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Prevalence and bacteriological identification of *Escherichia coli* isolated from bovine milk and workers' urine in dairy farms

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ABSTRACT

Various bacteria can cause mastitis in cows. *Escherichia coli* (E. coli) is the most common cause of both clinical and subclinical mastitis on dairy farms, leading to a decrease in milk production and economic losses. Also, *E. coli* is responsible for more than 81% of urinary tract infections (UTIs) in humans and animals, causes dysuria, and may lead to kidney damage. The purpose of this study was the detection and serotyping of *E. coli* isolated from bovines' milk and workers' urine in different dairy farms in Gharbia governorate, Egypt. A bacteriological examination of 158 samples (100 milk and 58 workers' urine) showed that the prevalence of *E. coli* in bovine milk samples was 5% and 6.9% in workers' urine samples. Serological identification of these isolates revealed that the predominant serotypes in samples from milk were *E. coli* O125 (3%) followed by *E. coli* O157 and *E. coli* O55 (1% for each), while in worker's urine samples, *E. coli* O157 was (3.4%) and *E. coli* O125 was (1.7%). The same *E. coli* serogroups were found in both bovine milk and workers' urine, posing a serious threat to both human and animal health due to zoonotic transmission of these important serotypes.

1. INTRODUCTION

Escherichia coli (*E. coli)* is a gram-negative bacterium that belongs to the family Enterobacteriaceae. It has a rod-shaped bacterium, is non-sporulated, flagellated, and facultative anaerobic (the best growth conditions are at 37 °C, but it can tolerate 49 °C) (Jang *et al*., 2017). One of the most common bacteria in the digestive tracts of most mammals, including humans, is *Escherichia coli*. It is known to be a good sign of feces contamination and the presence of enteropathogenic or toxigenic microbes that are bad for human health because it can be found in animal products and environmental samples like water (Koutsoumanis et al., 2020).

Environmental pathogens usually arise from the farm environment, including bedding materials, flies, the stable, and even the gastrointestinal tract of cattle, which is a popular reservoir for pathogens like *E. coli* (Klaas and Zadoks, 2018). Furthermore, antibiotics like cephalosporins and fluoroquinolones are frequently used to treat cows with severe *E. coli* mastitis, while many cows with less severe cases are treated within a week of the infection starting (Suojala et *al.*, 2013). *E. coli* can rapidly acquire new combinations of genes for virulence factors and antimicrobial resistance (AMR), despite the fact that the majority of strains are low-virulent and only cause infection when a predisposing condition affects an animal's natural immunity. Some *E. coli* strains cause primary intestinal and extra-intestinal infections as a result of acquired virulence factors (Mora *et al*., 2011). Many different microorganisms can cause mastitis in cows, but *E. coli* is one of the most common causes of both symptomatic and asymptomatic mastitis in dairy farms (Abebe *et al*., 2014; Bedasa *et al.*, 2018; Ismail and Butarbush, 2020). It is also one of the most common microorganisms contributing to the emergence of urinary tract infections (UTIs). Meanwhile, *E. coli* is responsible for more than 81% of UTIs (Cybulski *et al*., 2013; Jalilian *et al*., 2014). Uropathogenic *E. coli* (UPEC) is thought to be mainly found in the human intestines. This means that a lot of different types of virulence factors can move up into the urinary tract and infect it (Foxman, 2010). UTI symptoms may include dysuria, increased urinary frequency, urgency, suprapubic pain, fever, pyelonephritis with sepsis, and kidney damage in both humans and pets of all ages (Flores-Mireles *et al*., 2015).

E. coli have about 250 different serotypes based on the O, H, and K antigens, making them different serotypically (Sarantuya *et al*., 2004). Enteropathogenic *E. coli* was ascribed to sero-groups O26, O55, O86, O111, O114, O119, O125, O126, O127, O128, O142, and O158 by the World Health Organization in 1987 (Chen and Frankel 2005). In 1992, the infection of many people by *E. coli* O157:H7 in southern Africa and Swaziland was related to surface water contamination from animal carcasses and cow manure. Bovine and ovine dairy products (both pasteurized and unpasteurized) have

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been linked to verocytotoxigenic *Escherichia coli* (VTEC) infections. This has included several outbreaks among children who consume raw milk and dairy products (Zhang et al., 2000; Walsh et al., 2006). Reports say that an infection with *E. coli* O157:H7 can cause hemorrhagic colitis and hemolytic uremic syndrome. This type of infection is very hard to treat and keep under control (Karch et al., 2005).

This study aimed to investigate the prevalence of *E. coli* as an important pathogen in raw bovine milk and worker urine collected randomly from animals and humans in different farms in the Gharbia governorate. This pathogen's potential zoonotic hazards in terms of incidence and serotyping were also investigated.

2. MATERIAL AND METHODS

2.1 Collection of samples

A total of 158 random samples (100 raw bovines' milk and 58 workers' urine samples) were collected from different dairy farms in Gharbia governorate during the period from September 2022 to February 2023. Fifty ml of milk samples were taken after washing, drying, and swabbing the udder teat orifice with 70% ethyl alcohol, and discarding the first 3–4 streams of milk sterilized cups from apparently healthy cows. 50 ml of human urine samples were taken from midstream in sterilized cups without any chemical preservatives from workers without clinical signs. According to APHA (2001), all samples were transferred in an ice box to the laboratory for bacteriological examination under sterile conditions within an hour of being collected.

2.2 Isolation of E. coli

Twenty-five ml of human urine samples were centrifuged at 3000 x g for 5 minutes through Sigma apparatus (Germany), and the sediments were used for *E. coli* isolation according to Akter et al. (2014). According to APHA (2001), all milk samples and centrifuged urine samples were diluted at a rate of 1: 9 in nutrient broth (LabM, Heywood, U.K.) and incubated at 37 °C for 24 hours. A loopful from the milk broth was streaked on MacConkey agar (Oxoid) and incubated at 37 °C for 24 hours. The suspected lactose-fermenting pink colonies were sub-cultured on Levine's Eosin Methylene Blue agar (EMB) (Oxoid, Hampshire, U.K.) at 37 °C for 24 h. While urine broth was cultured on cystine–lactose–electrolyte-deficient agar (CLED agar) (Oxoid). Both suspected colonies on EMB and CLED media were picked and cultured on 5% sheep blood agar (for determination of hemolysis type), incubated at 37 °C for 24 h, purified by sub-culturing on new selective agar plates, and preserved in semisolid nutrient agar slants for further identification.

2.3. Morphological identification

Suspected colonies were identified by Gram's stain and motility test (by stabbing the bacterial isolate in the center of 0.5% semi-solid agar tubes, then incubated at 37°C for 24 hours) (Quinn et al., 2002).

2.4. Biochemical identification

Identification of *E. coli* isolates was confirmed biochemically according to Quinn et al. (2002) by sugar fermentation tests, indol, methyl red, Voges-Proskaur, citrate utilization, H2S production, urease, catalase, oxidase, and nitrate reduction tests.

2.5. Sero-grouping of E. coli isolates

Isolated *E. coli* from bovine milk and human urine samples were serotyped by using O-antisera (Group O-somatic antisera) and K-antisera (Group K-capsular antisera) (Denka Seiken-Co., Ltd., Tokyo, Japan), according to Edward and Ewing (1972).

3. RESULTS

2.1 Collection of samples

Out of 158 samples (100 raw bovines' milk and 58 workers' urine samples), five *E. coli* isolates were recovered from bovines' milk and four *E. coli* isolates were detected in workers' urine, with isolation rates of 5% and 6.9%, respectively (Table 1).

Table (1): Incidence rate of *E. coli* isolation from bovines' milk and workers' urine samples.

Samples	Number of examined samples	Number of positive samples	% of positive samples
Bovines' milk	100	5	5
Workers'urine	58	4	6.9
Total No	158	9	5.7

3.2. Culture characters of isolates

All isolates appeared as typical colonies of *E. coli* as follows: circular, non-pigmented colonies on nutrient agar medium; and rounded, bright pink colonies (lactose fermenter colonies) on MacConky's agar. On EMB media, there was a distinctive greenish metallic sheen colony, while yellowish colonies (lactose fermenter colonies) were on CLED agar. All isolates exhibited narrow colorless hemolysis zone β hemolysis (complete hemolysis) on blood agar.

3.3. Morphological identification

All *E. coli* isolates were gram-negative, medium-sized red rods, arranged singly, in pairs and groups, non-spore-forming, and motile.

3.4. Biochemical identification

Characteristic, identical biochemical reactions to *E. coli* appeared in all suspected isolates (Table 2).

Table (2): Biochemical identification of isolated *E. coli.*

Biochemical test	Reaction
Sugar fermentation	Positive (yellow color with gas)
Indole production	Positive (red ring)
Methyl red test	Positive (bright redcolour)
Voges-Proskaur test	Negative (yellow colour)
Citrate utilization test	Negative (green colour)
H_2S production test	Negative (no black colour)
Urease test	Negative (yellow colour)
Catalase test	Positive (gas bubbles and effervescence)
Oxidase test	Negative (no colour change)
Nitrate Reduction test	Positive (red colour)

3.5. Sero-grouping of *E. coli* isolates:

The detected serovars in all samples were *E. coli* (O125, O157, and O55). The predominant serotypes in milk samples (5 isolates) were *E. coli* O125 (3/5, 60% from the positive *E. coli* isolates with a final percent of 3% from all examined milk samples), followed by *E. coli* O157 (1/5, 20% from the positive

E. coli isolates with a final percent of 1% from all examined milk samples), and *E. coli* O55 (1/5, 20% from the positive *E. coli* isolates with a final percent of 1% from all examined milk samples). While *E. coli* O157 (2/4, 50% from the positive *E. coli* isolates with a final percent of 3.4% from all examined urine samples), then *E. coli* O125 (1/4, 25% from the positive *E. coli* isolates with a final percent of 1.7% from all examined urine samples), and an un-typed serotype (1/4, 25% from the positive *E. coli* isolates with a final percent of 1.7% from all examined urine samples) in workers' urine isolates. It was observed that the *E. coli* O55 serotype was detected in milk samples only (Table 3).

Table (3): Serological identification of *E. coli* and it's prevalence in samples.

Serotypes of *E.coli*	Total from total examined samples (158 samples)		Total from examined urine samples (58 samples)		Total from examined milk samples (100 samples)		Positive Workers' urine isolates (4 isolates)		Positive Bovines' milk isolates (5 isolates)	
	%	No.	%	No.	%	No.	%	No.	%	No.
O55	0.6	1	0	0	1	1	0	0	20	1
O125	2.5	4	1.7	1	3	3	25	1	60	3
O157	1.9	3	3.4	2	1	1	50	2	20	1
Un typed	0.6	1	1.7	1	0	0	25	1	0	0
Total	5.7	9	6.9	4	5	5	100	4	100	5

4. DISCUSSION

The overall prevalence of *E. coli* was 9/158 (5.7%) in the present study. The prevalence rate in bovines' milk samples was 5% (5/100), in agreement with Eldesoukey et al. (2022), who detected *E. coli* with a ratio of 5.3% (8/150) in milk samples. On the other hand, Elbehiry *et al*. (2021) recorded that the isolation rate was 12.10% from milk samples; also, Elmonir *et al*. (2018) found *E. coli* at an incidence of 13.2% in the Kafrelsheikh governorate, but a higher prevalence was 29.63% and 19.6%, respectively, as recorded by Rahman *et al*. (2018) and Rezatofighi *et al*. (2021), respectively. The highest proportions were (85.71%) from clinical mastitis cases and (80.48%) from subclinical mastitis cases from four Egyptian governorates (Giza, Menofia, Beheira, and Alexandria), according to El-Mohandes et al. (2022). Also, (80%) was reported by Sharaf et al. (2015) and (75%) in raw tested milk samples from different places in the Beni-Suef governorate recorded by Megawer et al. (2021). The big differences in the prevalence of *E. coli isolation from* bovines' milk may be related to the sampling time and region, udder fecal contamination, inadequate hygiene and sanitary practices that are followed in these farms, and delays in the diagnosis of diseases.

In this study, the prevalence rate of *E. coli* in workers' urine samples from the same dairy farms in Gharbia governorate was 4/58 (6.9%), in agreement with those obtained by Abujnah et al. (2015), who found that the *E. coli* isolation rate was 6.7% in Lybia. On the contrary, Selim et al. (2013) isolated *E. coli* from human urine, with a lower prevalence rate of 2.82% in Ismailia City. While higher results of *E. coli* isolation were recorded by Hassuna et al. (2020), who detected *E. coli* in incidence of 33.5%) from patients with UTIs from different hospitals at Minia and Kaliobia governorates, Essa (2022), who showed that *E. coli* isolation rate (42.9%) from humans affected by UTIs, which is similar to the recorded results for human patients by Ghavide et al. (2020), Mahdi et al. (2020), and Said et al. (2021), who found that *E. coli* was the most common bacteria isolated from different Egyptian hospitals and laboratories with a prevalence rate of 55.5%. The difference in the percentage of *E. coli* isolation from human urine in these results according to the sample collection localities, inadequate hygiene conditions, and disease stage of human cases (if samples were taken from an apparent healthy or clinically diseased human).

Colonies of isolated *E. coli* from bovines' milk and human urine appeared as round and non-pigmented colonies on nutrient agar, but their colonies appeared as round and pigmented lactose fermenters with non-mucoid vivid pink colonies on MacConkey's agar. On EMB, it showed greenish metallic sheen colonies for *E. coli* isolates from bovine milk and human urine. For human urine isolates on CLED agar, colonies appeared as yellowish colonies (lactose fermenter colonies); these were similar to the results recorded by Said et al. (2021).

The indole, methyl red, catalase, sugar fermentation, nitrate reduction, and Eijkman tests all yielded positive results for all nine isolates, proving that each one of them was *E. coli*. Additionally, oxidase, Voges-Proskauer, urease, citrate utilization, and H2S tests gave negative results; these were the same results that were recorded by Elshora (2019) and Essa (2022).

In the current work, serogroups for *E. coli* isolates were O125 (4/9); (3/5, 60% from the positive *E. coli* isolates with a final percent of 3% from all examined milk samples); and (1/4, 25% from the positive *E. coli* isolates with a final percent of 1.7% from all examined human urine samples). *E. coli* O157 serogroup in the prevalence of (3/9); (1/5, 20% from the positive *E. coli* isolates with a final percent of 1% from all examined milk samples; and 2/4, 50% from the positive *E. coli* isolates with a final percent of 3.4% from all examined urine samples). One isolate was typed as *E. coli* O55 (1/5, 1% of all tested bovine milk samples). These results are in agreement with Ombarak *et al.* (2019), who detected strain *E. coli* O55 in 1.3% of milk samples. However, the percentage of the O125 serogroup in the present study (3%) from milk samples was close to a previously recorded isolation rate of 2%. Younis *et al*. (2021) from milk samples, while Hassan et al. (2021) detected the O125 serogroup by a higher percentage (16.7%) from milk samples, but the detection rate of *E. coli* O125 in human urine samples in the present study was 1.7%, which is similar to results recorded by Elsayed et al. (2021), who detected the O125 strain in a ratio of 1.6% in human urine isolates. In current results, *E. coli* O157 was present in a lower proportion than other studies,

which showed that the prevalence rate of this serotype was only (2.9%) in cow milk samples of Ethiopia (Disassa et al., 2017), (2.3%) in marketed raw cow milk samples in Nigeria (Ghali-Mohammed et al., 2023) and (11.33%) in student urine samples in Nigeria (Saidu and Ebiala, 2022). However, the detection of *E. coli* O157in milk samples reached 22.5 percent, as recorded by Aliyu et al. (2021), respectively. Serotype O157: H7 can cause mild diarrhea or asymptomatic carriage, but it can also cause hemolytic uremic syndrome (HUS) and hemorrhagic colitis, two potentially serious consequences (Karch*et al*., 2005), which throws light on the public health risk of this serogroup.

5. CONCLUSIONS

The current study results drew maps for *E. coli* serogroups in most dairy farms in the Gharbia government. The most prevalent serotypes belong to enteropathogenic *E. coli O125 (2.5%)*, O55 (0.6%) from all samples, and enterohaemorrhagic *E. coli O157* (1.9%), which cause serious diseases and complications in both animals and humans. Milk contamination can occur from a variety of factors, including a contaminated udder, milk handlers with poor personal hygiene, contaminated water, and improperly cleaned and sanitized containers. So, we recommend adequate hygienic measures, either among animals, workers, or both, to avoid the spread of this important infection.

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