J. Dairy Sci. 98:2260–2267
http://dx.doi.org/10.3168/jds.2014-9057

Prevalence, distribution, and diversity of *Escherichia coli* in plants manufacturing goat milk powder in Shaanxi, China

Meili Xi,[1] Yuqing Feng,[1] Qiong Li, Qinnan Yang, Baigang Zhang, Guanghui Li, Chao Shi, and Xiaodong Xia[2]
College of Food Science and Engineering, Northwest A&F University, Yangling, Shaanxi 712100, China

ABSTRACT

The aim of the study was to investigate the prevalence, distribution, and diversity of *Escherichia coli* in goat-milk-powder plants in Shaanxi, China. Three plants manufacturing goat milk powder in Shaanxi province were visited once for sampling during 2012 and 2013. Samples were taken for isolation of *E. coli*. Isolates were characterized by antimicrobial susceptibility testing and detection of virulence genes. All isolates were further examined by pulsed-field gel electrophoresis analysis. In total, 53 *E. coli* strains were isolated from 32 positive samples out of 534 samples. Among *E. coli* isolates, resistance was most frequently observed to trimethoprim-sulfamethoxazole (75.5%), whereas all isolates were sensitive to gatifloxacin, kanamycin, amikacin, and amoxicillin-clavulanate. The 6 virulence genes of pathogenic *E. coli* were not detected. Pulsed-field gel electrophoresis results showed that *E. coli* strains in plants were genetically diverse and milk storage tank could be an important contamination source. This study could provide useful information for plants manufacturing goat milk powder to establish proper management practices that help minimize the chance of microbial contamination.

Key words: *Escherichia coli*, goat milk powder, antimicrobial resistance, virulence gene

INTRODUCTION

Goat milk accounted for about 2.3% of the worldwide milk production (Claeys et al., 2014). Goat farming is of vital importance for the national economy in many countries in the Mediterranean and Middle East region and is particularly well organized in France, Italy, Spain, and Greece (Park et al., 2007). Among the European countries, Greece has the largest goat population (6,000,000 animals) and produces about 450,000 t of goat milk per year (Anifantakis, 2001). Goat milk and its products have 3 significant benefits in human nutrition: (1) feeding more starving and malnourished people in the developing world than cow milk; (2) serving people afflicted with cow-milk allergies and gastrointestinal disorders, which is a significant segment in many populations of developed countries; and (3) meeting the gastronomic needs of connoisseur consumers, which has a growing market share in many developed countries.

Escherichia coli in raw milk is inactivated by pasteurization but may enter dairy powder after processing, such as during the drying process (IDF, 1991). *Escherichia coli* is regarded by the dairy industry as an important indicator of manufacturing hygiene, and this group, or the coliform subgroup, is widely included in microbial specifications or guidelines for dairy powders in various countries. Pathogenic *E. coli* in improperly pasteurized milk has caused foodborne outbreaks, and antimicrobial resistance of *E. coli* is another concern of the public. It was shown that when considering 60 outbreaks and 4 single cases described in the literature and implicating milk and milk products, pathogenic *E. coli* were responsible for 11 outbreaks (De Buyser et al., 2001). In 2009, a study revealed the presence of pathogenic *E. coli* in dairy powder factory environments (Duffy et al., 2009). A study from Ireland showed that the occurrence of the virulence gene of *E. coli* in raw milk was 36.0%, whereas the occurrence of isolates was 0.8% (Lynch et al., 2012).

Owing to the unique properties of goat milk, it has been widely consumed in some regions of the world and by a considerable portion of the population. And, goat milk-based infant formula is becoming more and more preferred by consumers. However, there is a paucity of data regarding *E. coli* contamination in goat milk and plants, except a study on the *E. coli* in dairy powder factory environments (Duffy et al., 2009). The objective of this study was to determine the prevalence of *E. coli* strains in goat milk powder in the year of 2012 and 2013 from 4 plants in Shaanxi province, China,

Received November 2, 2014.
Accepted January 4, 2015.
[1]These 2 authors contributed equally to this article.
[2]Corresponding author: foodscixiaodong@yahoo.com

and characterize these strains by antimicrobial susceptibility testing and toxin-gene detection. In addition, pulsed-field gel electrophoresis (**PFGE**) profiles of isolates were compared to identify the possible source of contamination at different stages and sampling sites of the processing line.

MATERIALS AND METHODS

Sampling Design and Collection

Three plants manufacturing goat milk powder in Weinan (plant A), Jingyang (plant B), and Yanliang (plant C) were selected in Shaanxi province, China. Plants were sampled once from December 2012 to March 2013, with an interval of about 1 mo between sampling of each plant. Latex gloves and disposable plastic boot covers were worn for sample collection. Gloves were disinfected with 75% ethanol before sample collection. A total of 534 various types of samples were collected, including milk powder and environmental swabs. Sites for sampling covered most procedures in the plant, and the nonprocessing and processing environments of the 3 plants were also considered. In detail, a total of 28, 29, and 19 sites were chosen for sampling in Weinan, Jingyang, and Yanliang, respectively. At each site, at least 3 samples were collected (Table 1).

Samples were collected as follows: 100 cm^2 of environmental surfaces were collected using a sterile cotton swab rehydrated with 10 mL of buffered peptone water; 500 g of goat-milk powder was collected using sterile sample bags; 500 mL of liquid goat milk was collected using a sterile bottle; and air samples were collected by exposing Luria-Bertani plates in the air for 5 min. All samples were transported on ice and processed within 24 h of collection.

Escherichia coli Enrichment and Isolation

All samples were used for the isolation and identification of *E. coli* (Duffy et al., 2009). Briefly, 10 mL (or 10 g) of samples was mixed with 90 mL of buffered peptone water. The solution was incubated at 37°C overnight. After preenrichment, a 1-mL aliquot was transferred to 10 mL of *E. coli* broth (EC; Beijing Land Bridge Technology Ltd., Beijing, China) and incubated at 44°C for 22 ± 2 h. Samples that were positive for the presence of gas were streaked onto eosin methylene blue agar (Beijing Land Bridge Technology Ltd.). Two presumptive *E. coli* colonies per sample were transferred to trypticase soy agar plates. Colonies were then confirmed as *E. coli* by PCR detection of the *uidA* gene. All isolates were stored at −80°C until use.

PCR Detection

Six virulence genes specific for each type of diarrheagenic *E. coli* were tested by PCR method: the *eae* gene for enteropathogenic *E. coli*, the *ipaH* gene for enteroinvasive *E. coli*, the *elt* and *est* gene for enterotoxigenic *E. coli*, the *aggR* gene for enteroaggregative *E. coli*, and the *stx* gene for Shiga toxin-producing *E. coli* (**STEC**). Primers for the PCR assays are list in Table 2. The PCR products were resolved by 1.0% (wt/vol) agarose gel electrophoresis in 0.5 × Tris-borate-EDTA buffer.

Serotyping

Pathogenic *E. coli* isolates were serotyped in the Henan Center for Disease Control and Prevention, China. O and H antigens were characterized using slide agglutination with hyperimmune sera (S&A Company, Bangkok, Thailand), and the serotype was assigned following the manufacturer's instructions.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing of *E. coli* was performed by the agar dilution method for ciprofloxacin, nalidixic acid, gatifloxacin, ampicillin, gentamicin, kanamycin, amikacin, streptomycin, cefoxitin, cefoperazone, ceftriaxone, trimethoprim-sulfamethoxazole, amoxicillin-clavulanate, chloramphenicol, and tetracycline. *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains in each run. The breakpoints used for *E. coli* were taken according to guidelines developed by the Clinical Laboratory Standard Institute (CLSI, 2013).

PFGE

Pulsed-field gel electrophoresis of *Xba*I (TaKaRa, Dalian, China)-digested genomic DNA of *E. coli* strains was carried out as described previously (Gautom, 1997). The PFGE conditions of *Xba*I macrorestriction analysis were 6 V/cm for 19 h with pulse times ranging from 2.16 to 54.17 s at a temperature of 14°C and an angle of 120°.

The gels were stained with ethidium bromide, and images were taken under UV transillumination (Bio-Rad, Hercules, CA). The images were analyzed using the BioNumerics Software (Applied-Maths, Kortrijk, Belgium) by using DICE coefficients and the unweighted-pair group method to achieve dendrograms with an optimization value of 0.5% and a 1.0% band position tolerance. The cluster cutoff was set at 85% similarity. Genomic DNA of *Salmonella* serotype Branderup strain H9812 digested with *Xba*I was used as a molecular-size marker.

Table 1. Sampling design for determining *Escherichia coli* contamination in 3 plants manufacturing goat milk powder in Shaanxi, China

Source of sample	Plant A	Plant B	Plant C	Total
Soil around the plants	5	10	10	25
Shoes	NT[1]	5	NT	5
Vents of packing rooms	5	5	5	15
Vents of fluidized beds	5	NT	NT	5
Wall of air-filter rooms	NT	NT	5	5
Wall of packing rooms	5	NT	5	10
Wall of spray-dryer rooms	NT	5	5	10
Ground of fluidized-bed rooms	5	NT	NT	5
Ground of spray-dryer rooms	5	5	5	15
Ground of fluidized beds	5	NT	NT	5
Ground of packing rooms	10	10	5	25
Ground of air filters	NT	NT	5	5
Windows of spray-dryer rooms	NT	5	NT	5
Windows of packing rooms	NT	5	NT	5
Air filters	5	10	10	25
Air of air-drying rooms	NT	4	NT	4
Air of packaging rooms	NT	2	NT	2
Air of spray-dryer rooms	NT	2	NT	2
Air of air-filter rooms	NT	4	NT	4
Water in the pipelines	NT	5	NT	5
Water for washing	NT	NT	5	5
Powder on the ground of packing rooms	NT	7	NT	7
Powder around the spray dryers	NT	13	NT	13
Powder on the ground of cooling-down machine rooms	NT	NT	4	4
Operation desks	4	20	NT	24
Packing material	8	NT	NT	8
Outside surface of packages	NT	6	NT	6
Inside surface of packages	NT	4	NT	4
Products	NT	27	41	68
Conveyor	10	10	5	25
Powder sifters	3	NT	5	8
Packing machines	15	NT	5	20
Milk clarifiers	2	NT	NT	2
Outside surface of milk clarifiers	3	NT	NT	3
Outside surface of balancing tanks	2	NT	NT	2
Inside surface of balancing tanks	2	NT	NT	2
Inside surface of fluidized beds	6	NT	NT	6
Outside surface of fluidized beds	4	NT	NT	4
Inside surface of drying towers	2	NT	NT	2
Evaporators	3	NT	NT	3
Inside surface of tankers	10	NT	NT	10
Inside surface of spray dryers	13	5	NT	18
Spray dryers	5	2	NT	7
Storage tanks	3	5	NT	8
Outside surface of spray dryers	5	13	5	23
Inside surface of powder sifters	5	NT	NT	5
Milk storage tanks	NT	5	5	10
Sealing machines	NT	5	NT	5
Inside surface of tanks	NT	5	NT	5
Cooling-down machines	NT	20	NT	20
Fluidized beds	NT	NT	5	5
Sieve beds	NT	NT	20	20
Total	155	224	155	534

[1]Not tested.

RESULTS

Isolation of E. coli

Of the 534 samples, 32 (6.0%) samples were positive for *E. coli*, including 24 (17.0%) of the 224 samples from the plant in Jingyang and 8 (9.0%) of the 155 samples from the plant in Yanliang. A total of 53 *E. coli* isolates were recovered from the 32 *E. coli*–positive samples (1 or 2 isolates per sample; Table 3).

The vast majority of *E. coli*–positive samples were obtained from the milk storage tanks (100% positive rate samples in Jingyang; 80.0% positive rate samples in Yanliang). The final milk powder in the package was contaminated at a rate of 4.0% with *E. coli*.

Table 2. Oligonucleotide primers for PCR

Forward primer sequence (5′–3′)	Reverse primer sequence (5′–3′)	Gene	PCR product (bp)	Reference
CGATTCCGTTTCAGGGTT	TTTCTGATAGGACCGAGCAT	*uidA*	194	This study
GTATACACAAAGAAGGAAGC	ACAGAATCGTCAGCATCAGC	*aggR*	254	Rúgeles et al. (2010)
GTTCCTTGACCGCCTTTCCGATACCGTC	GCCGGTCAGCCACCCTCTGAGAGTAC	*ipaH*	619	Sethabutr et al. (1993)
TTAATAGCACCCGGTACAAGCAGG	CCTGACTCTTCAAAAGAGAAAATTAC	*est*	147	Homes et al. (1991)
TCTCTATGTGCATACGGAGC	CCATACTGATTGCCGCAAT	*elt*	322	Tamanai-Shacoori et al. (1994)
CCCGAATTCGGCACAAGCATAAGC	CCCGGATCCGTCTCGCCAGTATTCG	*eae*	881	Oswald et al. (2000)
GAGCGAAATAATTTATATGTG	TGATGATGGCAATTCAGTAT	*stx*	518	Yamasaki et al. (1996)

Serotyping and Virulence Detection

Serotyping results showed that not all isolates could be typed by the available O antigens and H antigens. O antigens of 10 isolates and H antigens of 17 isolates could not be determined. Many isolates (11 of 35 isolates) belonged to serotype O125:H5, whereas the other 4 typeable isolates exhibited different serotypes: O1:H2, O125:H18, O27:H5, O27:H18, O121:H5, O18:H2, O27:H2, O63:H5, O103:H40, and O115:H40 (Figure 1). Different O types were observed in 8 H-nontypeable isolates, among which isolates 426A and 426B, 428A and 428B, 560A and 560B, and 679A and 679B reacted with the same O antiserums. Among 53 isolates of *E. coli*, none of the 6 virulence genes tested was detected.

Antimicrobial Susceptibility Testing

Of the 53 *E. coli* isolates, resistance was most frequently observed to trimethoprim-sulfamethoxazole (75.5%), followed by streptomycin (32.7%), and to a lesser extent ampicillin (20.8%), tetracycline (20.8%), nalidixic acid (7.6%), cefoxitin (7.6%), chloramphenicol (7.6%), cefoperazone (5.7%), gentamicin (5.7%), ciprofloxacin (3.8%), and ceftriaxone (3.8%). All *E. coli* isolates were susceptible to amikacin, gatifloxacin, kanamycin, and amoxicillin-clavulanic acid. Forty-three *E. coli* isolates (81.1%) were resistant to at least 1 antibiotic, and 13 *E. coli* isolates (24.5%) were resistant to at least 3 antibiotics (Table 4).

PFGE

All of 53 *E. coli* isolates were analyzed for genetic relatedness using PFGE with *Xba*I. Using a cutoff value of 85%, 20 different PFGE patterns were generated, including 13 clusters and 7 individual types (Figure 1). The 3 most predominant PFGE clusters were observed in 14 (26.4%; 14/53) isolates grouped in cluster J, 5 (9.4%; 5/53) isolates in cluster E, 4 (7.5%; 4/53) isolates in cluster C, and 4 (7.5%; 4/53) isolates in cluster M.

DISCUSSION

This study was carried out to assess *E. coli* contamination in goat-milk-powder plants in Shaanxi province, which is the largest goat-milk production area in China. Intervention based on the findings should help to reduce or eliminate microbial contamination from plants processing goat milk powder.

In the present study, isolates of *E. coli* came from 3 plants, and *E. coli* was not detected in the plant of Weinan. The overall contamination rate of *E. coli* in the

Table 3. Percentage occurrence of *Escherichia coli* in the environments of 3 milk-powder plants

Environment	% Samples (positive/number tested)		
	Plant B	Plant C	Total
Milk storage tanks	100.0 (5/5)	80.0 (4/5)	90.0 (9/10)
Vents of packing rooms	60.0 (3/5)	ND[1]	60.0 (3/5)
Soil around the plant	ND	40.0 (4/10)	40.0 (4/10)
Water in the pipelines	40.0 (2/5)	ND	40.0 (2/5)
Powder on the ground of packing rooms	28.6 (2/7)	ND	28.6 (2/7)
Operation desks	20.0 (4/20)	ND	20.0 (4/20)
Air filters	20.0 (2/10)	ND	20.0 (2/10)
Conveyor	10.0 (1/10)	ND	10.0 (1/10)
Ground of packing rooms	10.0 (1/10)	ND	10.0 (1/10)
Outside surface of spray dryers	7.7 (1/13)	ND	7.7 (1/13)
Powder around the spray dryers	7.7 (1/13)	ND	7.7 (1/13)
Cooling-down machines	5.0 (1/20)	ND	5.0 (1/20)
Products	3.7 (1/27)	ND	3.7 (1/27)
Total	16.6 (24/145)	53.3 (8/15)	20.0 (32/160)

[1]Not detected.

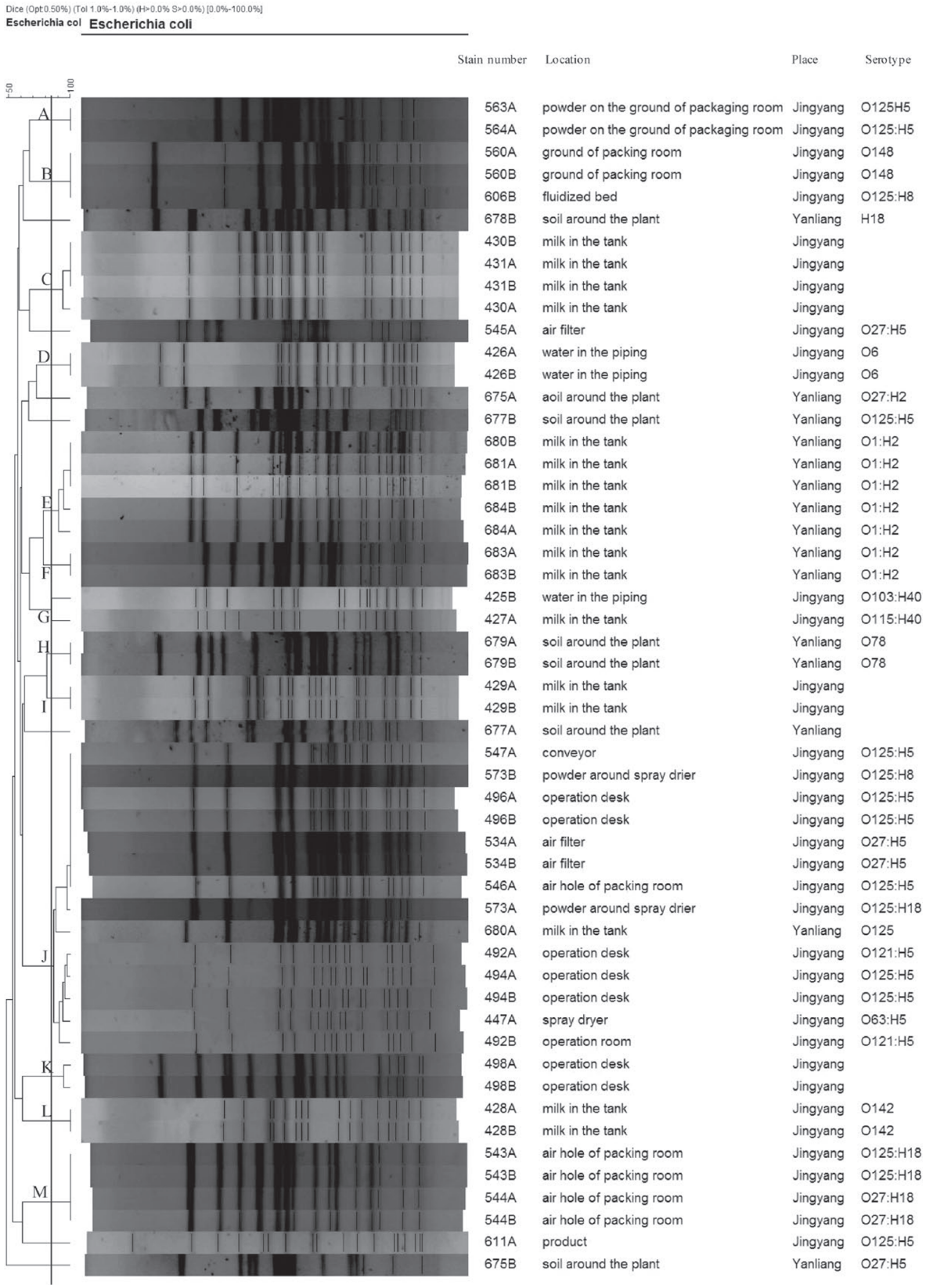
samples was 6.0% (32/534), which is much lower than that reported in the Australian dairy powder factory environments (Duffy et al., 2009). The difference in sampling design may account for the discrepancy. The study in Australian dairy powder factory environments focused on the surfaces of drains, gutters, and shoes.

Escherichia coli was isolated at a high frequency from milk storage tanks (90.0%, 9/10), which highlights the need for vigilance of preventing cross-contamination into spray-drying facilities. The packing-room environment was identified as an important contamination source. Operation desks, vents, ground, end products, and powder on the ground in the packing room were found to be contaminated.

In recent years, the emergence of multiple-antimicrobial-resistant strains of *E. coli*, particularly multidrug-resistant strains leading to either community-acquired and nosocomial infections, has become a major public-health concern (Xu et al., 2014). Many researchers have reported resistant *E. coli* isolated from various food samples in different countries (Yang et al., 2004; Mora et al., 2005; Ho et al., 2011), but antimicrobial resistance of *E. coli* in plants manufacturing goat milk powder has rarely been reported. In the current study, only 10 (18.9%) *E. coli* isolates were sensitive to all tested antibiotics, whereas 24.5% (13/53) of *E. coli* isolates showed multidrug resistance (resistant to at least 3 antimicrobials). Resistance to trimethoprim-sulfamethoxazole is common (75.5%) among *E. coli* isolates. Compared with antimicrobial-resistance rates of *E. coli* isolates from other food samples (Koo and Woo, 2011; Vieira et al., 2011), resistance to these antimicrobials in isolates from milk powder plants is generally lower. In Korea, a previous study showed that 67.8% of *E. coli* isolated from meat and meat products were resistant to streptomycin, 66.1% to ampicillin, 44.6% to ciprofloxacin, 41.3% to trimethoprim-sulfamethoxazole, and 26.4% to chloramphenicol. The antimicrobial resistance of isolates from goat-milk plants is different from that from other meats and meat products. This may be explained by the fact that generally, less antibiotics were used in goat farms than in pig or cow farms in China.

The 53 isolates were not found to carry any of the 6 virulence genes of *E. coli*, which may be due to the limited number of isolates tested. Duffy et al. (2009) showed that only 3 (1%) of all environmental *E. coli* isolates carried the virulence genes *escV* and *eaeA*. Several studies (Beutin et al., 1993; Blanco et al., 2003; Rey et al., 2003; Jacob et al., 2013) have shown that goat could be the reservoir of STEC. However, we did not find existence of STEC in the plants manufacturing goat milk powder. In the plants we selected, most frequent serotypes of *E. coli* isolates were O125:H5 and O1:H2, which differed from the serotypes of *E. coli* isolates reported in previous studies focusing on STEC (Cortés et al., 2010; Martin and Beutin, 2011; Scheutz et al., 2011).

The molecular typing of isolates by PFGE revealed the presence of multiple clones of *E. coli* within each plant. With one exception, these strains from different plants were grouped in different clusters. Only cluster J contained isolates from 2 plants with more than 85% similarity. As shown in Figure 1, PFGE grouped isolates from different samples and from different locations, indicating a certain degree of spread of specific clones in the dairy environment. Milk in different storage tanks was contaminated by *E. coli* of various PFGE patterns (such as strain 427, 429, 430), indicating that the possible contamination source is the storage tank, because milk samples were of the same lot when loaded into tanks. Cluster J included *E. coli* with an identical PFGE pattern isolated from a conveyor, powder around



Stain number	Location	Place	Serotype
563A	powder on the ground of packaging room	Jingyang	O125H5
564A	powder on the ground of packaging room	Jingyang	O125:H5
560A	ground of packing room	Jingyang	O148
560B	ground of packing room	Jingyang	O148
606B	fluidized bed	Jingyang	O125:H8
678B	soil around the plant	Yanliang	H18
430B	milk in the tank	Jingyang	
431A	milk in the tank	Jingyang	
431B	milk in the tank	Jingyang	
430A	milk in the tank	Jingyang	
545A	air filter	Jingyang	O27:H5
426A	water in the piping	Jingyang	O6
426B	water in the piping	Jingyang	O6
675A	aoil around the plant	Yanliang	O27:H2
677B	soil around the plant	Yanliang	O125:H5
680B	milk in the tank	Yanliang	O1:H2
681A	milk in the tank	Yanliang	O1:H2
681B	milk in the tank	Yanliang	O1:H2
684B	milk in the tank	Yanliang	O1:H2
684A	milk in the tank	Yanliang	O1:H2
683A	milk in the tank	Yanliang	O1:H2
683B	milk in the tank	Yanliang	O1:H2
425B	water in the piping	Jingyang	O103:H40
427A	milk in the tank	Jingyang	O115:H40
679A	soil around the plant	Yanliang	O78
679B	soil around the plant	Yanliang	O78
429A	milk in the tank	Jingyang	
429B	milk in the tank	Jingyang	
677A	soil around the plant	Yanliang	
547A	conveyor	Jingyang	O125:H5
573B	powder around spray drier	Jingyang	O125:H8
496A	operation desk	Jingyang	O125:H5
496B	operation desk	Jingyang	O125:H5
534A	air filter	Jingyang	O27:H5
534B	air filter	Jingyang	O27:H5
546A	air hole of packing room	Jingyang	O125:H5
573A	powder around spray drier	Jingyang	O125:H18
680A	milk in the tank	Yanliang	O125
492A	operation desk	Jingyang	O121:H5
494A	operation desk	Jingyang	O125:H5
494B	operation desk	Jingyang	O125:H5
447A	spray dryer	Jingyang	O63:H5
492B	operation room	Jingyang	O121:H5
498A	operation desk	Jingyang	
498B	operation desk	Jingyang	
428A	milk in the tank	Jingyang	O142
428B	milk in the tank	Jingyang	O142
543A	air hole of packing room	Jingyang	O125:H18
543B	air hole of packing room	Jingyang	O125:H18
544A	air hole of packing room	Jingyang	O27:H18
544B	air hole of packing room	Jingyang	O27:H18
611A	product	Jingyang	O125:H5
675B	soil around the plant	Yanliang	O27:H5

Figure 1. Dendrogram of pulsed-field gel electrophoresis patterns showing the relatedness of *Escherichia coli* isolated from milk powder plants. The cluster cutoff was set at 85% similarity.

Table 4. Multidrug resistance among *Escherichia coli* isolates recovered from the plants

No. of antimicrobials to which resistance was shown	Source [No. (%) of isolates]		
	Plant in Jingyang (n = 38)	Plant in Yanliang (n = 15)	Total (n = 53)
0	6 (15.8)	4 (1.6)	10 (18.9)
1	17 (44.7)	7 (46.7)	24 (45.3)
2	5 (13.2)	1 (6.7)	6 (11.3)
3	1 (2.6)	2 (13.3)	3 (5.7)
4	3 (7.9)	1 (6.7)	4 (7.5)
5	2 (5.3)	0 (0.0)	2 (3.8)
6	2 (5.3)	0 (0.0)	2 (3.8)
>6	2 (5.3)	0 (0.0)	2 (3.8)

spray drier, operation desk, and air filter and air hole of packing room, suggesting dissemination of one clone in the packing area. We speculate that *E. coli* may be transmitted by air, which has also been reported in an early study (Craven et al., 2010). This highlights the importance of maintaining good hygiene at this stage of packaging.

In summary, this study demonstrated the presence of *E. coli* in plants manufacturing goat milk powder in Shaanxi Province, China. Milk storage tank and air in the plant could be potential sources of contamination. These findings provide useful information for the milk-powder industry to establish proper management practices that help minimize the chance of microbial contamination.

ACKNOWLEDGMENTS

This work was supported in part by the Twelve-Five Science and Technology Support Program (No. 2012BAH30F03), the Science and Technology Development Plan Program of Shaanxi Province (2013KJXX-16), the Program for New Century Excellent Talent Support Plan (NCET-13-0448), and the International Science and Technology Collaboration Research Fund (No. 213021202) in Northwest A&F University.

REFERENCES

Anifantakis, E. M. 2001. Utilization of goat milk. Pages 2–7 in Dairy News. Natl. Greek Dairy Comm. Int. Dairy Fed., Brussels, Belgium.

Beutin, L., D. Geier, H. Steinrück, S. Zimmermann, and F. Scheutz. 1993. Prevalence and some properties of verotoxin (Shiga-like toxin)-producing *Escherichia coli* in seven different species of healthy domestic animals. J. Clin. Microbiol. 31:2483–2488.

Blanco, M., J. Blanco, A. Mora, J. Rey, J. Alonso, M. Hermoso, J. Hermoso, M. Alonso, G. Dahbi, and E. González. 2003. Serotypes, virulence genes, and intimin types of Shiga toxin (verotoxin)-producing *Escherichia coli* isolates from healthy sheep in Spain. J. Clin. Microbiol. 41:1351–1356.

Claeys, W. L., C. Verraes, S. Cardoen, J. De Block, A. Huyghebaert, K. Raes, K. Dewettinck, and L. Herman. 2014. Consumption of raw or heated milk from different species: An evaluation of the nutritional and potential health benefits. Food Contr. 42:188–201.

CLSI (Clinical and Laboratory Standards Institute). 2013. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-Second Informational Supplement, CLSI document M100–S23. Clin. Lab. Stand. Inst., Wayne, PA.

Cortés, P., V. Blanc, A. Mora, G. Dahbi, J. E. Blanco, M. Blanco, C. López, A. Andreu, F. Navarro, and M. P. Alonso. 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Appl. Environ. Microbiol. 76:2799–2805.

Craven, H. M., C. M. McAuley, L. L. Duffy, and N. Fegan. 2010. Distribution, prevalence and persistence of *Cronobacter* (*Enterobacter sakazakii*) in the nonprocessing and processing environments of five milk powder factories. J. Appl. Microbiol. 109:1044–1052.

De Buyser, M.-L., B. Dufour, M. Maire, and V. Lafarge. 2001. Implication of milk and milk products in food-borne diseases in France and in different industrialised countries. Int. J. Food Microbiol. 67:1–17.

Duffy, L. L., D. O'Callaghan, C. M. McAuley, N. Fegan, and H. M. Craven. 2009. Virulence properties of *Escherichia coli* isolated from Australian dairy powder factory environments. Int. Dairy J. 19:178–179.

Gautom, R. K. 1997. Rapid pulsed-field gel electrophoresis protocol for typing of *Escherichia coli* O157:H7 and other gram-negative organisms in 1 day. J. Clin. Microbiol. 35:2977–2980.

Ho, P.-L., K. Chow, E. L. Lai, W.-U. Lo, M. Yeung, J. Chan, P. Chan, and K. Yuen. 2011. Extensive dissemination of CTX-M-producing *Escherichia coli* with multidrug resistance to 'critically important' antibiotics among food animals in Hong Kong, 2008–10. J. Antimicrob. Chemother. 66:765–768.

Hornes, E., Y. Wasteson, and O. Olsvik. 1991. Detection of *Escherichia coli* heat-stable enterotoxin genes in pig stool specimens by an immobilized, colorimetric, nested polymerase chain reaction. J. Clin. Microbiol. 29:2375–2379.

IDF (International Dairy Federation). 1991. IDF Recommendations for the Hygienic Manufacture of Spray Dried Milk Powders. IDF Bulletin 267. Int. Dairy Fed., Brussels, Belgium.

Jacob, M., D. Foster, A. Rogers, C. Balcomb, X. Shi, and T. Nagaraja. 2013. Evidence of non-O157 Shiga toxin producing *Escherichia coli* in the feces of meat goats at a US slaughter plant. J. Food Prot. 76:1626–1629.

Koo, H. J., and G. J. Woo. 2011. Distribution and transferability of tetracycline resistance determinants in *Escherichia coli* isolated from meat and meat products. Int. J. Food Microbiol. 145:407–413.

Lynch, M., L. O'Connor, E. Fox, K. Jordan, and M. Murphy. 2012. Verocytotoxigenic *Escherichia coli* O157, O111, O26, O103, O145 in Irish dairy cattle and raw milk: Prevalence and epidemiology of emergent stains. Zoonoses Public Health 59:264–271.

Martin, A., and L. Beutin. 2011. Characteristics of Shiga toxin-producing *Escherichia coli* from meat and milk products of different origins and association with food producing animals as main contamination sources. Int. J. Food Microbiol. 146:99–104.

Mora, A., J. E. Blanco, M. Blanco, M. P. Alonso, G. Dhabi, A. Echeita, E. A. González, M. I. Bernárdez, and J. Blanco. 2005. Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Res. Microbiol. 156:793–806.

Oswald, E., H. Schmidt, S. Morabito, H. Karch, O. Marches, and A. Caprioli. 2000. Typing of intimin genes in human and animal enterohemorrhagic and enteropathogenic *Escherichia coli*: Characterization of a new intimin variant. Infect. Immun. 68:64–71.

Park, Y. W., M. Juárez, M. Ramos, and G. F. W. Haenlein. 2007. Physico-chemical characteristics of goat and sheep milk. Small Rumin. Res. 68:88–113.

Rey, J., J. E. Blanco, M. Blanco, A. Mora, G. Dahbi, J. M. Alonso, M. Hermoso, J. Hermoso, M. P. Alonso, and M. A. Usera. 2003. Serotypes, phage types and virulence genes of Shiga-producing *Escherichia coli* isolated from sheep in Spain. Vet. Microbiol. 94:47–56.

Rúgeles, L. C., J. Bai, A. J. Martínez, M. C. Vanegas, and O. G. Gómez-Duarte. 2010. Molecular characterization of diarrheagenic *Escherichia coli* strains from stools samples and food products in Colombia. Int. J. Food Microbiol. 138:282–286.

Scheutz, F., E. Møller Nielsen, J. Frimodt-Møller, N. Boisen, S. Morabito, R. Tozzoli, J. Nataro, and A. Caprioli. 2011. Characteristics of the enteroaggregative Shiga toxin/verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011. Euro Surveill. 16:19889.

Sethabutr, O., M. Venkatesan, G. S. Murphy, B. Eampokalap, C. W. Hoge, and P. Echeverria. 1993. Detection of Shigellae and enteroinvasive *Escherichia coli* by amplification of the invasion plasmid antigen H DNA sequence in patients with dysentery. J. Infect. Dis. 167:458–461.

Tamanai-Shacoori, Z., A. Jolivet-Gougeon, M. Pommepuy, M. Cormier, and R. Colwell. 1994. Detection of enterotoxigenic *Escherichia coli* in water by polymerase chain reaction amplification and hybridization. Can. J. Microbiol. 40:243–249.

Vieira, A. R., P. Collignon, F. M. Aarestrup, S. A. McEwen, R. S. Hendriksen, T. Hald, and H. C. Wegener. 2011. Association between antimicrobial resistance in *Escherichia coli* isolates from food animals and blood stream isolates from humans in Europe: An ecological study. Foodborne Pathog. Dis. 8:1295–1301.

Xu, Z.-Q., M. T. Flavin, and J. Flavin. 2014. Combating multidrug-resistant Gram-negative bacterial infections. Expert Opin. Investig. Drugs 23:163–182.

Yamasaki, S., Z. Lin, H. Shirai, A. Terai, Y. Oku, H. Ito, M. Ohmura, T. Karasawa, T. Tsukamoto, and H. Kurazono. 1996. Typing of verotoxins by DNA colony hybridization with poly- and oligonucleotide probes, a bead-enzyme-linked immunosorbent assay, and polymerase chain reaction. Microbiol. Immunol. 40:345–352.

Yang, H., S. Chen, D. G. White, S. Zhao, P. McDermott, R. Walker, and J. Meng. 2004. Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. J. Clin. Microbiol. 42:3483–3489.