCM acts as a source for the spread of potentially pathogenic *E. coli* strains that are resistant to many groups of antibiotics which may constitute a hazard for both public and animal health.
The contamination of milk by foodborne pathogens may be due to direct contact with contaminated sources from surrounding environment and/or excretions from infected udders (i.e., udders affected by mastitis) (1). Mastitis, in both clinical and subclinical forms, can pose a threat to human health due to bacterial contamination. In cases of clinical mastitis, milk abnormalities are easily observed, and the affected milk is discarded by the producer and therefore does not normally enter the food chain. However, in cases of subclinical mastitis (SCM), the milk that is produced possess no visible changes and can be mixed into the bulk milk; thus, such milk may enter the food chain and poses a public health threat to humans (2). Although the use of antibiotics has reduced the prevalence of contagious mastitis pathogens, environmental bacteria such as *Escherichia coli* continue to cause mammary infections (i.e., mastitis) and environmental *E. coli* are predominantly responsible for SCM (3).

Virulence factors are required for *E. coli* to induce infection, the roles of these factors include acting as toxins; promoting adhesion, invasion, and capsule production; and conferring the abilities to resist serum complement and scavenging iron (4).

In Egypt, dairy animals with suspected SCM is typically treated with antibiotics, moreover there is lack of stringent controls on antimicrobial use in animal production systems, which can lead to antibiotic resistance in bacteria. The characteristics of *E. coli* associated with SCM in Egypt such as dominant virulence factors and antimicrobial resistance (AMR) patterns have not been fully identified. Therefore, the objectives of this study were to characterize the serogroups, virulence factors and resistance patterns of *E. coli* isolated from SCM bovine milk samples in Egypt.

A total of 568 quarter milk samples, collected from three villages and two dairy farms located in Menoufia and Behira governorates in Egypt, were investigated for SCM using the California
mastitis test (CMT). Samples with a positive CMT result that showed no visible abnormalities were subjected to *E. coli* screening as described by Leininger et al. (5). The isolates were identified as *E. coli* based on colony morphology and biochemical tests as described by Ewing (6).

When multiple isolates were observed from the same sample, we randomly picked one of the colonies that exhibited same biochemical patterns.

The serotypes of the isolated *E. coli* strains were determined using an *E. coli* antiserum kit (Denka Seiken, Tokyo, Japan) in accordance with the manufacturer’s instructions.

Major virulence genes reported in pathogenic *E. coli* were screened by a colony hybridization assay, as described in a prior report (7). The previously described procedures (7) were used to prepare DNA probes for *stx1* and *stx2* (Shiga toxin 1 and 2), *eaeA* (intimin), *bfpA* (bundle-forming pilus), *elt* (heat-labile enterotoxin), *est* (heat-stable enterotoxin), *Eagg* (aggregate adherence), *astA* (EAEC heat-stable enterotoxin 1), *lpfA* (long polar fimbriae), *saa* (STEC autoagglutinating adhesion), *daaD* (fimbrial adhesin), *aer* (aerobactin), *afaI* (afimbrial adhesin), *cnf1* (cytotoxic necrotizing factor 1), *evaC* (colicin V), *fimH* (type 1 fimbrial adhesin), *fyuA* (yersiniabactin receptor for ferric yersiniabactin uptake), *hly* (alpha hemolysin), *ibeA* (invasion of brain endothelium), *iroN* (catecholate siderophore receptor), *iha* (iron-regulated gene A homologue adhesin), *kpsMT* (group 2 capsule), *ompT* (outer membrane protease T), *sfa/foc* (S/F1C fimbriae), *traT* (serum resistance associated) and *usp* (uropathogenic-specific protein).

The disk diffusion method was used for antimicrobial susceptibility testing for 15 antimicrobials in accordance with CLSI guidelines (8). The following antimicrobials (Becton Dickinson, USA) were used: ampicillin (10 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefotaxime/clavulanic acid (30/10 µg), ceftazidime/clavulanic acid (30/10 µg), gentamycin (10
µg), imipenem (30 µg), kanamycin (30 µg), streptomycin (10 µg), tetracycline (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), norfloxacin (10 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), chloramphenicol (30 µg), cefoxitin (30 µg) and fosfomycin (50 µg). Screening for extended-spectrum β-lactamase (ESBL) production was conducted according to CLSI criteria. ESBL genes (\textit{bla}_{CTX-M-1}, \textit{bla}_{CTX-M-2}, \textit{bla}_{CTX-M-8/25}, \textit{bla}_{CTX-M-9}, \textit{bla}_{TEM} and \textit{bla}_{SHV}) were detected using a multiplex PCR as described (9).

Out of 568 examined quarter-milk samples, 150 samples were found to be positive for SCM. \textit{E. coli} were isolated from 14 (9.3%) samples. The 14 \textit{E. coli} strains belonged to O55:H7 (2 isolates), O111:H4 (2 isolates), O127:H6 (2 isolates), O128:HUN (2 isolates), O26:HUN (1 isolate), O44:H18 (1 isolate), O114:H21 (1 isolate), O86:HUN (1 isolate), O124:HUN (1 isolate) and O127:H7 (1 isolate) serotypes (Table 1).

At least one of the examined virulence genes associated with pathogenic \textit{E. coli} was detected in 13 strains (93%). \textit{fimH}, \textit{traT}, \textit{kpsMT}, \textit{ibeA}, \textit{fyuA}, \textit{astA} and \textit{eaeA} virulence genes were detected in 13 (93%), 3 (21%), 2 (14%), 2 (14%), 2 (14%), 2 (14%) and 1 (7.1%) isolates, respectively (Table 1).

All isolates were subjected to antibiotic susceptibility testing, and 71% of these isolates (10 strains) were resistant to at least one of the tested antimicrobials; 50% (7/14) for tetracycline; 36% (5/14) for ampicillin; 21% (3/14) for kanamycin, trimethoprim/sulfamethoxazole and streptomycin; and 7.1% (1/14) each ceftazidime, cefotaxime and chloramphenicol. A strain showed ESBL production and possessed \textit{bla}_{CTX-M-1} and \textit{bla}_{TEM} (Table 1). Other 6 strains also possessed \textit{bla}_{TEM}. The antibiotic resistance patterns of the isolated \textit{E. coli} could be differentiated into 9 types and four strains (40%) were multidrug-resistant (Table 1).
Milk from animals with SCM has no visible abnormalities and can be mixed into bulk milk and thereby enter the food chain. If such milk contains potentially pathogenic bacteria such as pathogenic *E. coli*, it may constitute a public health threat particularly if it is used raw.

*E. coli* strains were isolated from 9.3% of the examined samples, this percentage was higher than the corresponding percentages reported in Sweden and Malaysia (around 1%) (10, 11), although higher prevalence of *E. coli* in SCM milk samples have been reported in Pakistan (16.2%) (12), Iran (9.4%) (13) and India (14.9%) (14). The isolated *E. coli* strains belonged to different O serogroups such as O26, O55, O111, O127 and O128, these O serogroups have been shown to be associated with foodborne illness and infections in humans (15).

The ability of *E. coli* to induce infections is attributed to several virulence factors, including factor involved in serum resistance which encoded by *traT* gene; the aerobactin encoded by *aer* gene; toxins (Shiga toxins, CNF1, *astA*, heat-labile and heat-stable enterotoxins); and the intimin encoded by *eae* gene (4). Ninety-three percent of the *E. coli* strains isolated in this study showed potential pathogenicity through possessing one or more virulence genes (Table 1). The gene encoding type 1 fimbriae (*fimH*) was the most prevalent virulence gene, with 93% of the isolates positive for this gene. A research has demonstrated that these fimbriae play an important role in the initial colonization of tissues and are expressed by more than 90% of uropathogenic *E. coli* strains (16).

AMR is a problem with respect to public and animal health. However, antimicrobials are one of the most important tools for controlling mastitis, and the wide use of antimicrobials can cause antibiotic residues in milk, leading to the risk of bacteria developing AMR in consumers of milk or milk product (17).
The findings of AMR in our study differ from those reported by Persson et al. (10), who found that most *E. coli* strains isolated from SCM cases in Sweden are generally susceptible to common antimicrobials used in mastitis therapy. This difference may be associated with the wide use of long acting oxytetracycline; trimethoprim and sulfonamide combinations; gentamycin; penicillin; streptomycin; cefquinome; cefotaxime and sometimes florfenicol as drug therapy for mastitis treatment and control in Egypt.

Some of the multidrug resistant strains detected in this study were also found to be possessing virulence genes (Table 1). *E. coli* strains carrying resistance and virulence genes are of great concern as this could enhance the emergence of pathogens that might be difficult to be treated with antibiotics (7).

In conclusion, the data presented here emphasize that SCM acts as an invisible potential source for the spread of potentially pathogenic *E. coli* strains that are resistant to many groups of antibiotics and may constitute a hazard for both public and animal health. Therefore, appropriate diagnosis of SCM is crucial to prevent the entry of SCM milk into the food chain.

**Conflict of interest**

None to declare

**References**


Table 1. Serotypes, virulence genes, antimicrobial resistance phenotypes and ESBL-gene groups of *E. coli* strains isolated from subclinical mastitis milk samples

<table>
<thead>
<tr>
<th>ID</th>
<th>Serotype</th>
<th>Virulence genes</th>
<th>Antimicrobial resistance phenotype(^1)</th>
<th>ESBL group(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>O55:H7</td>
<td><em>fimH</em></td>
<td>KAN</td>
<td>-</td>
</tr>
<tr>
<td>M2</td>
<td>O144:H21</td>
<td><em>astA, fimH, kpsMT, ibeA</em></td>
<td>AMP, STR, TET</td>
<td>TEM</td>
</tr>
<tr>
<td>M3</td>
<td>O127:H6</td>
<td><em>fimH, fyuA, ompT</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M4</td>
<td>O86:HUN</td>
<td><em>fimH</em></td>
<td>TET</td>
<td>-</td>
</tr>
<tr>
<td>M5</td>
<td>O128:HUN</td>
<td><em>fimH</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M6</td>
<td>O26:HUN</td>
<td><em>astA, fimH, kpsMT, ibeA</em></td>
<td>TET</td>
<td>TEM</td>
</tr>
<tr>
<td>M7</td>
<td>O111:H4</td>
<td><em>fimH</em></td>
<td>AMP, TET</td>
<td>TEM</td>
</tr>
<tr>
<td>M8</td>
<td>O127:H6</td>
<td>-</td>
<td>AMP, STR, TET, SXT (NAL-intermediate)</td>
<td>TEM</td>
</tr>
<tr>
<td>M9</td>
<td>O124:HUN</td>
<td><em>fimH</em></td>
<td>KAN, CHL, TET (STR-intermediate)</td>
<td>TEM</td>
</tr>
<tr>
<td>M10</td>
<td>O55:H7</td>
<td><em>fimH</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M11</td>
<td>O128:HUN</td>
<td><em>eaeA, fimH, traT</em></td>
<td>AMP, KAN</td>
<td>TEM</td>
</tr>
<tr>
<td>M12</td>
<td>O127:H7</td>
<td><em>fimH, fyuA, traT</em></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>M13</td>
<td>O44:H18</td>
<td><em>fimH, traT</em></td>
<td>SXT</td>
<td>-</td>
</tr>
<tr>
<td>M14</td>
<td>O111:H4</td>
<td><em>fimH</em></td>
<td>AMP, CTX, CAZ, STR, TET, SXT (ESBL+ve)</td>
<td>TEM/CTX-M-1</td>
</tr>
</tbody>
</table>

\(^1\) AMP = Ampicillin (10 µg), CHL = Chloramphenicol (30 µg), CAZ = Ceftazidime (30 µg), CTX = Cefotaxime (30 µg), KAN = Kanamycin (30 µg), NAL = Nalidixic acid (30 µg), STR = Streptomycin (10 µg), SXT = Trimethoprim-Sulfamethoxazole (1.25/23.75 µg), TET = Tetracycline (30 µg). No resistance showed against gentamycin (10 µg), imipenem (30 µg), ciprofloxacin (5 µg), norfloxacin (10 µg), cefoxitin (30 µg) and fosfomycin (50 µg).

\(^2\) ESBL-gene grouping was carried out by multiplex PCR according to Le *et al.* 2015