COMPARATIVE STUDY ON PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI* AND *LISTERIA MONOCYTOGENES* ISOLATED FROM RAW MILK IN EGYPT

BY
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ABSTRACT
The current study aimed to study the prevalence of *Escherichia coli* (*E. coli*) and *Listeria monocytogenes* (*L. monocytogenes*) in raw market and investigate their antimicrobial susceptibility to different antimicrobial agents. Nowadays, since the multidrug resistances of pathogens represent a severe problem due to the misuse of antibacterial agents, this study determine the multi resistances of both types of bacteria to different antimicrobial classes. In the current study, out of 100 milk samples, 15 *E. coli* isolates were isolated and biochemically identified. Through PCR technique, all of these isolates were positive for eae gene which encodes intimin. Moreover, 13 *L. monocytogenes* were isolated and biochemically identified. Then, the presence of hlyA gene, which encodes listeriolysin O, was detected in these isolates via PCR technique. Furthermore, the susceptibility to 10 antimicrobial agents was determined among all isolated pathogens. *E. coli* isolates exhibited high resistance rate to sulfamethoxazole-trimethoprim, and amoxicillin while they showed high sensitivities to gentamicin, chloramphenicol, and norfloxacin. However, *L. monocytogenes* isolates showed high resistance rate to neomycin, nalidixic acid, oxytetracycline and chloramphenicol. However, they were highly sensitive to gentamicin and norfloxacin.

The comparative sensitivity study to the investigated antimicrobial agents showed that both types of bacterial spp. exhibited high levels of sensitivity to gentamicin and norfloxacin. On contrast, both types of bacteria were either completely or highly resistant to neomycin, amoxicillin, oxytetracycline, and erythromycin. Additionally, About 93% of the isolated *E. coli* showed multi resistance against at least 5 classes of the antimicrobial agents. Likewise, all the isolated *L. monocytogenes* exhibited multiple resistances against at least 4 antimicrobial agent classes. In conclusion, there is high risk for consumption of unpasteurized milk as this may result in food-borne illnesses. Additionally, the food-borne pathogens, such as *E. coli* and *L. monocytogenes*, isolated from raw milk were high multi-drug resistant (MDR) to several classes of antimicrobial agents.
INTRODUCTION

Food safety defects may result in high risks of food-borne diseases that are considered as an important public health problems with high social and economic effects. In the United States, food-borne illness accounts for morbidity and mortality. Reports suggest that each year about 48 million Americans suffer from food-borne illnesses and this result in 128000 hospitalizations and 3000 deaths. In addition, these illnesses have high effects on the food manufacturing. It is highly sophisticated to determine the extent of food-borne diseases. For food-borne illnesses, there are highly susceptible population that includes infant, children less than 5 years old, aged people, immuno-compromised people and pregnant women. What is more, many factors may increase the severity or risk of food-borne illnesses including random consumption of antimicrobial agents, hospitalization and surgical removal of parts of stomach or intestine (Foegeding and Roberts, 1996).

There is no doubt that there is a correlation between food-borne illnesses and animal products. Consumption of raw animal origin food like raw milk, undercooked chicken, refrigerated ready-to-eat meat, poultry products and/or raw shellfish is the most common cause of human infection with E. coli, L. monocytogenes, Clostridium perfringens, and Campylobacter jejuni.

Milk is nutritious product and represents a very important daily diet part for growing children and expectant mothers (Javaid et al., 2009). Unfortunately, milk serves as a good growth medium for several microorganisms. The origins of raw milk contamination with bacteria may include different sources such as equipments of milking, air, soil and faeces. The pathogenic bacteria presence in milk is considered as a one of the major public health problems, specifically for those people who are raw milk consumers (Mubarack et al., 2010). Diseases associated with the consumption of milk are caused mainly by bacteria including pathogenic E. coli, L. monocytogenes, Staphylococcus aureus, Campylobacter, Yersinia, Salmonella and Clostridium botulinum (Hahn, 1996).

E. coli is a Gram-negative, facultative anaerobe, and within the family Enterobacteriaceae. There are several types of E. coli; few of them that possess specific virulence factors, colonization factors and pathogenic genes can cause a variety of diarrhoeal diseases with symptoms varying according to the strain of E. coli and the resistance of the individual to such illness. The locus of enterocyte effacement (LEE) is one of the other important virulence determinants in E. coli and responsible for the formation of attaching and effacing (A/E) lesions on intestinal epithelial cells (Nataro and Kaper, 1998). It contains the eae gene encoding intimin. As a result, some pathogenic E. coli that transferred to healthy human through contaminated raw milk can cause many consequences including hemolytic uraemic syndrome (HUS), haemorrhagic diarrhea, and haemorrhagic colitis (HC) (Croxen and Finlay, 2010; Gyles and Fairbrother, 2010).

On the other hand, the consumption of milk contaminated with L. monocytogenes can lead to serious diseases such as listeriosis particularly affecting the risk populations. This disease manifests clinically as septicemia, encephalitis, and meningitis (Pesavento et al., 2010). The listeriolysin O, encoded by the hlyA gene, plays a role in the lysis of the host cell phagosomes; this results consequently in the possibility of the growth of Listeria cells (Marquis et al., 1995; Sibelius et al., 1999).
Generally, the treatment of bacterial infections requires usually therapy with antimicrobial agents. The misuse of antimicrobial agents has resulted in the occurrence of multiple antimicrobial resistances; consequently, this renders the treatment, by using antimicrobial agents, ineffective (Johnston et al., 1983). The severity and period of diseases caused by antimicrobial resistant bacterial strains is more than those caused by antimicrobial susceptible counterparts. The resistant microorganisms that present in food products of animal origin may cause human infections difficult to be treated. Incidence of antimicrobial resistant bacteria is still common in milk (Farzana et al., 2009; Sharma and Malik, 2012). Several studies reported the increasing of antimicrobial resistances in E. coli strains (Lehtolainen et al., 2003; Srinivasan et al., 2007; Suojala et al., 2011).

The increase in administration of antimicrobial agents to animals may be the cause of the detected antimicrobial resistances of L. monocytogenes (Schwartz et al., 2003). Therefore, this work aimed at isolation of some food-borne pathogens from raw market milk and detection their prevalences and the antimicrobial susceptibilities of these pathogens.

**MATERIALS AND METHODS**

**I- Samples collection:**

In Mansoura city, Dakahlia, Egypt, 100 raw milk samples were randomly collected from different supermarkets. These samples were immediately tested after their delivery to laboratory.

**II-Isolation of some pathogens from raw milk:**

1) **E. coli:**

The isolation of *E. coli* was carried out by enrichment technique. This was achieved through the inoculation of 25 ml of each sample into 225 ml of tryptone soya broth (TSB) which was then incubated for 24 h at 37 °C; 0.1 ml of this incubated enriched broth suspension was streaked onto sorbitol MacConkey agar supplemented with tellurite and cefixime (SMAC-TC) plates and then incubated for 24 h at 37 °C (Roberts et al., 1995). The incubated plates were examined for sorbitol fermenter colony appearing with pink colour and non sorbitol fermenter colony that is characterized by having almost colourless appearance. Five characteristic colonies were picked up and subcultured on tryptone soya agar (TSA) and then incubated for 24 h at 37 °C. The biochemical identification was then carried out for all the isolates prescribed by Bergey’s Manual of systematic Bacteriology, 1st edition.

2) **L. monocytogenes:**

Listeria species were isolated using enrichment technique in which 25 ml of each sample were transferred into 225 ml of TSB and then the incubation for 24 h at 37 °C was done; 10 ml of incubated broth were added to 90 ml of Listeria selective broth base supplemented with Listeria selective enrichment supplement and then incubated for 48 h at 37 °C; 0.1 ml of incubated homogenate was plated onto Oxford agar plates supplemented with Listeria selective supplement which were then incubated for 48 h at 37°C (Roberts et al., 1995). The representative colonies for *L. monocytogenes* are grey-green colonies surrounded by black zones of asculine hydrolysis. The characteristic colonies were picked from incubated Oxford agar plates and then
purified by using TSA. Afterwards, all the isolates were confirmed as *L. monocytogenes* by the biochemical identification prescribed by Bergey’s Manual of systematic Bacteriology, 1st edition.

**III- Genotypic detection of some virulence factors:**

The screening was carried out for *E. coli* and *L. monocytogenes* isolates by colony PCR amplification of *eae* and *hlyA* genes, respectively, using the oligonucleotide primer sets in table 1). Genomic DNA was prepared by picking up two or three colonies grown on nutrient agar medium and incubated for 24 hours at 37 ºC. Then, these colonies were suspended into 0.1 ml of nuclease free water and its temperature was raised to 95 ºC for 10 minutes. By Techne progene thermocycler, PCR was performed in 25 µl reaction volume that contained 12.5 µl of Dream Tag Green PCR Master Mix (Fermentas), 1 µl of DNA of the tested bacteria, 1 µl (10 µM) of forward *eae* gene primer and 1 µl (10 µM) of reverse *eae* gene primer in case of *E. coli* while 1 µl (10µM) of forward *hlyA* gene primer and 1 µl (10µM) of reverse *hlyA* gene primer in case of *L. monocytogenes*. PCR reactions were performed under cycling conditions including the following: initial denaturation at 95 ºC for 5 minutes; 40 cycles of denaturation at 95ºC for 30 seconds; annealing at 60ºC for 30 seconds; extension at 72ºC for 1 minute; a final extension at 72ºC for 5 minutes. All PCR amplified products were then analyzed by using gel electrophoresis (1.2% agarose containing ethidium bromide) and compared with 100 bp DNA molecular weight ladder in order to verify the success of PCR reaction. The amplified products were visualized by using an UV transilluminator and photography.

### Table (1): Specific amplification primer sets for the tested virulence genes among *E. coli* and *L. monocytogenes* isolates.

<table>
<thead>
<tr>
<th>Gene name</th>
<th>Type</th>
<th>Sequence (5’-3’)</th>
<th>Product size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>eae</td>
<td>Fw</td>
<td>GCAACATGACCGATGACAAG</td>
<td>180</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>Rv</td>
<td>ACCTCTGCCGTCCATAATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hlyA</td>
<td>Fw</td>
<td>ACTTCGGCGCAATCAGTGA</td>
<td>136</td>
<td>AL-Ashmawy <em>et al.</em> (2014)</td>
</tr>
<tr>
<td></td>
<td>Rv</td>
<td>TGCAGCTGCTCTTTAGTAACAGCTT</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fw: forward primer Rv: reverse primer

**IV-Antimicrobial susceptibility testing:**

The antimicrobial testing was performed using agar disc diffusion method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2006a; CLSI, 2006b). Antimicrobial discs (Oxoid) were used for the determination of the susceptibility of both *E. coli* and *L. monocytogenes* isolates on Mueller–Hinton agar. The antimicrobial agents that were used included amoxicillin (30 µg), ampicillin (10 µg), erythromycin (15 µg), gentamicin (10 µg), chloramphenicol (30 µg), nalidixic acid (30 µg), norfloxacin (10 µg), oxytetracycline (30 µg), neomycin (30 µg), and sulfamethoxazole-trimethoprim (23.75/1.25 µg).
RESULTS

I- Isolation of some pathogens from raw milk:

1) *E. coli:*

In the present study, from 9 samples with a prevalence of 9% (9/100), 15 *E. coli* strains were isolated and biochemically identified. Outer membrane protein intimin, encoded by the *eae* gene, represents one of the virulence factors commonly present in pathogenic STEC strains. Via PCR technique, all the isolated *E. coli* strains were positive for *eae* gene (Figure 1a).

2) *L. monocytogenes:*

During the current study, from 100 raw milk samples, 13 *L. monocytogenes* were isolated out of 100 milk samples. These strains were biochemically identified. Then, PCR detection of the *hlyA* gene was applied (Figure 1b).

![Agarose gel electrophoresis of a) eae gene amplicones of *E. coli*. Lanes from 1 to 15 represent positive isolates. Lane C-, negative control; Lane C+, positive control; Lane M, 100 bp DNA molecular weight marker(100-1000). b) hlyA gene amplicones of *L. monocytogenes*. Lanes from 1 to 13 represent positive isolates. Lane C-, negative control; Lane C+, positive control; Lane M, 100 bp DNA molecular weight marker (100-1000).](image)

II-Antimicrobial susceptibility testing:

Susceptibility patterns of different *E. coli* isolates to 10 antimicrobial agents were illustrated in (Figure 2). In this study, *E. coli* isolates showed 100% resistance rates to sulfamethoxazole-trimethoprim, and amoxicillin as well as high resistances against neomycin, oxytetracycline and ampicillin were detected. However, high susceptibilities were observed against gentamicin, chloramphenicol and norfloxacin.
Fig. (2): Antimicrobial susceptibility pattern of *E. coli* isolates (n=15).

Susceptibility patterns of *L. monocytogenes* isolates to different antimicrobial agents were illustrated in Figure 3; 100% resistance rates of *L. monocytogenes* isolates to neomycin, nalidixic acid, oxytetracycline and chloramphenicol were identified. High resistances were determined against erythromycin, amoxicillin and sulfa- methoxazole-trimethoprim while high levels of sensitivity were detected against gentamicin and norfloxacin.

Fig. (3): Antimicrobial susceptibility pattern of *L. monocytogenes* isolates (n=13).
The comparative sensitivity study to the tested antimicrobial agents among *E. coli* and *L. monocytogenes* isolates showed that both types of bacterial isolates were highly sensitive to gentamicin and norfloxacin. Chloramphenicol was highly effective on *E. coli* isolates however it gave no effect on *L. monocytogenes* isolates. Nalidixic acid gave intermediate effect on *E. coli* isolates while all *L. monocytogenes* isolates showed complete resistances against it. Ampicillin showed higher effectiveness against *L. monocytogenes* isolates than *E. coli* isolates. *L. monocytogenes* isolates showed some sensitivity to sulfamethoxazole-trimethoprim. In contrast, *E. coli* isolates were completely resistant to the previous antimicrobial agent. Both types of bacteria exhibited nearly similar resistances to neomycin, amoxicillin, oxytetracycline, and erythromycin (Figure 4).

![Antimicrobial agents sensitivity graph](image)

**Fig. (4):** The comparative sensitivity study to the tested antimicrobial agents among *E. coli* and *L. monocytogenes* isolates.

In the current study, 14 out of 15 *E. coli* isolates were MDR (resistant to 3 or more antimicrobial classes). Twenty percent of isolates exhibited multi resistances to 7 antimicrobial classes (table 2).
Table (2): Antimicrobial resistance profile of *E. coli* (n=15)

<table>
<thead>
<tr>
<th>No of isolates (%)</th>
<th>Antimicrobial resistance profile</th>
<th>Multiple antimicrobial resistance (classes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT, AMP, NA, NOR, C</td>
<td>7</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT, AMP, NA, NOR</td>
<td>6</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT, AMP, NA</td>
<td>6</td>
</tr>
<tr>
<td>3 (20%)</td>
<td>AMX, SXT, E, N, OT, AMP</td>
<td>5</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT, AMP, NA, NOR, C, CN</td>
<td>7</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT, AMP, NA, NOR, C</td>
<td>7</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT, AMP, NA</td>
<td>6</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT, AMP</td>
<td>5</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT, AMP</td>
<td>5</td>
</tr>
<tr>
<td>2 (13.3%)</td>
<td>AMX, SXT, E, N, OT</td>
<td>5</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT, E, N, OT</td>
<td>5</td>
</tr>
<tr>
<td>1 (6.67%)</td>
<td>AMX, SXT</td>
<td>2</td>
</tr>
</tbody>
</table>

OT: Oxytetracycline; NA: Nalidixic acid; AMX: Amoxicillin; SXT: Sulfamethoxazole-trimethoprim; AMP: Ampicillin; S: Streptomycin; N: Neomycin; C: Chloramphenicol; NOR: Norfloxacin; CN: Gentamicin.

Moreover, all the isolated *L. monocytogenes* exhibited multiple resistances to at least 4 classes of antimicrobial agents. About 69% of the isolate were resistant to 7 different classes of antimicrobial agents in the present study (Table 3).

Table (3): Antimicrobial resistance profile of *L. monocytogenes* (n=13)

<table>
<thead>
<tr>
<th>No of isolates (%)</th>
<th>Antimicrobial resistance profile</th>
<th>Multiple antimicrobial resistance (classes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (7.69 %)</td>
<td>OT, N, NA, C, E, AMX, SXT, AMP, CN, NOR</td>
<td>7</td>
</tr>
<tr>
<td>3 (23.07 %)</td>
<td>OT, N, NA, C, E, AMX, SXT, AMP</td>
<td>7</td>
</tr>
<tr>
<td>5 (38.46%)</td>
<td>OT, N, NA, C, E, AMX, SXT</td>
<td>7</td>
</tr>
<tr>
<td>3 (23.07 %)</td>
<td>OT, N, NA, C, E, AMX</td>
<td>6</td>
</tr>
<tr>
<td>1 (23.07 %)</td>
<td>OT, N, NA, C</td>
<td>4</td>
</tr>
</tbody>
</table>

OT: Oxytetracycline; NA: Nalidixic acid; AMX: Amoxicillin; SXT: Sulfamethoxazole-trimethoprim; AMP: Ampicillin; S: Streptomycin; N: Neomycin; C: Chloramphenicol; NOR: Norfloxacin; CN: Gentamicin.
DISCUSSION

In the current study, out of 100 milk samples, 15 E. coli isolates were isolated with a prevalence of 9%. On the other hand, lower prevalences of E. coli were identified in other studies. For example, in Ireland, out of 120 raw cow's milk samples, 0.8% were positive for E. coli (Lynch et al., 2012). In Germany, 1.5% of 337 raw cow's milk samples containing E. coli (EFSA, 2011). Additionally, in France, 4.8% of 205 raw cow's milk samples were positive for different E. coli serotypes (Perelle et al., 2007). Outer membrane protein intimin, encoded by the eae gene, represents one of the virulence factors commonly present in pathogenic STEC strains (Paton and Paton, 1998). Via PCR technique, all the isolated E. coli strains were positive for eae gene.

Thirteen L. monocytogenes isolates were isolated out of 100 milk samples. PCR detection of the hlyA gene was applied. The presence of hlyA gene, encoding Listeriolysin O (LLO) (Mengaud et al., 1988), is a characteristic of L. monocytogenes. In other studies, L. monocytogenes isolates were detected in 10% of raw milk samples (El-marrakchi et al., 1993) and were similarly identified in 10.53% of raw milk samples (Minea et al., 2005). On contrast, lower prevalence percentages were reported in other countries such as Sweden where a prevalence of 1%, out of 294 samples, was found (Waak et al., 2002) and Austria where a prevalence of 1.5% was estimated (Deutz et al., 1999). However, in Switzerland, there was no detection for L. monocytogenes in 310 raw milk samples (Stephan and Buehler, 2001).

In the current study, all E. coli isolates were resistant to sulfamethoxazole-trimethoprim, and amoxicillin as well as high resistances against erythromycin, neomycin, oxytetracycline, and ampicillin were detected. However, high sensitivities were observed to gentamicin, chloramphenicol, and norfloxacin. Other study in Bangladesh agreed with ours as all isolated E. coli from raw milk were resistant to tetracycline while resistance of 50% against nalidixic acid was determined (Uddin et al., 2011). On contrast, in Dehradun, E. coli isolates from raw milk samples were sensitive to erythromycin. However, an intermediate effect was identified against chloramphenicol while there is an agreement with our results in that a high level of resistance was identified against tetracycline (Pant et al., 2013).

On the other hand, complete resistances of L. monocytogenes isolates to neomycin, nalidixic acid, oxytetracycline, and chloramphenicol were identified while high levels of sensitivity were detected to gentamicin and norfloxacin. These results are in agreement with a previous study in Iran where all L. monocytogenes isolates from raw milk were susceptible to gentamicin while high resistances were determined against tetracycline (Jamali et al., 2013). In a previous study in Egypt, L. monocytogenes isolates exhibited high resistances against tetracycline. However, intermediate effects against gentamicin were identified (AL-Ashmawy et al., 2014).

As a result of the misuse of antimicrobial agents, there is an expectation that the emergence rate of antimicrobial resistance will increase (WHO, 2001). In the current study, about 93% of the isolated E. coli were multi resistant against at least 5 classes of the antimicrobial agents. However, 12% of non-O157 E. coli strains in Spain were resistant to five or more antimicrobial agents (Mora et al., 2005). Moreover, the treatment of L. monocytogenes becomes difficult as a result of its resistance to
multiple antibiotics (Charpentier et al., 1995). All the isolated L. monocytogenes exhibited multiple resistances to at least 4 classes of antimicrobial agents in the present study. In another study in Egypt, 100% of L. monocytogenes isolates were MDR to at least 4 antimicrobial agent classes; 25% of the isolates showed resistances to 8 classes of antimicrobial agents; 25% of the strains also were multi resistant to 7 classes of agents (AL-Ashmawy et al., 2014). Consequently, it is very important to reduce the misuse of antimicrobial agents. One of the ways for limiting the increasing of antimicrobial resistances is the reduction of the application of antimicrobial agents to both people and animals. This consideration resulted in the ban of the use of antimicrobial agents as feed additives in 1999 in Switzerland (Arnold et al., 2004) and also in the European Union from January 2006 (Castanon, 2007). On contrast, in some countries, for example, in the United States, antimicrobial agents are still used for growth promotion with no control by veterinarians (Jones and Ricke, 2003). The stopping of the application of the antibiotics as growth promoters has been suggested by an announcement of the Food and Drug Administration (FDA) from April 2012 (www.fda.gov).

In conclusion, this study revealed that raw milk is considered to be hazards for the consumption without being heat treated. Some food-borne pathogens are highly resistant to several types of antimicrobial agents. Moreover, multi resistances of these pathogens to different antimicrobial agent classes have highly increased and these resistances are mainly due to the misuse of these agents. Consequently, it is a very difficult mission to treat patients infected by these pathogens. Finally, further investigations are required for the isolation of other pathogens from raw milk by different technique of isolation, the detection of various virulence genes, and the determination of the susceptibility of these isolated pathogens to several antimicrobial agents. Moreover, the studying of the use of some commercial herbal products, as natural food additives for milk, is important as these products could be successful in controlling of some food-borne pathogens; these natural products would also be healthier than the synthetic food additives. All of these studies will help in the choice of the best antimicrobial agent for treatment of infected patients, in reducing the multi resistances of these pathogens, and in finding new natural antimicrobial agents that would be safe and effective.

REFERENCES


دراسة مقارنة بين مدى تواجد و الحساسية للمضادات الحيوية لكل من الإشيزريخيا كولاي و الليستريا مونوسويتويجنس المعزولة من الحليب الغير مبستر في مصر

للدكتورة مهسا العشماوي - محمد عادل السكرى - خالد عبد الجليل - رشا بروة

تهدف هذه الدراسة إلى عزل بعض مسببات الأمراض المنقولة عبر الغذاء والتي تتضمن الإشيزريخيا كولاي و الليستريا مونوسويتويجنس من عينات الحليب الغير مبستر في السوق المصري بالإضافة إلى إجراء مقارنة بين مدى تواجد هذه البكتيريا في عينات الألبان المستخدمة و المقارنة أيضا بين حساسية هذه البكتيريا للمضادات الميكروبية المختلفة، حيث أن مقاومة البكتيريا للعديد من مضادات ميكروبية تتمثل مشكلة كبيرة في الأيام الحالية و هذه المشكلة تنتج من الاستخدام الخاطئ لهذه المضادات. قامت هذه الدراسة على تعدين المقاومة لكل نوع البكتيريا للعديد من المضادات الميكروبية التي تنتمي لأقسام مختلفة.

جرت الدراسة على 100 عينة من حليب السوق وقد تم عزل 15 عزلة من الإشيزريخيا كولاي، وتحديد بعض عزل من البكتيريا المونوسويتويجنس باستخدام الطريقة البيوكيميائية (Biochemical method). كما أوضح استخدام تفاعل البممره المتسمسل (PCR) أن كل هذه العزل تحتوي على جين eae مونوسويتويجنس و تحديدهم عبر الطرق البيوكيميائية. ثم تعدين تواجد جين hlyA في هذه العزلات عن طريق تفاعل اللمبر المتسلسل. علامة على ذلك، تم دراسة حساسية جميع العزلات بعد عشر مضادات ميكروبية. كانت كل عزلات الإشيزريخيا كولاي مقاومة للسلفاميزين، البروميدير وأميبوكسيدين. بينما أظهرت هذه العزلات حساسية عالية للجينتاميسين، الكلورامفينيكول، و النيوسيتون. على الصعيد الأخر، أظهرت جميع عزلات الليستريا مونوسويتويجنس مقاومة لكل من نيميسين، حمض النيميديد، الثياميسين، الأوكسيترياسيكلين، والكلورامفينيكول. لكنها لديها حساسية عالية للجينتاميسين، النيوسيتون.

أظهرت المقارنة بين الحساسيات للمضادات الميكروبية المستخدمة في هذه الدراسة أن كل من البكتيريا المعزولة من النوعين لديها حساسية عالية نحو الجينتاميسين، و النيوسيتون. وعلى النقيض، أظهرت هذه البكتيريا مقاومة عالية للنيميسين، الأوكسيترياسيكلين، والأزاثيميدين. بالإضافة إلى ذلك، يقرب من 93% من عزلات الإشيزريخيا كولاي كانت مقاومة على الأققل ل 4 أنواع من أقسام المضادات الميكروبية. كذلك كل عزلات الليستريا مونوسويتويجنس أظهرت مقاومة على الأقل ل 4 أنواع من أقسام المضادات الميكروبية. في الختام، هناك خطرة عالية من تناول الحليب الغير مبستر حيث أن قد يسبب العديد من الأمراض التي تنتقل عن طريق الأغذية، بالإضافة إلى ذلك، وجد أن مسببات الأمراض المنقولة عن طريق الأغذية، التي تشمل كل من الإشيزريخيا كولاي و الليستريا مونوسويتويجنس، لديها مقاومة للعديد من المضادات الميكروبية من الأقسام المختلفة.