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Prevalence of antibiotic resistant pathogenic *E. coli* from animals, retail and humans diagnosed with Gastroenteritis

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ABSTRACT

Foodborne pathogens represent one of the major challenges to health systems around the world. This risk is exacerbated by the presence of antimicrobial resistant (AMR) pathogens. Knowledge of the presence of these pathogens in the food supply chain would help in establishing intervention strategies to mitigate their risk. The objective of this study was to detect AMR among serotypes of *Escherichia coli (E. coli*) food adulterants serotypes of *E. coli* in the food supply chain and among isolates from gastroenteritis cases. *E. coli* isolates recovered from animals, meat processing plants, retail, and humans were examined for the presence of AMR using phenotypic and genotypic approaches. AMR to aminoglycosides, β-lactams, and tetracycline were detected in all isolates recovered from these sources at different levels. Similarly, presence of the bla-Tem, bla-SHV, aadA, and strAB genes were detected in isolates from these sources but there was no significant correlation between the genetic detection and phenotypic expression AMR.

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1.Introduction

Foodborne illnesses represent major health burdens worldwide. In the United States of America (USA), it is estimated that 48 million people become ill annually due to foodborne diseases and 128,000 of those cases being hospitalized resulting in 2 % case-fatality rate (1). The World Health Organization (WHO) estimates a case-fatality from foodborne diseases 0.07 annually worldwide (2). Although data on individual countries is available, information on the global burden of foodborne diseases is lacking, but estimated cost per individual nation is high (3-5).

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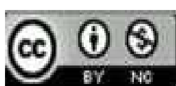

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Foodborne Diseases Burden Epidemiology Reference Group (FERG) along with WHO are currently undertaking the estimation of the worldwide burden of foodborne disease, listing *Campylobacter* and *Salmonella spp*. among the top challenges (2).

Antibiotics are chemical compounds used as chemotherapeutic agents against bacterial infections in humans and in animals. This type of antimicrobial began to revolutionize the medical world when the first antibiotic, Penicillin was discovered in 1928 by Alexander Fleming. Since the discovery of penicillin, many others antibiotics have been developed. The main usage of these antimicrobials is to aid in the fight against bacterial infections. However, in recent years the worldwide abuse of antibiotics as growth promoters in the food-producing industry as feed-additives for livestock and other food-producing animals has become a problem. As a result, bacteria have become less susceptible while developing resistance to these antimicrobials due to their extensive genetic plasticity (6,7).

Because the rumen microbiome of animals is a reservoir for microbes, especially antibiotic resistant pathogens, there is a high risk of zoonotic transmission of antibiotic resistant pathogens via contaminated water and food production and manipulation to human populations and this has been widely characterized (8,9). One of the most common pathogens responsible for bacterial infections in animals and humans is a gram-negative bacterium *E. coli* that can cause watery diarrhea, urinary tract infections (UTI), respiratory illnesses and even life-threatening infections depending on the severity of the infection and bacterial strain. The *E. coli* bacterial serogroup responsible for most hospitalizations in the US due to bloody diarrhea is *E. coli* O157:H7. This strain produces a Shiga toxin, thus, it is known as Shiga toxin-producing *E. coli* (STEC) that affects intestinal epithelial cells leading to inflammation of the stomach lining thus causing watery diarrhea and even lethal systemic complications such as haemolytic uraemic syndrome (HUS) and hemorrhagic colitis (10,11) Therefore, it is important to define the antimicrobial resistance of bacterial populations coming from food-producing animals as it can be used to understand the best approach to treat these bacterial infections.

The WHO advices against the usage of antibiotics in healthy animals to prevent disease, as it is seen as an unnecessary use in animals (12). Because of this, the general population has tended towards the consumption of commercially advertised products as organically grown as well as raised without antibiotics. Many studies have been done comparing the antibiotic susceptibility of different antimicrobials in bacterial isolates including *E. coli* and *Salmonella* by comparing the antimicrobial susceptibility rates of bacterial isolates obtained from milk and poultry samples that have been organically grown and raised without antibiotics to those bacterial isolates coming from conventional/traditional farms (13,14). These findings show that not only are products marketed as organic and raised without antibiotics harbor bacterial pathogens but are also carriers of antibiotic resistant strains after subjecting bacterial isolates obtained from these samples to a disc diffusion assay to analyze their phenotypic effects after exposure to a variety of antimicrobials (13).

The objective of this study was to describe the antimicrobial susceptibility of foodborne pathogenic *E. coli* isolates from food animals, retail, and from humans diagnosed with gastroenteritis to assess the potential correlation among these isolates.

2. Materials and Methods

2.1. Source of samples

A total of 36 *E. coli* isolates were recovered from humans diagnosed with gastroenteritis at Hamad Medical Corporation (HMC), Qatar (15). Retail isolates, 56 samples, were recovered from retail stores in Qatar from beef, mutton, chicken, and seafood (16). Additionally, 108 isolates were recovered from samples that were collected previously from Organic and Conventional dairy operations located in New York State (NYS) (17). Detailed information of sample collection can be found in previously published sources (15-17). Following the manufacturer's instructions, *E. coli* was detected using the BAX® System Real-Time PCR assay (DuPont, DE). Utilizing the same molecular method, screening for the presence of STEC and pathogenic serotypes O26, O111, O121, O45, O103, O145 and O157:H7 as well as STEC virulence genes *stx*, and *eae* were performed. Positive samples were stored at -80°C in 30% glycerol.

2.2. Isolates

Pure *E. coli* isolates were obtained by a series of molecular and biochemical testing. In brief, 1mL Brain-Heart Infused (BHI) broth media was inoculated with 200 μL *E. coli* stock in 30% glycerol. Samples were incubated overnight at 37°C for 24 h. Then, applying sterile techniques, McConkey plates (Hardy Diagnostics, CA) were inoculated with a loopful of primary enrichment by streaking the plate 4 times, rotating the plate 90° each time. The plates were sealed in parafilm and incubated overnight at 37°C for 24 h. Next, 3-5 isolated colonies from each sample were used to inoculate 2-3 mL BHI broth media and incubated at 37°C for 2 h.

2.3. Antibiotic sensitivity test

Antimicrobial susceptibility test was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (18). The Kirby-Bauer disk diffusion on Muller Hilton agar plates was used the method for exposure test. First, the turbidity of the pure fresh isolates was adjusted by diluting 200-250 μL of each bacterial sample in 2-3 mL BHI broth media adjusting the volume of inoculum until it reached the McFarland Latex Standard 0.5 (Hardy Diagnostics, CA) concentration. Secondly, a sterile swab was submerged into the standardized inoculum until it was completely soaked. Mueller-Hinton agar plates (Hardy Diagnostics, CA) were streaked with the soaked swab three times by rotating the plate 45°C in between each strake. Thirdly, standardized isolates were subjected to five antibiotic discs: Tetracycline (30 μg), Erythromycin (15 μg), Penicillin (10 U), Streptomycin (10 μg) and Neomycin (30 μg). Fourthly, using sterile tweezers, the five antibiotic discs were placed on the plate surface. The plates were then sealed in parafilm paper and then incubated at 37°C for 16-18 h. After the incubation period, the inhibition zones were recorded by measuring the diameter across the disc. These results were interpreted according to the CLSI antibiotic guidelines (18) given in Table 1.

2.4. Antibiotic resistance genes

Samples displaying phenotypic resistance to penicillin and streptomycin were subjected to molecular screening for the antibiotic resistance determinants blaTEM, blaSHV, aadA and strA-strB via PCR. Primers and PCR conditions were taken from previously published work (19,20). Briefly, all PCRs were performed in a final volume of 25 µL: each reaction consisted of 2.5 µL 10X DreamTaq buffer, 0.5 µL dNTPs (200µM), 0.24 µL of forward and reverse primer to a final concentration of 25 pmol each (Integrated DNA Technologies, Coralville, IA), 0.20 µL Taq DNA polymerase, 5 U/µL (Thermo Scientific, Waltham, MA), and DNA to a final concentration of 5 ng/µL.

2.5. Statistical analysis

Descriptive statistics and measure of central tendency and dispersions were computed using the SPSS statistical version 25 (IBM statistical software, White Plain NY, USA). Comparisons of the proportion of resistant strains isolated from the different sources by each antibiotic was performed using the analysis of variance (ANOVA) statistical technique. Significance of differences of proportion resistance by source were evaluated using Tukey Post-hoc test in the SPSS. The significance of association between the presence of the resistant genes for penicillin and streptomycin and the phenotypic expression was evaluated using the chi-square test in SPSS.

2. Results

A total of 200 isolates were selected from the pool of samples from different sources. Figure 1 shows the distribution of the different genes detected in these isolates by source. The STEC gene was most common among isolates from conventional dairy farms (78%) and absent from the samples recovered human diagnosed gastroenteritis cases. The *stx* gene was most common among isolates recovered from organic dairy operations (53%) and least prevalent among isolates recovered from retail samples (8.9%). Isolated from organic and conventional dairy operations had the highest prevalence of the *eae* gene, 86 and 67% respectively. Conventional dairy operations had the highest proportion of the O157:H7 serotype (48%) while organic operation and humans had the same serotype at a much lower proportion, 6 and 5% respectively. This serotype was not detected in isolates recovered from retail samples (Figure 1).

E. coli serotype O26 was detected in 27% of the isolates recovered from organic operations and was neither detected among isolates recovered from conventional operations or retail (Figure 2). Serotype O45 was identified at proportions of 41 and 26%, respectively, in isolates recovered conventional and organic dairy operations, 41 and 26 % respectively. Serotype O103 was detected at a higher proportion among isolates recovered from organic operations. Isolates recovered from organic and conventional dairy operations had relative higher prevalence of O121, 37 and 33% respectively, in comparison to isolates recovered from retail and human samples. Serotype O145 was not common among all the isolates recovered (Figure 2).

Table 1. CLSI Inhibition Zone interpretation criteria for *E. coli* (26)

Antibiotic Disk	Disk Concentration	Diameter Inhibition Zones (mm)		
		Susceptible	Intermediate	Resistant
Erythromycin (D)	15 µg	n/a	n/a	n/a
Neomycin (C)	30 µg	≥ 17	13-16	≤ 12
Penicillin	10 U	≥ 13	n/a	≤ 14
Streptomycin (C)	10 µg	≥ 15	12-14	≤ 11
Tetracycline (C)	30 µg	≥ 15	12-14	≤ 11

Abbreviations:
C: caution – antibiotic considered only when category D have not been clinically effective.
D: prudence – first line treatment, antibiotic used only when medically needed.

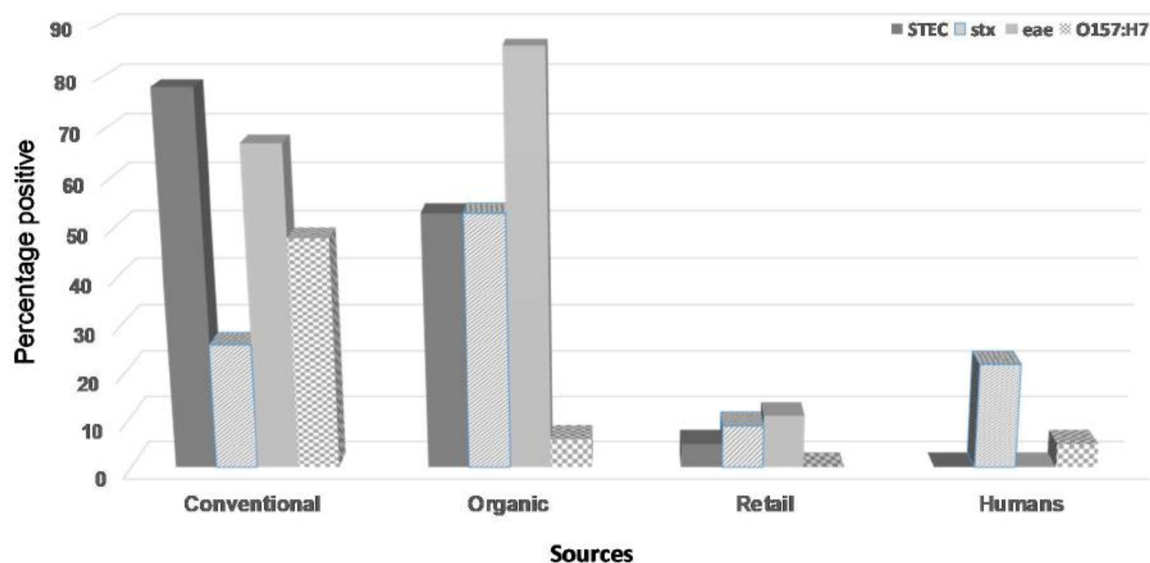

Figure 1. The percent distribution of the samples used in the evaluation by source of the sample

Table 2. The distribution of the inhibition zone and the percent resistance among *E. coli* by different antibiotics and by sources

Antibiotic Source	Tetracycline	Erythromycin	Penicillin	Streptomycin	Neomycin
Dairy (conventional)	13.1 (2.3) [24%][¥,a]	7.4 (4.5 [[100%][a]	7.6 (1.1) [44%][b]	8.8 (1.2) [88%][a]	17.3 (1.4) [10%][a]
Dairy Organic	18.2 (0.7) [21%][a]	8.0 (0.2) [98%][a]	6.8 (0.4) [70%][c]	13.4 (0.5) [44%][bc]	17.0 (0.4) [6%][a]
Retail	18.9 (1.1) [21%][a]	11.1 (0.5) [91%][a]	7.8 (0.5) [88%][a]	13.8 (0.6) [25%][bd]	18.2 (0.6) [8%][a]
Humans	14.5 (1.2) [42%][a]	8.3 (0.4) [100%][a]	6.1a (0.03) [100%][a]	10.9 (0.6) [53%][a]	16.1 (0.3) [3%][a]

: Standard error
¥: Percent resistant
na: not applicable
a: Proportion with the same letter within and antibiotic are not significantly different

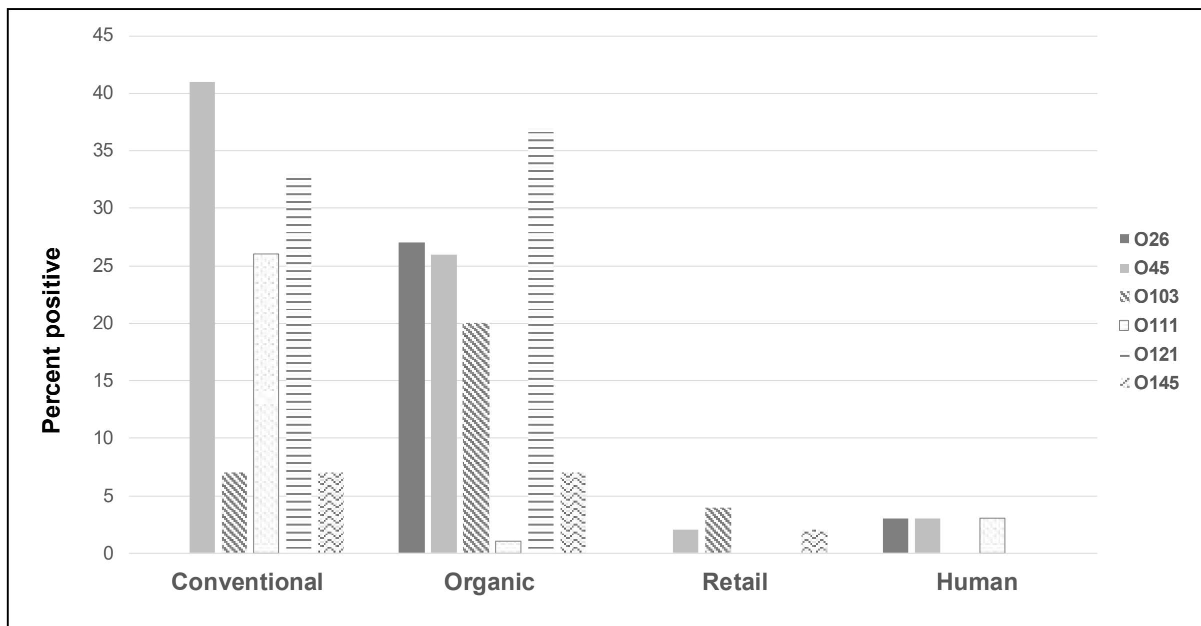

Figure 2. The percent distribution of the samples revaluated for antibiotic resistance by serotype of *E. coli*

In addition, following the CLSI disk interpretation guidelines the classification of the size of the inhibition zone the isolates were classified as resistant or susceptible (Table 1). Table 2 shows the results of the results of the phenotypic inhibin of the five antibiotics evaluated in the study by the source of the sample. Isolates recovered from humans had the largest percentage of resistant isolated to tetracycline (42%), however there was no significant difference among the four sources with respect to the proportion that were resistant (Table 2).

Isolates from conventional and organic dairy operations as well as retail had relatively had half of the percentage resistant isolated in comparison to the isolates from humans (Table 2) but the differences were not significant.

All isolates from different sources showed a relatively high proportion of resistant strains with respect to penicillin; isolates from human had the highest proportion (100%) (Table 2). All the isolates investigated in this study showed high proportions of resistance to erythromycin (Table 2). There was no significant difference in the resistance levels among the different sources. Isolates from organic dairy, and conventional operations showed significantly different proportion of resistance to penicillin at 44, 70%, respectively than humans. Isolates from humans with gastroenteritis showed a resistant proportion of 53% to streptomycin (Table 2). This proportion was significantly different from the isolates recovered from organic operations and from retail. `

Isolates recovered from conventional dairy operations had the highest proportion that expressed phenotypic resistance (88%) to streptomycin (Table 2). This proportion was significantly higher than the proportions expressed by isolates recovered from organic dairy operations and retail samples which they showed resistance to streptomycin at proportion of 44 and 25%, respectively. The resistance among isolates recovered from organic dairy and retail operations were significantly different from each other's. All the isolates recovered from the 4 different sources showed a higher relative degree of susceptibility to neomycin (Table 2).

Table 3 shows the resistant proportion of isolates detected with virulence gene among serotypes evaluated in the study. The majority of the isolates that had the STEC gene showed phenotypic resistance to streptomycin and penicillin, 73 and 64% respectively. Isolates that were detected with stx and eae genes showed high resistance to penicillin (Table 3). Isolates that belong to O157:H serotype showed high resistance to streptomycin at a proportion of 94%. On the other hand, isolates belonging to the non-O157 serotypes showed higher resistance to penicillin, all showed resistance at 77% or higher (Table 3). The same non-O157 isolates showed high sensitivity to tetracycline, streptomycin, and neomycin at proportions higher than 81%.

E. coli isolates demonstrated to have phenotypic resistance to penicilin were investigated for the presence of the extended-spectrum β-lactamases genes (ESBL), blaTEN and blaSHV. The blaTEM and blaSHV genes were detected in 17 and 38% proportion of the samples, respectively (Figure 3). There was no significant association between the presence of the respective gene and demonstration of phenotypic inhibition. Similarly, isolates demonstrated phenotypic to streptomycin were further investigated for the presence of the aadA and the strA genes (Figure 3).

Streptomycin antibiotic resistance genes aadA and strA were investigated in isolates demonstrating phenotypic inhibition to this antibiotic. The aadA gene was detected in 30% of the isolates and the strA was detected in 59% of the isolates that showed phenotypic resistance (Figure 3). There was a significant association between the phenotypic resistance and the detection of either of these genes among the isolates.

We evaluated the relationship between the detection of the resistant genes for penicilin (Bla-TEM and Bla-SHV) and expression of phenotypic resistance by sources of the isolates (Table 4). About 42% of the human isolates demonstrated the presence of the bla-TEM gene while 57% of the conventional dairy isolates demonstrated the presence of the same gene (Table 4). This gene was identified at a lower proportion among isolates from organic dairy and from retail, 6 and 5% respectively. However, the bla-SHV gene was detected in isolates from conventional dairy, organic operations, and retail that demonstrated phenotypic resistance at the proportion of 63, 29, and 57%, respectively. The Bla-SHV was detected in 6% of the isolates recovered from humans that demonstrated phenotypic resistance to penicillin (Table 4).

The tetracyclines resistant genes aadA and strA were investigated in isolates recovered from humans, conventional dairies, organic dairies, and retail isolates that demonstrated phenotypic resistance to the antibiotic (Table 4). The aadA gene was discovered at a proportion of 25, 11, 8, and 50%, respectively among the isolates recovered from these sources (Table 4). While the strA-strAB gene was detected in all isolates recovered from humans, conventional, organic, and retail isolates that demonstrated phenotypic resistance to tetracyclines at the proportion of 75, 47, 10, and 78%, respectively (Table 4).

Table 5 shows the relationship of the presence of the different classes of antibiotics with focus on the β-lactamase and introgens. Among the isolates from cases of gastroenteritis the strAB and bla-TEM were the most common. The bla-SHV was the most the common among isolates recovered from dairy operations. The other genes that were assessed were not common. Isolates recovered from retail samples had relatively high detection of the genes except for the bla-TEM gene (Table 5). There were no significant correlations between the use of these antibiotics and AMR genes that were evaluated in this study (Table 6).

Table 3. The distribution of resistance to different types of antibiotic by the gene and serotypes

Gene and Serotype	Tetracycline	Erythromycin	Streptomycin	Neomycin	Penicillin
STEC	25%	98%	73%	31%	64%
stx	21%	98%	70%	33%	73%
eae	28%	99%	73%	35%	65%
O157:H7	22%	100%	94%	43%	33%
O26	12%	100%	12%	14%	77%
O45	16%	100%	17%	10%	83%
O103	10%	100%	10%	7%	80%
O111	5%	100%	5%	3%	78%
O121	19%	97%	19%	15%	80%
O145	5%	100%	5%	5%	77%

Table 4. The proportions of resistant genes for different antibiotics among the isolates recovered from different sources

Source	bla-TEM	bla-SHV	aadA	strAB
Penicillin	17%	38%	32%	69%
	(146)	(124)	(34)	(26)
Tetracycline	21%	22%	30%	59%%
	(42)	(37)	(27)	(22)

Table 5. The proportions of resistant genes for different antibiotics among the isolates recovered from different sources

Source	Bla-TEM	Bla-SHV	aadA	strAB
Humans	42%	6%	25%	75%
	(36)	(36)	(8)	(8)
Dairy conventional	57	63%	11%	47%
	(7)	(7)	(19)	(19)
Dairy Organic	6%	29%	8%	10%
	(54)	(52)	(26)	(21)
Retail	5%	57%	50%	78%
	(30)	(30)	(12)	(9)

Table 6. Correlation of phenotypic and genotypic antimicrobial resistance among the different antibiotics investigated in the study

	Antibiotic								
	Penicillin	Erythro mycin	Neomycin	Tetra-cycline	Strepto-mycin	blaTEM	blaSHV	aadA	strAB
Penicillin	1.000	0.058	0.145	0.286	0.122	0.080	-0.125	0.243	0.273
Erythromycin	0.058	1.000	0.160	0.061	0.004	0.091	0.061	-0.26	0.
Neomycin	0.145	0.160	1.000	-0.376	0.080	0.097	-0.133	0.375	0.503
Tetracycline	0.286	0.061	-0.376	1.000	0.352	0.218	-0.156	-0.07	0.
Streptomycin	0.122	0.004	0.080	0.352	1.000	0.148	-0.143	0.085	-0.05
blaTEM	0.080	0.091	0.097	0.218	0.148	1.000	0.015	-0.19	0.127
blaSHV	-0.125	0.061	-0.133	-0.156	-0.143	0.015	1.000	-0.39	-0.27
aadA	0.243	-0.263	0.375	-0.078	0.085	-0.193	-0.385	1.000	0.322
strAB	0.273	0.	0.503	0.	-0.048	0.127	-0.265	0.322	1.000

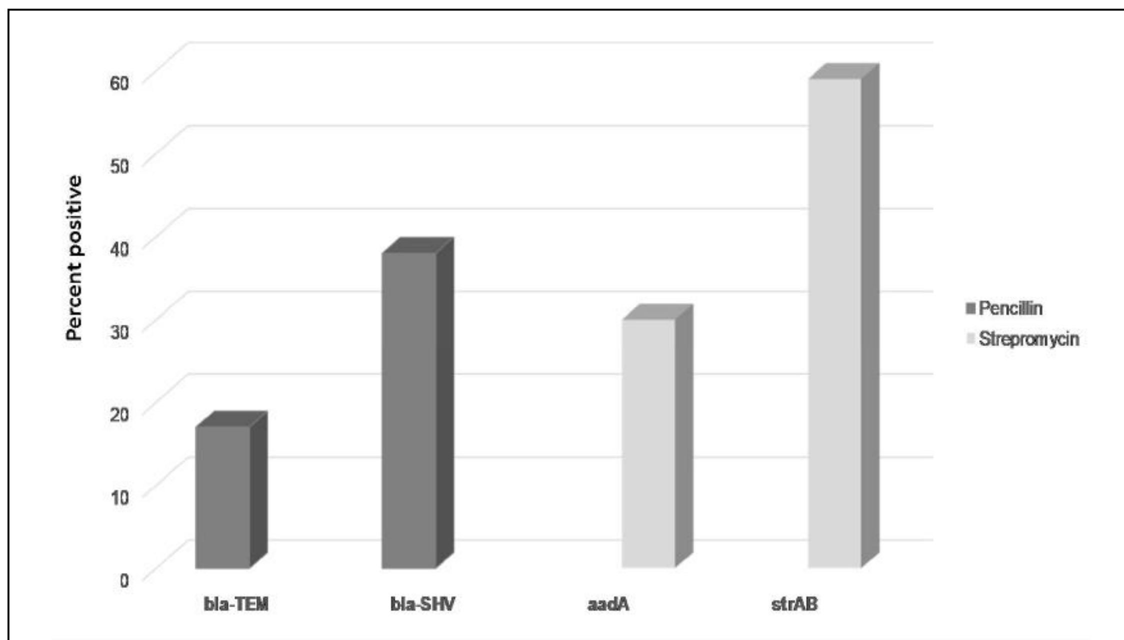

Figure 3. The distribution of specific resistance gene of penicillin and streptomycin among the isolates recovered in the study

4.Discussion

The wide use of antibiotics in in food animal production systems and the treatment of many human health conditions has resulted in the emergence of zoonotic resistant bacterial foodborne pathogens. Infections with these pathogens has posed a challenge to public health systems due to increased consequences of treatment failure and severity of disease (6).

If effective intervention strategies tare to be instituted to mitigate the risk associated with AMR foodborne pathogens it is important to identify the sources of these pathogens and their dynamic in ecological systems. It was the intent of this paper in the context of shedding more light on the dynamic of these foodborne pathogens along the food supply chain and among isolates from gastroenteritis patients.

As a part of our long term objectives to highlight the consequences of infection with foodborne pathogens, we carried out the current study to describe the antimicrobial susceptibility of foodborne pathogenic isolates of *E. coli* from food animals, retail, and from humans diagnosed with gastroenteritis. The hope was to shed light on the dynamic of these pathogens in such ecosystem might provide clues in the sequelae of long-term infection with these pathogens. The focus was the most common foodborne pathogen, *E. coli* and its food adulterant serotypes (21). Other studies have attempted to characterize non-O157 from animals and from humans (22,23). Unlike our study, other studies focused on either animals, environment, or humans.

In our approach we adapted a systematic approach where the presence of *E. coli* O157:H7 and serotypes of non-O157 that are characterized as food adulterants along the food chain and in patients diagnosed with gastroenteritis were studied (15). To our knowledge although there have been some efforts to shed light on AMR among these non-O157 serotypes but the focus has been limited in a number of these isolates (24-26). We focused on the presence of AMR resistance in these 7 serotypes, including O157:H7, at different levels along the food chain (source (animal), retail (environment), and humans diagnosed with gastroenteritis) hoping to shed more light on the presence of these AMR serotypes of *E. coli* that would contribute to the body of knowledge on potential critical control points that might help in mitigating the associated risk with these pathogens. The isolates evaluated in this study represented a random and proportional sample of the isolates recovered during our investigations of the food supply chain and the risk in humans). The intent was also to explore a potential relatedness among those isolates with respect to AMR. Similar studies were carried out in other parts of the world; however, we focused on the entire food supply chain from the food source to humans and with a different technical approach (23,27).

The distribution of the of the serotypes included in this samples that were evaluated for AMR are similar to the distribution of those serotypes recovered from the original sources (15-17) Therefore, the isolates evaluated in this study are representative of the original isolates recovered and hence the extrapolation of the results from this study are valid. The antibiotic considered in this investigation were the ones that we found commonly used in the assessment AMR in similar populations as the populations considered in this study (21- 23, 28, 29). However, we also included the five earlier mentioned antibiotic because we are

investigating the whole food supply chain and the host of interest, humans.

This investigation showed that there was considerable variation with respect to the resistance to isolates recovered from different sources. For example, there was no significant differences in the resistance among the isolates to tetracycline and neomycin there was significant variation in the resistance to penicillin and streptomycin. Similar variability in the resistance have been reported by other studies carried out on isolates recovered from animal and humans (29-32). The level of resistance to tetracyclines observed in our studies among isolates recovered from conventional and organic dairy operations are similar to what have been reported previously (33,31). However, the isolates assessed in the aforementioned studies are from different targeted populations than in our studies with respect to the sources and the antibiotic that were used. On the other hand, we noticed significant differences in the AMR among the isolates from different sources to penicilin and streptomycin. In the cases of the penicilin, these differences could be attributed to the use of the penicilin among the sources, which it is used commonly against a broad range of bacterial infections in Qatar, where all the isolates are recovered from humans with gastroenteritis diagnoses. The high rate of resistance among isolates from retail could be attributed to circulation of these resistant isolates due to cross contamination from handlers of the retail products (34). The food handlers in Qatar could have played a role by exacerbating the risk of cross contamination (34-36). The observed high resistance proportion among the isolates in our study to *E. coli* serotype is consistent to what have been reported in the literature (37,38).

As a part of our objective we examined AMR in relation to the genetic and serotypes of *E. coli* and genetic variability. As has been reported in the literature there is variability in AMR among the different serotypes of this pathogens (29,31). For example, in this study *E. coli* O157:H7 were most resistance phenotypically to streptomycin. The majority of these isolates were recovered from human with gastroenteritis. This finding is different from the finding reported earlier from the sample population (39). The difference could be attributed to the difference in the populations been investigated where we included both adult and children the other investigators focused on isolates recovered from children. However, there are similarities with respect to the penicillin family (39). However, an earlier study among food handlers found that AMR among *E. coli* isolates at a lower rate than was observed among children (40).

One of the aims of the study was to investigate the relationship between the genotypic and the expression of the phenotypic AMR among the *E. coli* serotypes including O157:H7 and non-O157:H7 that are classified as food adulterants. We focused on the perceived commonly used antibiotics among animal food production, retail, and among humans with the diagnosis of gastroenteritis and subset of the gene acquired through the production of β-lactamase (*bla*SHV, *bla*TEM), Class I integrons (aadA), and aminoglycosides streptomycin resistant gene (strAB) (41, 42). These genes were targeted because they are common among the 5 antibiotics that were evaluated.

Our data were not able to establish if there is correlation between the genotypic characteristic of the isolates investigated and the phenotypic expression. This finding is consistent with many finding in the literature (36,42,43). Many factors play a role in this poor relationship including the potential environmental factors that play role in the expression of AMR. It is known that phenotypic characteristics are the function of the genes and the environment (43). Knowledge on the environmental factors that led to the evolution of the expression of AMR could help in controlling the growth of MDR microorganisms and minimizing the transmission/expression of AMR genes in the food chain ecosystem (21).

5.Conclusion

In conclusion investigating the presence of MDR microorganism in the food supply chain and the potential risk to human health is a daunting task because of the complex nature of the food supply system. However, since the ultimate goal is to sustain the health of the food supply system, the search for AMR bacteria is unescapable. Other additional complementary studies are required to develop a comprehensive picture of AMR bacteria in the food supply chain for humans.

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