

Antibiotic Susceptibility of Commensal Bacteria from Human Milk

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Abstract Recent studies have focused on foodborne or commensal bacteria as vehicles of antibiotic resistance. However, the antibiotic resistance of milk bacteria from healthy donors is still vague in Taiwan. For this purpose, human milk samples were obtained from randomly recruited 19 healthy women between 3 and 360 days postpartum. Antibiotic susceptibility profile of bacteria from milk samples was determined. About 20 bacterial species were isolated from milk samples including *Staphylococcus* (6 species), *Streptococcus* (4 species), *Enterococcus* (2 species), *Lactobacillus* (1 species), and bacteria belonging to other genera (7 species). Some opportunistic or potentially pathogenic bacteria including *Kluyvera ascorbata*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Actinomyces bovis*, and *Staphylococcus aureus* were also isolated. Intriguingly, *Staphylococcus* isolates (22 strains) were resistant to 2–8 of 8 antibiotics, while *Streptococcus* isolates (3 strains) were resistant to 3–7 of 9 antibiotics, and members of the genus *Enterococcus* (5 strains) were resistant to 3–8 of 9 antibiotics. Notably,

Staphylococcus lugdunensis, *S. aureus*, *Streptococcus parasanguinis*, *Streptococcus pneumoniae*, and *Enterococcus faecalis* were resistant to vancomycin, which is considered as the last-resort antibiotic. Therefore, this study shows that most bacterial strains in human milk demonstrate mild to strong antibiotic resistance. Whether commensal bacteria in milk could serve as vehicles of antibiotic resistance should be further investigated.

Introduction

Bacterial antibiotic resistance has been well documented. For instance, an increase in the prevalence of antibiotic-resistant bacteria such as *Staphylococcus aureus* [6, 16], *Acinetobacter baumannii* [2, 19, 20], *Klebsiella* spp., and *Enterobacter* spp. has been frequently reported [4, 18, 26]. Previously, the selection and dissemination of antibiotic resistance was mainly studied in clinically relevant bacterial species. However, recent studies have suggested that foodborne or commensal bacteria may also function as reservoirs of antibiotic resistance genes, similar to those found in pathogens [8, 14, 25]. For example, human milk has been implicated as a reservoir for several antibiotic-resistant bacteria in that the strains of *Staphylococcus epidermidis* isolated from human milk (from healthy mothers) have shown resistance to gentamicin, tetracycline, erythromycin, clindamycin, or vancomycin, with several isolates demonstrating multi-drug resistance as well [3]. Moreover, *S. epidermidis* isolated from human milk of a healthy donor was also reported to exhibit multiple antibiotic resistances [7]. Additionally, *Enterococcus* spp. isolated from milk of healthy women, as well as that of porcine, canine, ovine, and feline origin, showed resistance to various clinically relevant antibiotics. Notably, two

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Enterococcus faecalis isolates from human milk displayed resistance to five of eight tested antibiotics, including gentamicin, streptomycin, quinupristin/dalfopristin, tetracycline, and chloramphenicol [11]. Since *E. faecalis* and *E. faecium* isolated from milk contained virulence genes and antibiotic resistance, they can serve as a reservoir of antibiotic resistance in infants or offspring [11]. Collectively, these reports indicate that some isolates of *Staphylococcus* or *Enterococcus* in milk (from healthy donors) exert resistance to various antibiotics. However, the antibiotic resistance profile of milk bacteria from local area in Taiwan is not clear. Therefore, human milk samples were collected from healthy women, and the bacterial antibiotic susceptibility profile of isolates from milk samples was determined.

Materials and Methods

Milk Samples

This study has been approved by the Institutional Review Board (IRB) at Saint Mary's Hospital, Lundong (SMHIRB102007), and informed consent was obtained from all individual participants included in the study. Healthy, lactating mothers ($n = 19$) between 3 and 360 days post-partum who visited the Saint Mary's Hospital, Lundong, were randomly recruited, and milk samples were collected as previously described [1]. For mothers who delivered by cesarean section, Keflex (cephalexin) had been treated as a prophylactic antibiotic. To collect milk sample, the breast was cleaned using sterile warm water or saline, and the first 0.5 mL of milk was discarded to avoid contamination from the skin. Then, 5–10 mL of milk was collected in sterile tubes by manual expression using sterile gloves. The samples were immediately processed in the laboratory and incubated in different culture media under both anaerobic and aerobic conditions.

Bacterial Isolation

Milk samples were immediately transferred to the laboratory, and the samples were centrifuged briefly at 6000 rpm for 10 min to collect all bacteria. Then, a certain amount of supernatant was discarded, and the centrifuged bacteria were re-suspended in the same milk. Bacteria were isolated by inoculating different culture plates with centrifuged samples. For instance, blood agar plate (BAP)/eosin-methylene blue (EMB) bi-plates and chocolate agar plates were incubated under aerobic conditions at 37 °C for 24 h. For anaerobic species, centrifuged samples were plated onto de Man, Rogosa and Sharpe (MRS), Bacteroides-bile-

esculin (BBE), and kanamycin-vancomycin laked blood agar (KVLB) plates and incubated under anaerobic conditions at 37 °C for 48 h in a Bugbox anaerobic workstation (Ruskin Technology, Ltd., Pencoed, UK; atmospheric composition: 80 % N₂, 10 % CO₂, and 10 % H₂). The obtained bacterial colonies were further purified and examined using phenotypic identification. Pure colonies were identified using both phenotypic and genotypic identification methods as indicated below.

Identification of Bacterial Isolates

The bacterial isolates were identified by Gram staining and 16S rRNA sequence analysis. Morphological characteristics of the bacterial isolates were examined, followed by phenotypic and genotypic identification methods. Phenotype was characterized using the analytical profile index (API) [15, 17] and the Biolog Microstation system [12, 21]. For genotypic identification, chromosomal DNA of the isolates was extracted and used as a template in polymerase chain reactions (PCR) [23] as previously described, using primer sequences for the 16S rRNA gene [22, 24]. The PCR sequences were confirmed by BLAST searches against the GenBank database at the National Center for Biotechnology Information, USA (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

PCR Amplification

PCR was carried out in a final volume of 50 µL containing 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM dNTP, 100 ng chromosomal DNA template, 20 pM primer, and 2 U Taq DNA polymerase (Promega, Madison, WI, USA). Thermal cycling was carried out as follows: initial denaturation at 94 °C for 30 s, followed by 30 cycles at 50 °C for 1 min, 72 °C for 1.5 min, and 94 °C for 1.5 min, and a final extension at 72 °C for 5 min in a PerkinElmer GeneAmp 9600 PCR system (Applied Biosystems, Foster City, CA, USA). Finally, the sequences were submitted to GenBank via Sequin software (<http://www.ncbi.nlm.nih.gov/Sequin/index.html>).

Antimicrobial Susceptibility Testing by Disk Diffusion Assay

Antibiotic susceptibility of isolates was determined using Kirby-Bauer's disk diffusion method. The results were analyzed and interpreted according to CLSI guidelines using the disk diffusion technique (NCCLS 2012). All antimicrobial disks were purchased from Oxoid Ltd (Oxoid, Basingstoke, UK). Antibiotics, such as oxacillin (1 µg), ampicillin (10 µg), amoxicillin (25 µg), cefazolin (30 µg), cephalothin (30 µg), vancomycin (30 µg),

sulfamethoxazole–trimethoprim (25 µg), and ampicillin/sulbactam (20 µg), were selected to test the resistance of *Staphylococcus* spp. to these antibiotics. Moreover, antibiotics such as ampicillin (10 µg), cefazolin (30 µg), gentamicin (10 µg), erythromycin (15 µg), clindamycin (2 µg), oxacillin (1 µg), ampicillin/sulbactam (20 µg), sulfamethoxazole–trimethoprim (25 µg), and vancomycin (30 µg) were used to test resistance of *Enterococcus* and *Streptococcus* spp. Similarly, amikacin (30 µg), ampicillin (10 µg), cefotaxime (30 µg), cefazolin (30 µg), gentamicin (10 µg), ampicillin/sulbactam (20 µg), trimethoprim/sulfamethoxazole (25 µg), ciprofloxacin (5 µg), imipenem (10 µg), and meropenem (10 µg) were used for *Kluyvera* spp. As for *A. baumannii*, amikacin (30 µg), ertapenem (10 µg), ceftriaxone (30 µg), ciprofloxacin (5 µg), imipenem (10 µg), meropenem (10 µg), ampicillin/sulbactam (20 µg), cefotaxime (30 µg), ampicillin (10 µg), and cefazolin (30 µg) were used. Otherwise, tetracycline (30 µg), ampicillin (10 µg), amoxicillin (25 µg), chloramphenicol (30 µg), and erythromycin (15 µg) were used for *Lactobacillus gasseri*.

Moreover, the same assay was routinely conducted using the quality control organisms recommended by NCCLS, including *E. coli* ATCC 25922, *S. aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Haemophilus influenzae* ATCC 49766, and *Streptococcus pneumoniae* ATCC49619, to validate the disk diffusion assay and the effectiveness of antibiotic disks.

Results and Discussion

Distribution of Bacteria in Human Breast Milk

The presence of bacteria in human milk has been acknowledged since the 1970s, with studies focusing on pathogens [10]. In this study, we evaluated the antibiotic susceptibility of bacteria isolated from milk of healthy mothers. In Table 1, approximately four groups of bacterial species were isolated from milk samples, including *Staphylococcus* (6 species), *Streptococcus* (4 species), *Enterococcus* (2 species), *Lactobacillus* (1 species), and bacteria belonging to other genera (7 species). As described in the paragraph “Identification of Bacterial Isolates,” the bacterial isolates were identified by phenotypic (Gram staining, API, and Biolog Microstation system) and genotypic identification methods (16S rRNA sequence analysis). Staphylococcal and streptococcal cells were the most common, wherein *S. epidermidis* (in 9 samples) and *Staphylococcus lugdunensis* (in 6 samples) were frequently isolated (Table 1).

The bacterial species, supplements, and duration of lactation are shown in Table 2. These data indicate that

Table 1 Bacterial isolates from human milk

Species	Occurrence in mothers
<i>Staphylococcus</i>	
<i>Staphylococcus epidermidis</i>	9
<i>Staphylococcus haemolyticus</i>	1
<i>Staphylococcus pasteurii</i>	3
<i>Staphylococcus lugdunensis</i>	6
<i>Staphylococcus aureus</i>	1
<i>Staphylococcus</i> sp.	1
<i>Streptococcus</i>	
<i>Streptococcus parasanguinis</i>	1
<i>Streptococcus pneumoniae</i>	2
<i>Streptococcus mitis</i>	1
<i>Streptococcus</i> sp.	2
<i>Enterococcus</i>	
<i>Enterococcus faecalis</i>	4
<i>Enterococcus malodoratus</i>	1
<i>Lactobacillus</i>	
<i>Lactobacillus gasseri</i>	1
Other microorganisms	
<i>Kluyvera ascorbata</i>	1
<i>Klebsiella oxytoca</i>	1
<i>Klebsiella pneumoniae</i>	2
<i>Acinetobacter baumannii</i>	2
<i>Actinomyces bovis</i>	1
Yeast-like	1
<i>Bacterium</i> sp.	1

each milk sample had a distinct and heterogeneous bacterial profile. These bacterial strains could be roughly classified into four groups according to their pathogenicity or basic characterizations: commensals (or low-pathogenic), opportunistic pathogens, pathogens, and *Lactobacillus* species. In summary, commensal bacteria were found in 78 % (15 out of 19) of samples, and opportunistic bacteria were found in 37 % (7 out of 19) of samples. Furthermore, *Lactobacillus* spp. or potential lactic acid species (such as *E. faecalis*) were found in 31 % (5) of samples. Thus, most milk-derived isolates were found to be mildly pathogenic or non-pathogenic bacteria, comprising about 94 % of isolates.

Antibiotic Sensitivity Testing for Bacterial Isolates from Human Milk

As indicated in the introduction section, some isolates of *Staphylococcus* or *Enterococcus* in milk from healthy donors exert resistance to various antibiotics [3, 7, 11]. In this study, a total of 22 bacterial isolates of *Staphylococcus*

Table 2 Bacterial species, supplements, and lactation days between mothers

Mother	Bacterial species	Duration of lactation (day)
M1	<i>Staphylococcus epidermidis</i>	≤7
M2	<i>Enterococcus faecalis</i> , <i>Acinetobacter baumannii</i>	≤7
M3	<i>Staphylococcus lugdunensis</i>	≤7
M4	<i>Staphylococcus aureus</i> , <i>Enterococcus malodoratus</i>	≤7
M5	<i>Staphylococcus epidermidis</i> , <i>Enterococcus faecalis</i>	≤7
M6	<i>Staphylococcus epidermidis</i>	≤7
M7	<i>Staphylococcus haemolyticus</i> , <i>Enterococcus faecalis</i>	≤7
M8	<i>Kluyvera ascorbata</i> , <i>Acinetobacter baumannii</i>	≤7
M9	<i>Staphylococcus pasteurii</i>	≤7
M10	<i>Staphylococcus lugdunensis</i> , <i>Staphylococcus epidermidis</i>	≤7
M11	<i>Streptococcus mitis</i> , <i>Staphylococcus epidermidis</i>	≤7
M12	<i>Staphylococcus epidermidis</i> , <i>Klebsiella pneumoniae</i> , <i>Streptococcus pneumoniae</i> , <i>Staphylococcus lugdunensis</i>	30
M13	<i>Klebsiella oxytoca</i> , <i>Klebsiella pneumoniae</i> , <i>Actinomyces bovis</i>	60
M14	<i>Enterococcus faecalis</i> , <i>Staphylococcus lugdunensis</i>	90
M15	<i>Staphylococcus pasteurii</i> , <i>Staphylococcus epidermidis</i>	120
M16	<i>Staphylococcus lugdunensis</i> , <i>Staphylococcus</i> sp., <i>Streptococcus</i> sp.	120
M17	<i>Staphylococcus pasteurii</i> , <i>Staphylococcus epidermidis</i>	190
M18	<i>Lactobacillus gasseri</i> , <i>Streptococcus parasanguinis</i> , <i>Streptococcus</i> sp., <i>Bacterium</i> sp.	300
M19	Yeast-like, <i>Staphylococcus lugdunensis</i> , <i>Staphylococcus epidermidis</i>	360

spp. (Table 3) were also evaluated for their susceptibility to several antibiotics. Similar to previous reports, most of these bacteria showed resistance to multiple antibiotics, and these bacteria were found to be resistant to two to eight out of eight antibiotics tested, while two isolates, *S. lugdunensis* (M3) and *S. aureus* (M4), were resistant to vancomycin. The resistance rates were also calculated, and a high resistance rate, including intermediate resistance, was observed against oxacillin (82 %) and ampicillin (100 %). Resistance rates of these bacteria to other antibiotics tested were below 17 %.

Antibiotic resistance of *Staphylococcus* strains from milk samples of healthy donors has been occasionally reported [3, 5]. For instance, the disk diffusion assay found a high rate of antibiotic resistance of *S. epidermidis*, *S. warneri*, *S. haemolyticus*, and *S. aureus* isolated from milk to penicillin (87 %) and erythromycin (59.3 %) [5]. Notably, two *S. haemolyticus* isolates from the same study were considered to be intermediately resistant to vancomycin based on the minimum inhibitory concentration assay [5]. That was the first report to indicate that *Staphylococcus* strains from human milk express reduced susceptibility to vancomycin [5]. More recently, *S. epidermidis* strains from the milk of healthy mothers have shown resistance to tetracycline, erythromycin, clindamycin, and vancomycin as well [3]. Interestingly, our

study is the first to report antibiotic resistance of *S. lugdunensis* and *Sporosarcina pasteurii* from milk samples. Collectively, data from previous (from different countries) and the present study indicate that several *Staphylococcus* spp. isolated from milk from healthy mothers show multiple antibiotic resistances, including that against vancomycin. These data imply that more milk samples from healthy donors should be studied for their antibiotic susceptibility.

Both *Enterococcus* and *Streptococcus* spp. show resistance against the selected antibiotics (Table 4). For example, the majority of *E. faecalis* strains displayed resistance (including intermediate resistance) against three to eight of the nine antibiotics tested, while most *Streptococcus*-related strains also displayed resistance or intermediate resistance against three to seven out of nine antibiotics tested. Importantly, one *Streptococcus parasanguinis* strain (M18) was resistant to vancomycin, while another four strains including *E. faecalis* and *S. pneumoniae* (samples M2, M5, M12, and M14) also displayed intermediate resistance to vancomycin. The antibiotic resistance rates were also evaluated for these strains. Higher resistance rates for *Enterococcus*-related strains (total of six strains; Table 3), including intermediate resistance, were observed against cefazolin (66.6 %), gentamicin (83.3 %), erythromycin (100 %), clindamycin

Table 3 Antibiotic sensitivity testing for *Staphylococcus* spp. of human milk

Mothers	Bacterial isolates	Antibiotics								
		OX	AMP	AMC	CZ	CF	VA	SXT	SAM	
M1	<i>Staphylococcus epidermidis</i>	R	R	S	S	I ^a	I	S	R	
M5	<i>Staphylococcus epidermidis</i>	R	R	S	S	I	S	S	R	
M6	<i>Staphylococcus epidermidis</i>	R	R	R	R	R	R	R	S	
M6	<i>Staphylococcus epidermidis</i>	R	R	S	S	S	S	R	S	
M11	<i>Staphylococcus epidermidis</i>	S	R	S	S	S	S	S	S	
M12	<i>Staphylococcus epidermidis</i>	R	R	S	S	S	S	R	S	
M15	<i>Staphylococcus epidermidis</i>	R	R	S	S	S	S	S	S	
M17	<i>Staphylococcus epidermidis</i>	R	R	S	S	S	S	S	S	
M19	<i>Staphylococcus epidermidis</i>	S	R	S	S	S	S	S	S	
M7	<i>Staphylococcus haemolyticus</i>	R	R	S	S	S	S	S	S	
M9	<i>Staphylococcus pasteurii</i>	R	R	S	S	S	S	S	S	
M15	<i>Staphylococcus pasteurii</i>	R	R	S	S	S	S	S	S	
M17	<i>Staphylococcus pasteurii</i>	S	R	S	S	S	S	S	S	
M3	<i>Staphylococcus lugdunensis</i>	R	R	R	I	R	I	I	R	
M10	<i>Staphylococcus lugdunensis</i>	R	R	S	S	S	S	S	S	
M10	<i>Staphylococcus epidermidis</i>	R	R	S	I	R	S	S	S	
M12	<i>Staphylococcus lugdunensis</i>	R	R	S	S	S	S	S	S	
M16	<i>Staphylococcus</i> sp.	R	R	S	S	S	S	S	S	
M16	<i>Staphylococcus lugdunensis</i>	I	R	S	S	S	S	S	S	
M14	<i>Staphylococcus lugdunensis</i>	R	R	S	S	S	S	S	S	
M19	<i>Staphylococcus lugdunensis</i>	R	R	S	S	S	S	S	S	
M4	<i>Staphylococcus aureus</i>	S	R	S	S	S	R	S	R	

OX Oxacillin, AMP/AM ampicillin, AMC amoxicillin, CZ/KZ cefazolin, CF cephalothin, VA vancomycin, SXT sulphamethoxazole–trimethoprim, SAM ampicillin/sulbactam, R resistant, S sensitive

^a I Intermediate, the diameter of the zone of inhibition around a particular antibiotic ranged between the criteria of “susceptible” and “resistant.” This indicates either a technical problem that should be resolved by repeat testing or a lack of clinical experience in treating organisms with these zones (NCCLS, 2012)

Table 4 Antibiotic sensitivity testing for *Enterococcus* and *Streptococcus* spp. of human milk

Mothers	Bacterial isolates	Antibiotics								
		AM	CZ	GM	E	CC	OX	SAM	SXT	VA
M2	<i>Enterococcus faecalis</i>	S	R	R	R	R	R	R	S	I ^a
M5	<i>Enterococcus faecalis</i>	I	R	R	R	R	R	R	R	I
M7	<i>Enterococcus faecalis</i>	S	S	R	R	R	R	S	S	S
M14	<i>Enterococcus faecalis</i>	S	R	R	R	R	R	S	S	I
M4	<i>Enterococcus malodoratus</i>	S	S	S	R	R	I	S	S	S
M12	<i>Streptococcus pneumoniae</i>	S	R	R	R	R	R	S	R	I
M18	<i>Streptococcus parasanguinis</i>	S	R	R	R	R	R	S	R	R
M18	<i>Streptococcus</i> sp.	R	S	S	R	S	R	R	R	S

AM Ampicillin, CZ cefazolin, GM gentamicin, E erythromycin, CC clindamycin, OX Oxacillin, SAM ampicillin/sulbactam, SXT sulphamethoxazole–trimethoprim, VA vancomycin, S sensitive, R resistant

^a I Intermediate, the diameter of the zone of inhibition around a particular antibiotic ranged between the criteria of “susceptible” and “resistant.” This indicates either a technical problem that should be resolved by repeat testing or a lack of clinical experience in treating organisms with these zones (NCCLS, 2012)

(100 %), and oxacillin (100 %). Moreover, all *Streptococcus*-related strains were resistant to oxacillin and sulphamethoxazole–trimethoprim. In comparison to a previous

report, *Enterococcus* spp. isolated from milk of healthy women, including that of porcine, canine, ovine, and feline origin, have been observed to display resistance against

Table 5 Antibiotic sensitivity testing for *Kluyvera* spp. and *Acinetobacter baumannii* of human milk

Mothers	Bacterial isolates	Antibiotics												
		AN	AM	CTX	CZ	GM	SAM	MEM	CIP	IPM	SXT	ETP	CRO	
M8	<i>Kluyvera ascorbata</i>	S	R	S	R	S	S	S	S	S	S	ND	ND	
M13	<i>Klebsiella oxytoca</i>	S	R	S	S	S	S	S	S	S	S	ND	ND	
M13	<i>Klebsiella pneumoniae</i>	S	R	R	R	S	S	S	S	S	S	ND	ND	
M12	<i>Klebsiella pneumoniae</i>	S	R	S	S	S	S	S	S	S	S	ND	ND	
M2	<i>Acinetobacter baumannii</i>	S	R	R	R	S	S	S	S	S	ND	R	R	
M8	<i>Acinetobacter baumannii</i>	S	R	R	R	S	S	S	S	S	ND	R	R	

AN Amikacin, AM ampicillin, CTX cefotaxime, CZ cefazolin, GM gentamicin, SAM ampicillin/sulbactam, SXT trimethoprim/sulfamethoxazole, CIP ciprofloxacin, IPM imipenem, MEM meropenem, S sensitive, R resistant, ND non-determined

various clinically relevant antibiotics [11]. Of note, two *E. faecalis* isolates from human milk displayed resistance to five of the eight antibiotics tested, including gentamicin, streptomycin, quinupristin/dalfopristin, tetracycline, and chloramphenicol [11]. Altogether, the present and previous data indicate that *Enterococcus* or *Streptococcus* strains isolated from milk show wide-spectrum antibiotic resistance.

Kluyvera and *Klebsiella* (from M8, M12, or M13) strains were resistant to one to three of 10 antibiotics tested (Table 5). Notably, all strains were resistant to ampicillin. In contrast, *A. baumannii* (two strains) was resistant to five of 11 antibiotics tested, including ertapenem, ceftriaxone, cefotaxime, ampicillin, and cefazolin. Thus, compared to *Kluyvera* and *Klebsiella*, an increased antibiotic resistance was noted for *A. baumannii*. Conversely, *L. gasseri* was sensitive to all antibiotics tested and resistant to erythromycin.

Taken together, our data provide evidence that most isolates obtained from the milk of healthy donors showed resistance to various antibiotics. Notably, resistance of bacteria to a given antibiotic can be intrinsic or acquired. However, we did not screen for virulence determinants or antibiotic resistance genes in this study; this will be further evaluated in a subsequent study.

The Role of Commensal Bacteria in Human Milk

The mothers recruited in this study continuously breast-fed their babies for the duration of the study. Although our data show that most bacterial isolates possessed resistance to multiple antibiotics, we believe that these milk-borne bacteria may not pose a risk to the breast-fed infants. Our and previous data only indicate that milk-borne bacteria could be a reservoir or a vehicle for the dissemination of antibiotic resistance [3, 5]. Moreover, the possible threat

associated with these bacteria is that they may transfer resistance genes to pathogenic bacteria.

Previous findings have reported the presence of various *Lactobacillus* or *Bifidobacterium* species within human milk [9, 10, 13]. Similar to previous studies, one milk sample in our study was colonized with *L. gasseri*. However, no *Bifidobacterium* species were isolated from our milk samples. The lack of *Bifidobacterium* species may be due to the lesser number of milk samples harvested in the present study. Therefore, more milk samples from healthy donors will be studied in our next study. In contrast, the supernatants produced by selected milk isolates, *L. gasseri* and *E. faecalis*, exhibited anti-bacterial activity against foodborne pathogens including *Escherichia coli* HER 1255, *Salmonella enterica* ATCC 19430, and *Staphylococcus aureus* ATCC 25923 (data not shown). These data may partially explain the roles of *Lactobacillus* or potential lactic acid bacteria within human milk.

In conclusion, this study demonstrates that human milk from healthy donors contains various antibiotic-resistant bacteria and that several opportunistic pathogens can colonize the milk as well.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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