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Original article

Prevalence of *Escherichia coli* strains resistance to antibiotics in wound infections and raw milk

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ABSTRACT

Antibiotic-resistant *Escherichia coli* strains including extended-spectrum β-lactamase (ESBL) isolates are globally widespread in medical, food, and environmental sources. Some of these strains are considered the most pathogenic bacteria in humans. The present work examined the predominance of antibiotic resistance in *E. coli* strains in wound infections comparing with *E. coli* strains isolated from a raw milk as a potential source of those strains. The wound infections included abdomen, anus, arm, back, buttock, chest, foot, hand, head, leg, lung, mouth, neck, penis, thigh, toe, and vagina infections. In total, 161 and 153 isolates identified as *E. coli* were obtained from wound infections and raw milk, respectively. A Vitek 2 system innovated by bioMérieux, France was applied to perform the identification and susceptibility tests. The *E. coli* isolates that have ability to produce ESBL were detected by an ESBL panel and NO45 card (bioMérieux). Over half of the *E. coli* were from abdomen, back, and buttock wound infections. More than 50%of the *E. coli* isolates obtained from wound infections were resistant to cefazolin, ampicillin, cefuroxime, ciprofloxacin, mezlocillin, moxifloxacin, piperacillin, and tetracycline; 70% of the isolates from wound infections and 0% of the isolates from raw milk were *E. coli* isolates produced ESBL. The data showed that the strains resistance to multi-antibiotic and produced ESBL are more widespread among wound infections than in raw milk.

1. Introduction

Escherichia coli is a gram-negative bacterium. Strains of *E. coli* are typically not pathogenic to humans. However, several *E. coli* strains have ability to cause several diseases in different sites including the renal system, gastrointestinal tract, and the central nervous system (Nataro and Kaper, 1998). Numerous strains of *E. coli* have evolved as opportunistic and commensal pathogens (Groisman and Ochman, 1996). Regrettably, several bacterial strains have become progressively resistant to antimicrobial agents over the last several decades (Guilfoile and Alcamo, 2007). Many international health institutions, such as World Health Organization and the United States Institute of Medicine, have formally expressed concern about the environmental and health hazards of antibiotic-resistant bacteria that pose health and economical risks (Meyer et al., 2010; Wise et al., 1998).

E. coli strains that are resistant to various antibiotics are of particular concern for global health so they are the most common uropathogenic and enteropathogenic bacteria. Unpasteurized milk is considered a main source of *E. coli* isolates resistance to multi-antibiotics. It has been confirmed that approximately 7% of *E. coli* isolates identified in raw milk are multi-drug resistant (Rasheed et al., 2014). A retrospective study that included more than 8900 bacterial isolates from milk reported an increasing prevalence of *E. coli* strains resistance to erythromycin (Makovec and Ruegg, 2003). The infections diagnosed in skin and soft tissue of patients almost at all ages are the most common among other microbial infections. Many cases of clinical wound infections may require

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treatment with antibiotics or parenteral therapy (Moet et al., 2007). It has been reported that 46%, 25%, and 21% of *E. coli* isolates that cause wound infections are resistant to ampicillin, tetracycline, and fluoroquinolones, respectively. *E. coli* isolates have many virulence factors that include outer membrane protease, cytotoxic necrotizing factor 1, drug resistance, and hemolysin (Petkovšek et al., 2009; Welch, 2016). A genetic analysis of *E. coli* isolates obtained from surgical incisions, traumatic injuries, and foot ulcers reported that 97.5%, 97.5%, 4%, 12.5%, 12.5%, and 2.5% of those isolates harbored the *fimH*, *iutA*, *papC*, *hlyA*, *cnf1*, and *neuC* gene, respectively (Chakraborty et al., 2017b). Most (90%) uropathogenic *E. coli* isolates possessed the *fimH* gene, followed by *iutA* (n = 98; 63%), *papC* (n = 76; 49%), *cnf1* (n = 46; 29.5%), *hlyA* (n = 45; 29%), and *neuC* (n = 8; 5%), respectively (Chakraborty et al., 2017a).

The present work analyzed the prevalent patterns of *E. coli* strains resistance to multi-antibiotic from wound infections in patients treated at King Saud University Medical City (KSUMC) Riyadh, Saudi Arabia. The antibiotic resistance patterns were compared with *E. coli* strains that isolated from raw milk produced in the Riyadh region.

2. Material and methods

2.1. Microbial strains

The collection of *E. coli* isolates was performed between 02 October 2016 and 02 June 2017 at King Khaled University Hospital. Throughout this period, 227 wound infections samples were cultivated and identified agreeing with the directions of manufacturer using the full automatic system (Vitek 2 system). The types of wound infection and antibiotic resistance patterns were analyzed and compared with these data that obtained from bacterial isolates obtained from raw milk. The total number of milk samples collected in this study were 240. During the same time, the raw milk samples were collected weekly in a sterilized container at random from one of the companies producing milk in Riyadh, Saudi Arabia, identified here as company R. MacConkey agar (MCA), nutrient agar (NA), blood agar (BA), and violet red bile agar (VRBA) (Oxoid, UK) were used to isolate the bacterial strains (*E. coli*) based on culture characteristics. The primary identification was carried out using API 20 E (bioMerieux) and the identification was completed using the Vitek ®2 GN ID.

2.2. Determination of antimicrobial susceptibility

antibiotic susceptibility tests were done by the Vitek 2 system (bioMérieux) using several of cards including AST-GN69, AST-XN06, and AST-GN69, according to the instructions of manufacturer. One colony of each pure isolate on Blood medium (bloog agar base medium with 5% sheep blood) was tested. The purification of isolates was performed on previously described culture media at 35 °C for 20 h (Bobenchik et al., 2014).

2.3. Determination of extended-spectrum-β-lactamase (ESBL)

The Vitek 2 system was used to test ESBL in all the bacterial strains isolated in this work. A ESBL panel (NO45 card, bioMérieux) was applied. The panel consists of six wells, the first contains cefepime (1.0 μg/ml), the second contains cefotaxime (0.5 μg/ml), the third contains ceftazidime (0.5 μg/ml), the fourth contains cefepime + clavulanate (1.0 + 10 μg/ml), the fifth contains cefotaxime + clavulanate (0.5 + 4 μg/ml), and the sixth contains ceftazidime + clavulanate (0.5 + 4 μg/ml). Bacterial growth was recorded using spectrophotometric scanner. Decreasing in bacterial growth was determined by comparison between antibiotic alone with antibiotics + clavulanate. To perform the control test, *E. coli* ATCC 35,218 and ATCC 25,922 were employed.

2.4. Analysis of data

The statistical analysis of data was done by the Ward method, odds ratio and relative risk using SPSS statistical software. Correlation coefficients were determined as the square of the Pearson product using the Excel program.

3. Results

The results showed that 161 of 227 wound infections samples were identified as *E. coli*. The sources of wound infections included abdomen, anus, arm, back, buttock, chest, foot, hand, head, leg, lung, mouth, neck, penis, thigh, toe, and vagina. 39.8%, 14.2%, 14.2%, 8.5%, and 6.3% of the *E. coli* strains were obtained from the abdomen, buttock, back, foot, and anus, respectively, while other *E. coli* isolates were obtained in less than 5% of cases from wounds in the arm, chest, hand, head, leg, lung, mouth, neck, penis, thigh, toe, and vagina (Fig. 1). The data obtained from antibiotic

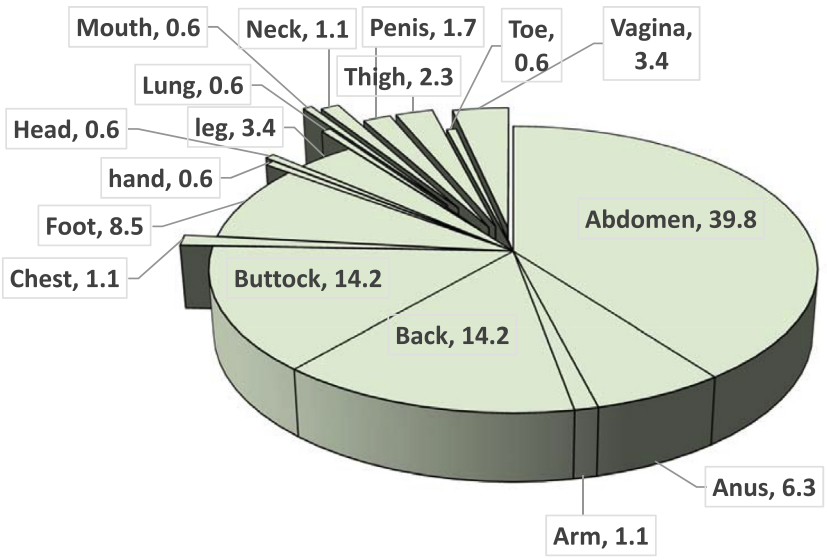

Fig. 1. Percentage of *E. coli* isolates obtained from several different wound infection sites in patients treated at King Khaled University Hospital. (N = 161).

Table 1
Antibiotic-resistant *E. coli* isolates from wound infections compared with the isolates from raw milk.

	From patients			From raw milk			
Antibiotics	S	R	I	S	R	I	RSQ
Amoxicillin/Clavulanic Acid	62.4	23.0	14.6	100.0	0.0	0.0	0.97
Ampicillin	15.2	84.8	0.0	90.9	9.1	0.0	0.07
Cefepime	50.6	47.8	1.7	91.6	0.0	8.4	0.22
Cefotaxime	42.7	9.0	0.6	91.6	0.0	8.4	0.93
Cefoxitin	79.8	19.7	0.6	100.0	0.0	0.0	0.95
Ceftazidime	46.6	10.1	3.4	91.6	0.0	8.4	0.95
Cefuroxime	37.1	51.7	3.4	91.6	8.4	0.0	0.09
Ciprofloxacin	43.3	55.1	1.7	100.0	0.0	0.0	0.09
Gentamicin	74.2	25.8	0.0	100.0	0.0	0.0	0.88
Imipenem	99.4	0.0	0.0	100.0	0.0	0.0	1.00
Meropenem	100.0	0.0	0.0	100.0	0.0	0.0	1.00
Piperacillin/Tazobactam	87.6	7.3	5.1	100.0	0.0	0.0	1.00
Tigecycline	90.4	1.7	0.0	100.0	0.0	0.0	1.00
Trimethoprim/sulfamethoxazole	39.9	56.7	0.0	91.6	8.4	0.0	0.10

RSQ, Pearson correlation coefficient.

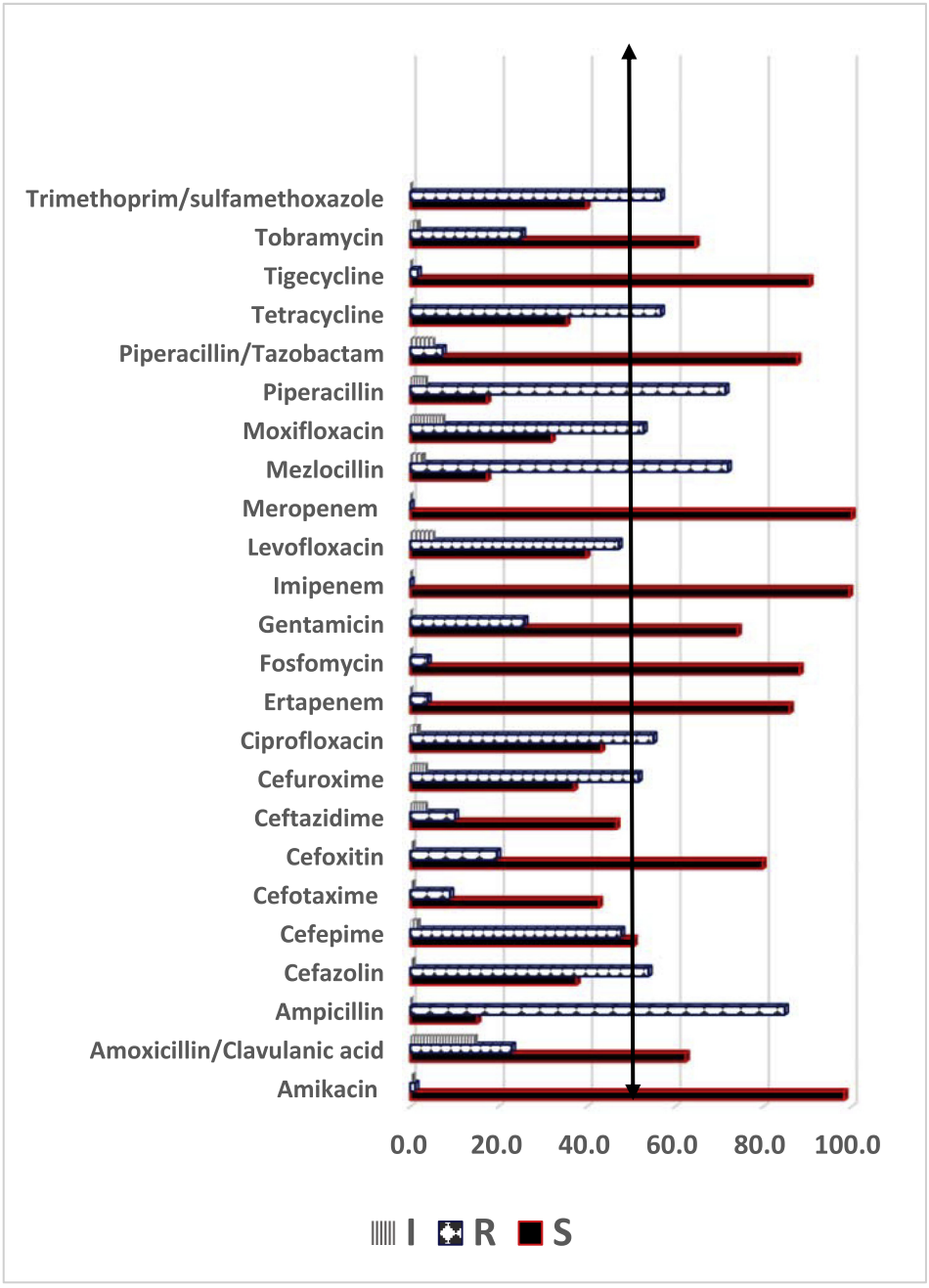

Fig. 2. Percentage of antibiotic-resistant *E. coli* isolates from wound infections in patients treated at King Khaled University Hospital. (N = 161).

susceptibility testing revealed that more than half of the *E. coli* strains from wound infections have the ability to resist the ampicillin, cefazolin, cefuroxime, ciprofloxacin, mezlocillin, moxifloxacin, piperacillin, and tetracycline, while more than half of the isolates were susceptible to amikacin, cefepime, cefoxitin, ertapenem, fosfomycin, gentamicin, imipenem, meropenem, tigecycline, tobramycin, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, and amoxicillin/clavulanic acid. The data presented in Table 1 indicate that 81–100% of *E. coli* strains were susceptible to the standard antibiotics that applied in this research. However, while the isolates from raw milk showed susceptibility to most the tested standard antibiotics, it is conceivable that raw milk could

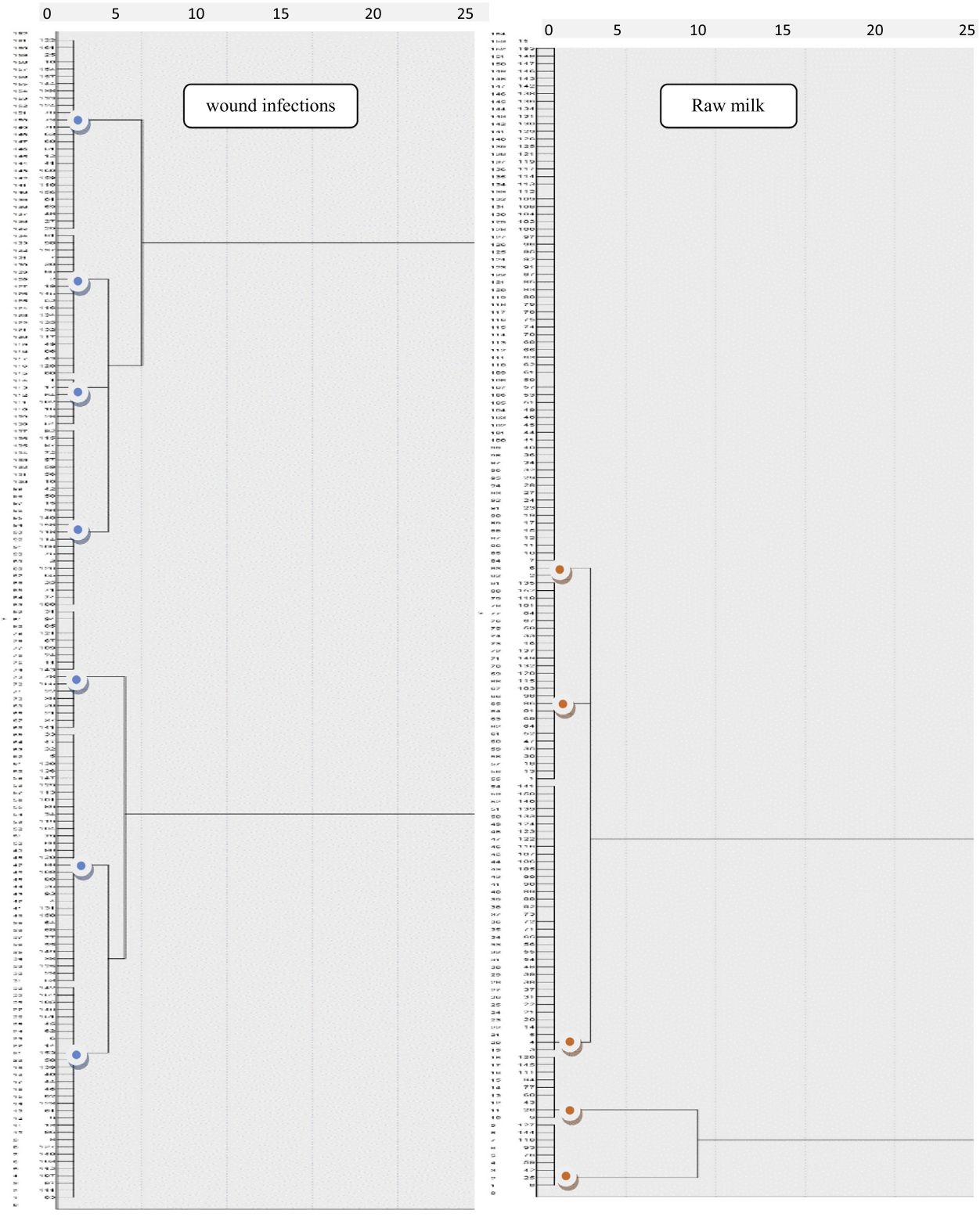

Fig. 3. Dendrogram of a hierarchical cluster analysis of the antibiotic-resistant *E. coli* isolates from wound infections (N = 161) and raw milk (N = 153) using Ward's methods.

Table 2
Risk estimate between ampicillin resistant *E coli* and the part of body.

Part of body	Odds ratio	95% confidence interval (lower–upper)
Abdomen	5.33	3.8–7.4
Back	4.65	0.6–35.97
Buttock	2.36	0.52–10.63
Foot	1.11	0. 23–5.25
Anus	0.26	0.07-0.975
Vagina	0.028	0.003–0.24
Leg	0.32	0.056–1.85
Thigh	0.05	0.05–4.99
Chest	0.16	0.01–2.75
Penis	0.98 (Cohort)*	0.95–1.003
Neck	0.98 (Cohort)*	0.96–1.005
Toe	0.99 (Cohort)*	0.981–1.006
Hand	0.99 (Cohort)*	0.981–1.006
Head	0.99 (Cohort)*	0.981–1.006

If one of value is zero the odds ratio cannot apply, for this reason the cohort test analysis is sued to identify relationships between the ampicillin resistant *E coli* and the part of human body.

still be a potential source of ampicillin, cefuroxime, and trimethoprim/sulfamethoxazole resistant *E. coli* isolates (see Fig. 2).

A dendrogram of a hierarchical cluster analysis of the antibiotic resistance patterns of bacterial strains isolated from wound infections and raw milk using Ward's method is presented in Fig. 3. *E. coli* isolates from wound infections were classified into seven groups. The first group (16.8% of the strains) was susceptible to almost all the standard antibiotics. The second group (12.4% of the strains) showed resistance to ampicillin, ciprofloxacin, gentamicin, levofloxacin, mezlocillin, moxifloxacin, piperacillin, tetracycline, and tobramycin. The third group (4.7% of the bacterial strains) showed resistance to amoxicillin/clavulanic acid, ampicillin, cefazolin, cefotaxime, and cefuroxime. The fourth group (15.5% of the bacterial strains) displayed resistance to ampicillin, mezlocillin, piperacillin, and tetracycline. The fifth group (10.6% of the bacterial strains) showed resistance to amoxicillin/clavulanic acid, ampicillin, cefazolin, cefepime, cefotaxime, ceftazidim, cefuroxime, mezlocillin, and piperacillin. The sixth group (21.7% of the bacterial isolates) showed resistance to ampicillin, cefazolin, cefepime, ceftazidim, cefuroxime, ciprofloxacin, gentamicin, mezlocillin, amoxicillin, piperacillin, tetracycline, and tobramycin. The seventh group (18.6% of the isolates) displayed resistance to ampicillin, cefazolin, cefepime, cefoxitin, ceftazidime, cefuroxime, levofloxacin, ciprofloxacin, mezlocillin, moxifloxacin, and piperacillin.

The analysis of the raw milk revealed five groups of *E. coli* isolates. Bacteria in the first group (47.1% of the bacterial strains) had no resistance to all tested antibiotics. The second group (17.6%) displayed intermediate resistance to amikacin. The third group (23.5%) displayed intermediate resistance to cefalotin. The fourth group (5.9%) displayed resistance to amoxicillin, ampicillin, cefalotin, cefixime, and aztreonam. The fifth group (5.9%) displayed resistance to trimethoprim/sulfamethoxazole, amoxicillin, and ampicillin. The data in Table 1 revealed high correlation coefficients between the *E. coli* isolates from wound infections and raw milk concerning the resistance and susceptible patterns for imipenem, meropenem, piperacillin/tazobactam, and tigecycline. Conversely, low correlation coefficients were evident for ampicillin, cefuroxime, and ciprofloxacin. Risk estimate between ampicillin resistant *E coli* and the part of body have been presented in Table 2, which show that odds ratios of abdomen, back, buttock and foot were 5.3, 4.65, 2.3 and 1.1 respectively. The Table 3 shows odds ratios and relative risk of the *E coli* strains isolated from raw milk that have resistance to ampicillin, cefuroxime and Trimethoprim/sulfamethoxazole. The data indicated that there is relative risk ranged from 6.66 to 10.01 and that the odds ratio ranged from 12.9 to 20.4.

4. Discussion

The present research aimed to evaluate the prevalence of *E. coli* strains resistance to the standard antibiotic isolated from wound infections, and studied the correlation between those isolates and the antibiotic-resistant *E. coli* strains isolated from raw milk. Microbial infections caused by *E. coli* strains can cause illness and the bacteria can be transmitted through contaminated food or through contact with animals or people. In dairy factories, the workers often contact animals and raw milk. Furthermore, in Saudi Arabia and elsewhere, many dairy farmers are in daily contact with raw milk. For this reason, the study included raw milk as a source of antibiotic-resistant *E. coli* isolates. In recent years, a dramatic decline in the antibacterial susceptibility of medical *E. coli* isolates has been reported (Johnson and Russo, 2002; Petkovšek et al., 2009). The present data completely agree with these reports. The majority (70%) of the isolates from wound infections were ESBL-producing strains of *E. coli* compared with 0% of the bacterial strains that isolated from raw milk. The findings indicate that raw milk produced in the Riyadh Region is not a potential source of ESBL-producing strains of *E. coli*. It has been confirmed that the tetracycline, streptomycin, and ampicillin resistant *E. coli* strains identified in humans and bovines, with more than 50% of resistant isolates being multidrug resistant. The present findings concerning *E. coli* strains isolated from wound infections are consistent with prior observations (Wilkerson et al., 2004).

Antibiotic-resistant and *E. coli* isolates produced ESBL have been identified and investigated worldwide from several medical and environmental sources (Lautenbach et al., 2001; Rodríguez-Bano et al., 2004; Santman-Berends et al., 2017; Tansawai et al., 2018). The present work confirms that the strains of *E. coli* identified in wound infections and from raw milk displayed resistance to broad range of standard antimicrobial agents, and that the *E. coli* strains produced ESBL were prevalent in wound infections and not in raw milk. The majority (68.2%) of wound infections were from abdomen, back, and buttock infections. The percentage of multi-antibiotic resistant *E. coli* isolates from wound infections was more than that isolated from raw milk. ESBL-producing strains of *E. coli* were not identified in the tested raw milk. The present work concluded that antibiotics resistant *E coli* isolates infected the wounds in abdomen, back, buttock more than others body parties. In addition, the results confirmed that the raw milk could be resource of *E coli* isolates resistance to cefuroxime and the isolates that resistance to trimethoprim/sulfamethoxazole.

Table 3
Risk estimate for antibiotics resistant *E coli* in wound infection and milk.

Part of body	Odds ratio	95% confidence interval (lower–upper)	Relative risk	95% confidence interval (lower–upper
Ampicillin resistant *E coli*	12.92	7.14–23.37	6.66	3.98–11.15
Cefuroxime resistant *E coli*	20.4	10.876–38.38	10.017	5.03–17.293
Trimethoprim/sulfamethoxazole resistant *E coli*	18.462	9.863–34.55	9.149	5.312–15.756

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Disclosure Statement

There are No competing financial interests.

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