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# Antibiotic resistance and sensitivity patterns of bacteria isolated from mastitis cases in dairy cows in Honduras

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### SUMMARY

Additional keywords

Antimicrobial resistance. Mastitis therapy. Milk bacteria. Udder health

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#### INFORMATION

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#### INTRODUCTION

The worldwide growth of bacterial resistance to antibiotics has been identified as a major public health threat (Roca et al., 2015; World Health Organization, 2014). Animal production has been considered one of the main sources of resistant or multiresistant bacterial strains (Angulo et al., 2014; Eng, 2015; Ventola, 2015). The massive and routine use of antibiotics in animal production, either for prevention or therapy, exerts a selection pressure over

Mastitis is a major problem in dairy farms around the world and is usually treated with antibiotics. However, bacteria - the usual causative agents - often become resistant to commonly used antibiotics, hindering milk production, and generating a possible public health problem. With the purpose of generating information on this situation in Honduras, we carried out a retrospective documentary study examining official records of bacterial isolates and antibiotic resistance tests carried out to milk samples coming from mastitis cases in dairy cows from 2013 to 2018. We examined 235 cases of bacterial isolation and sensitivity test to 15 antibiotics (amoxicillin + clavulanic acid, ampicillin, ceftiofur, ceftriaxone, enrofloxacin, ciprofloxacin, oxacillin, doxycycline, neomycin, trimethoprim + sulphamethoxazole, gentamicin, tetracycline, erythromycin, and penicillin). The more frequently isolated bacteria taxon was Staphylococcus spp. (168 cases) followed by *Streptococcus* spp. (31 cases), *Corynebacterium* spp. (14 cases), *Escherichia* spp. (13 cases), *Klebsiella* spp. (five cases), *Enterobacter* sp. (one case), *Pasteurella* sp. (one case), and *Pseudomonas* sp. (one case). We found antibiotic resistance cases to practically all tested antibiotics, however, patterns in resistance and sensitivity were uncommon.

#### Resistencia antibiótica y sensibilidad en aislamientos de bacterias en mastitis en vacas lecheras en Honduras

#### RESUMEN

La mastitis es un importante problema en granjas de producción lechera alrededor del mundo y usualmente se trata con antibióticos. Sin embargo, las bacterias –que son los agentes causales usuales– van volviéndose resistentes a los antibióticos de uso común. Con el objetivo de generar información sobre esta situación en Honduras, realizamos un estudio retrospectivo documental examinando los registros oficiales de aislamientos de bacterias y pruebas de resistencia antibiótica llevados a cabo en muestras de leche de casos de mastitis en vacas, entre 2013 y 2018. Examinamos 235 casos de aislamiento bacteriano y pruebas de sensibilidad a 15 antibióticos (amoxicilina + ácido clavulánico, ampicilina, ceftiofur, ceftriaxona, enrofloxacina, ciprofloxacina, oxacilina, doxiciclina, neomicina, trimetoprim + sulfametoxazol, gentamicina, tetraciclina, eritromicina y penicilina). Los taxones más frecuentemente aislados fueron Staphylococcus spp. (168 casos), seguido por Streptococcus spp. (31 casos), Corynebacterium spp. (14 casos), Escherichia coli (13 casos), Klebsiella spp. (cinco casos), Enterobacter sp. (un caso), Pasteurella sp. (un caso) y Pseudomonas sp. (un caso). Encontramos casos de resistencia a prácticamente todos los antibióticos, aunque los patrones de resistencia fueron poco comunes.

> bacterial populations thus promoting the appearance of super-resistant strains (Aarestrup, 2012; Dahms et al., 2014). It has been estimated that the global consumption of antibiotics in animal production will increase by 67% between 2010 and 2030 (Van Boeckel et al., 2015) and this, of course, could aggravate the problem.

> Cow milk production is one of the most common farm activities around the world (Faye & Konuspayeva, 2012) and mastitis is one of the most common

diseases and certainly a frequent reason for antibiotic use (Barlow, 2011). This means that not only could the milk of a cow with mastitis harbor bacteria, but these bacteria could be antibiotic-resistant. In a developing country like Honduras, where the government is not able to control all the milk or dairy products that are sold on the markets, there is an increased risk of acquiring antibiotic-resistant bacteria via the consumption of these products.

Despite Honduras is a traditional dairy farmer country (Molina, 2010), the publications about mastitis-causing bacteria and their antibioticresistant patterns are practically inexistent. Therefore, our objectives in this research were to analyze official records of laboratory culture and sensitivity tests of mastitis cases in dairy cows in Honduras from 2013 to 2018.

#### MATERIAL AND METHODS

#### STUDY SITE AND SAMPLING

We conducted a retrospective study based on records of the Honduran Institute of Veterinary Medical Research (HIVMR), National Service of Agrifood Health and Safety, Republic of Honduras. We examined the outcomes of microbiologic cultures of milk samples from mastitis cases in dairy cows, from years 2013 to 2018. We looked for bacterial isolation and antibiotic sensitivity tests. The samples came from 12 Honduran provinces (Atlántida, Choluteca, Colón, Comayagua, Cortés, El Paraíso, Francisco Morazán, Intibucá, La Paz, Olancho, Santa Bárbara y Yoro).

The isolation and identification of bacteria in the HIVMR was carried out according to the ISO 6579 International Standard Procedures (International Organization for Standardization, 2002) and the antimicrobial susceptibility tests were performed following the methods and criteria of the M100S standards of the Clinical and Laboratory Standards Institute (Patel et al., 2017).

#### DATA PROCESSING

Positive cases were examined and the isolated species (or taxon) of bacteria was registered as well as the sensitivity to 15 common use antibiotics (Amoxicillin + Clavulanic Acid, Ampicillin, Ceftiofur, Ceftriaxone, Enrofloxacin, Ciprofloxacin, Oxacillin, Doxycycline, Neomycin, Trimethoprim + Sulphamethoxazole, Gentamicin, Tetracycline, Erythromycin, and Penicillin). We arranged the data by the species of bacteria isolated, province of origin, year of sample processing and sensitivity test outcomes.

#### Statistical analysis

We looked for patterns in isolations as well as antibiotic resistance and sensitivity throughout the six years of data using heterogeneity G-tests (Sokal & Rohlf, 1995). All tests were performed in the software R. Tests were not performed for periods that had less than two years of data.

#### RESULTS

We examined 263 mastitis clinical case records and found 235 positives to bacterial isolation and sensitivity test. Thirteen cases corresponded to Atlántida, seven to Choluteca, 13 to Colón, 11 to Comayagua, 15 to Cortés, 19 to El Paraíso, 78 to Francisco Morazán, three to Intibucá, 40 to La Paz, 17 to Olancho, 16 to Santa Bárbara and three to Yoro.

The more frequently isolated bacteria taxon was *Staphylococcus* spp. (168 cases) followed by *Strepto-coccus* spp. (31 cases), *Corynebacterium* spp. (14 cases), *Escherichia* spp. (13 cases), *Klebsiella* sp. (five cases), *Enterobacter* sp. (one case), *Pasteurella* sp. (one case) and *Pseudomonas* sp. (one case). We found antibiotic resistance cases to practically all tested antibiotics. The sensitivity and resistance patterns shown by the four most isolated taxa to 15 common use antibiotics from 2013 to 2018 are shown in tables I to IV. The summation of resistance and sensitivity frequencies for these taxa is shown in **Table V**.

In general, patterns in resistance or sensitivity were infrequent. However, for the most isolated taxon (*Staphylococcus* spp.), we observed just a few patterns and these patterns did not show any strong tendency towards sensitivity or resistance (**Table I**). For the least isolated taxa, the observed patterns are probably not conclusive.

#### DISCUSSION

The genus *Staphylococcus* is a common etiologic agent of intramammary infection in cows (Sutra & Poutrel, 1994). Several species have been identified in clinical and subclinical cases (Hosseinzadeh & Dastmalchi-Saei, 2014; Rall et al., 2014). Their presence is ubiquitous in the environment, as well as the skin and mucous membranes of cows, so it can easily invade the nipple and the mammary gland (Dego et al., 2002). According to our data, *Staphylococcus* spp. were involved in 168 of 234 mastitis records. Furthermore, 685 of the 1836 antibiotic sensitivity tests performed for this taxon yielded resistance outcomes.

Although our data are probably not representative of the Honduran dairy cow population, they do suggest that antibiotic treatment of mastitis caused by *Staphylococcus* spp. could fail 37% of the time. Antibiotic resistance of *Staphylococcus* spp has been previously reported in Algeria (Saidi et al., 2019), Argentina (Gentilini et al., 2000), Brazil (Coelho et al., 2009), Germany (Lüthje & Schwarz, 2006), Iran (Jamali et al., 2014), Korea (Moon et al., 2007), Switzerland (Frey et al., 2013) and Turkey (Güler et al.,

Table I. Antibiotic sensitivity and resistance frequencies of *Staphylococcus* spp. isolated from bovine mastitis cases submitted to the Honduran Institute of Medical Veterinary Research (IHIMV), 2013 to 2018 (Sensibilidad frente antibióticos y frecuencias de resistencia de *Staphylococcus* spp. aisladas de casos de mastitis bovina presentadas al Instituto Hondureño de Investigaciones Médico Veterinarias (IHIMV), 2013 a 2018).

No.	Antibiotic		2013	2014	2015	2016	2017	2018	Total
		S	7	12	0	6	26	20	72
1	Amoxicillin + Clavulanic Acid**	IS	0	0	0	0	0	0	0
		R	2	13	11	3	9	4	42
		S	6	8	0	0	14	21	49
2	Ampicillin**	IS	0	0	0	0	0	0	0
		R	20	36	11	0	22	9	100
		S	14	8	7	5	19	27	82
3	Ceftiofur**	IS	0	10	4	3	0	3	20
		R	7	18	0	2	7	0	34
		S	16	20	0	0	12	20	69
1	Ceftriaxone**	IS	0	16	11	0	0	4	31
		R	8	6	0	0	6	2	22
		S	0	5	0	0	0	0	5
5	Cefuroxime <sup>⊥</sup>	IS	0	9	0	0	0	1	10
		R	0	0	0	0	0	0	0
		S	13	24	5	10	23	11	87
6	Enrofloxacin*	IS	3	3	0	0	0	3	9
		R	4	2	0	0	0	2	8
		S	- 16	15	0	0	14	20	66
7	Ciprofloxacin∔	IS	2	2	0	0	1	1	6
	Olpronoxaonte	R	8	15	0	0	4	4	31
		S	6	20	7	7	- 19	- 19	80
3	Oxacillin**	IS	0	20	0	0	0	0	2
,	C Addinin	R	17	21	0	3	12	7	60
		S	2	13	3	7	14	, 13	53
)	Doxycycline**	IS	0	3	8	0	1	0	13
,	Doxyoyomio	R	3	8	0	3	10	3	27
		S	7	16	0	4	14	10	51
10	Neomycin**	IS	0	9	7	4	2	0	23
	Noonyon	R	15	17	0	0	5	5	42
		S	17	27	11	9	23	17	106
11	Trimethoprim + Sulfamethoxazole**	IS	0	5	0	0	0	0	5
		R	8	6	0	1	8	10	33
		S	0 16	29	3	10	29	27	114
12	Gentamicin**	IS	10	1	0	0	0	0	2
	Gentamon	R	10	20	4	0	1	3	38
		S	14	20	3	5	18	13	74
13	<b>Tetracycline</b> <sup>↓</sup>	IS	0	7	0	2	4	4	17
10	Totradyonne.	R	10	22	8	3	- 10	6	60
		S	6	22 17	0	3 1	7	16	48
14	Erythromycin**	IS	3	7	11	4	2	8	40 36
14	Liyunoniyoni	R	3 17	23	0	4 1	2 8	о З	50 52
		к S							
4.5	Dec: 10110-000		4	11	0	3	13	17	48
15	Penicillin**	IS	0	4	0	0	2	0	6

Note: S = sensitive, IS = intermediate sensitivity, R = resistant,  $\frac{1}{2}$  = pattern (homogeneity), \*= significant heterogeneity, \*\*= highly significant heterogeneity.

**Table II.** Antibiotic sensitivity and resistance frequencies of *Streptococcus* spp. isolated from bovine mastitis cases submitted to the Honduran Institute of Medical Veterinary Research (IHIMV), 2013 to 2018 (Sensibilidad frente antibióticos y frecuencias de resistencia de *Streptococcus* spp. aisladas de casos de mastitis bovina presentadas al Instituto Hondureño de Investigaciones Médico Veterinarias (IHIMV), 2013 a 2018).

No.	Antibiotic		2013	2014	2015	2016	2017	2018	Tota
		S	8	3		1	5	5	22
	Amoxicillin + Clavulanic Acid	IS	0	0		0	0	0	0
		R	3	0		2	0	0	5
		S	6	1		1	5	1	14
2	Ampicillin	IS	1	0		0	1	3	5
		R	4	2		0	0	2	8
		S	7	1		2	3	6	19
3	Ceftiofur <sup>□</sup>	IS	2	0		0	0	0	2
		R	0	2		0	0	0	2
		S	11	1		0	4	0	16
ŀ	Ceftriaxone**	IS	0	4		0	0	3	7
		R	0	0		0	0	1	1
		S	6	1		0	0	0	7
5	Cefuroxime	IS	2	0		0	0	2	4
		R	3	0		0	0	0	3
		S	7	0		2	2	0	11
6	Enrofloxacin□	IS	2	0		1	1	0	4
		R	2	2		0	0	0	4
		S	5	1		0	3	0	9
,	Ciprofloxacin*	IS	2	0		0	0	3	5
		R	4	2		0	0	0	6
		S	9	1		0	5	0	15
3	Oxacillin*	IS	0	0		0	0	0	0
		R	2	2		3	1	5	13
		S	5	1		1	3	2	12
)	Doxycycline	IS	0	0		0	0	0	0
		R	4	2		2	0	3	11
		S	0	1		0	3	0	4
0	Neomycin	IS	0	2		0	0	0	2
		R	0	2		1	1	5	9
		S	4	0		0	5	0	9
1	Trimethoprim + Sulfamethoxazole**	IS	2	0		0	1	0	3
		R	5	4		3	0	6	18
		S	11	1		2	5	0	19
2	Gentamicin**	IS	0	0		0	0	1	1
		R	0	2		1	1	5	9
		S	5	3		0	0	2	10
3	Tetracycline**	IS	0	1		0	5	0	6
		R	6	1		3	1	4	15
		S	4	2		1	3	3	13
14	Erythromycin <sup>□</sup>	IS	2	1		0	0	2	5
	-	R	5	2		2	0	1	10
		S	3	3		0	5	1	12
15	Penicillin**	IS	4	0		0	0	0	4
		R	4	2		3	0	5	4 14

Table III. Antibiotic sensitivity and resistance frequencies of <i>Corynebacterium</i> sp. isolated from bovine mastitis cases submitted
to the Honduran Institute of Medical Veterinary Research (IHIMV), 2013 to 2018 (Sensibilidad a los antibióticos y frecuencias de resistencia
de Corynebacterium sp. aisladas de casos de mastitis bovina presentadas al Instituto Hondureño de Investigaciónes Médico Veterinarias (IHIMV), 2013 a 2018).

No.	Antibiotic		2013	2014	2015	2016	2017	2018	Total
		S	1			0	0	20	4
	Amoxicillin + Clavulanic Acid**	IS	0			0	0	0	0
		R	0			6	0	4	0
		S	1			0	0	21	3
2	Ampicillin∔	IS	0			0	0	0	0
		R	0			0	0	9	2
		S	0			0	0	27	5
}	Ceftiofur**	IS	0			4	0	3	0
		R	0			2	0	0	0
	C - their and	S	1			0	0	20	4
1	Ceftriaxone↓	IS	0			0	0	4	0
		R S	0			0	0	2	0 0
	Cefuroxime↓	IS	0 0			0 0	0 0	0 1	0
5	Celuloxime	R	0			0	0	0	0
		S	0			4	2	11	4
6	Enrofloxacin∔	IS	1			4 2	0	3	4 0
,	Linoloxaolin	R	0			0	0	2	0
		S	1			0	0	20	4
7	Ciprofloxacin <sup>↓</sup>	IS	0			0	0	1	0
	Cipronovadini	R	0			0	0	4	0
		S	0			0	0	19	0
3	Oxacillin*	IS	0			0	0	0	0
		R	0			4	2	7	2
		S	0			0	0	13	3
)	Doxycycline*	IS	0			0	0	0	0
	, , , ,	R	0			6	0	3	0
		S	0			2	2	10	2
10	Neomycin∔	IS							
0			0			0	0	0	1
		R	0			0	0	5	0
		S	0			4	0	17	4
1	Trimethoprim + Sulfamethoxazole <sup>‡</sup>	IS	0			0	0	0	0
		R	1			2	2	10	1
		S	11	1		2	5	0	19
2	Gentamicin**	IS	0	0		0	0	1	1
		R	0	2		1	1	5	9
		S	5	3		0	0	2	10
3	Tetracycline**	IS	0	1		0	5	0	6
	-	R	6	1		3	1	4	15
		S	4	2		1	3	3	13
4	Erythromycin∔	IS	2	1		0	0	2	5
	· ·	R	5	2		2	0	1	10
		S	3	3		0	5	1	12
15	Penicillin**	IS	4	0		0	0	0	4
		R	4	2		3	0	5	- 14
	sensitive, IS = intermediate sensitivity, R = resista								

Table IV. Antibiotic sensitivity and resistance frequencies of *Escherichia* spp. isolated from bovine mastitis cases submitted to the Honduran Institute of Medical Veterinary Research (IHIMV), 2013 to 2018 (Sensibilidad a los antibióticos y frecuencias de resistencia de *Escherichia* spp. aisladas de casos de mastitis bovina presentadas al Instituto Hondureño de Investigaciones Médico Veterinarias (IHIMV), 2013 a 2018).

No.	Antibiotic		2013	2014	2015	2016	2017	2018	Total
	Amoxicillin + Clavulanic Acid <sup>↓</sup>	S	5	0			1		6
		IS	0	0			0		0
		R	0	1			0		1
	Ampicillin*	S	0	3			1		4
		IS	0	0			0		0
		R	5	2			0		7
3	Ceftiofur↓	S	0	0			0		0
		IS	0	3			0		3
		R	0	0			0		0
	Ceftriaxone <sup>↓</sup>	S	5	5			1		11
		IS	0	0			0		0
		R	0	2			0		2
5	Cefuroxime <sup>↓</sup>	S	2	3			0		5
		IS	0	0			0		0
		R	0	0			0		0
6	Enrofloxacin <sup>↓</sup>	S	5	2			0		7
		IS	0	0			0		0
		R	0	0			0		0
	Ciprofloxacin <sup>↓</sup>	S	2	3			1		6
		IS	0	0			0		0
		R	0	2			0		2
	Oxacillin∔	S	5	1			0		6
		IS	0	0			0		0
		R	0	2			0		2
I	Doxycycline*	S	2	3			0		5
		IS	0	4			0		4
0	Neomycin <sup>‡</sup>	R S	3 0	0 2			0		3 2
0	Neomych*						0		
		IS	2	5			1		8
	<b>T</b>	R	0	0			0		0
1	Trimethoprim + Sulfamethoxazole**	S	0	4			1		5
		IS	2	0			0		2
		R	3	0			0		3
2	Gentamicin∔	S	5	3			1		9
		IS	0	0			0		0
		R	0	0			0		0
3	Tetracycline <sup>↓</sup>	S	0	5			0		5
		IS	0	0			0		0
		R	5	2			1		8
4	Erythromycin↓	S	0	3			0		3
		IS	0	0			0		0
		R	0	4			0		4
5	Penicillin <sup>‡</sup>	S	0	1			0		1
		IS	0	0			0		0
		R	0	2			0		2

Note: S = sensitive, IS = intermediate sensitivity, R = resistant,  $\downarrow$  = pattern (homogeneity), \* = significant heterogeneity, \*\* = highly significant heterogeneity.

Table V. Antibiotic sensitivity and resistance number of outcomes for the more frequently isolated taxa frombovine mastitis cases submitted to the Honduran Institute of Medical Veterinary Research (IHIMV), 2013 to2018 (Sensibilidad a los antibióticos y número de resistencia de los resultados para los taxones más frecuentemente aislados de loscasos de mastitis bovina presentados al Instituto Hondureño de Investigaciones Médico Veterinarias (IHIMV), 2013 a 2018).

			Taxon				
Antibiotic	Challenge outcome	Staphylococcus	Streptococcus	Corynebacterium	Escherichia		
Amoxicillin + Clavulanic Acid	S	72	22	5	6		
	IS	0	0	0	0		
	R	42	5	6	1		
Ampicillin	S	49	14	4	4		
	IS	0	5	0	0		
	R	100	8	2	7		
Ceftiofur	S	82	19	5	0		
	IS	20	2	4	3		
	R	34	2	2	0		
Ceftriaxone	S	69	16	5	11		
	IS	31	7	0	0		
	R	22	1	0	2		
Cefuroxime	S	5	7	0	5		
	IS	10	4	0	7		
	R	0	3	0	0		
Enrofloxacin	S	87	11	10	7		
	IS	9	4	3	0		
	R	8	4	0	0		
Ciprofloxacin	S	66	9	5	6		
	IS	6	5	0	0		
	R	31	6	0	2		
Oxacillin	S	80	15	0	6		
	IS	2	0	0	0		
	R	60	13	8	2		
Doxycycline	S	53	12	3	5		
	IS	13	0	0	4		
	R	27	11	6	3		
Neomycin	S	51	4	6	2		
	IS	23	2	1	8		
	R	42	9	0	0		
Trimethoprim + Sulfamethoxazole	S	106	9	8	5		
	IS	5	3	0	2		
	R	33	18	6	3		
Gentamicin	S	114	19	8	9		
	IS	2	1	1	0		
	R	38	9	3	0		
Tetracycline	S	74	10	2	5		
	IS	17	6	1	0		
	R	60	15	8	8		
Erythromycin	S	48	13	1	3		
	IS	36	5	0	0		
	R	52	10	9	4		
Penicillim	S	48	12	1	1		
Note: S = sensitive, IS = intermediat	IS	6	4	1	0		

2005) among others. The high frequencies of resistance showed by this taxon in this study to ampicillin, ciprofloxacin, oxacillin, doxycycline, neomycin, and tetracycline is noteworthy, and had not been previously reported.

*Streptococcus* spp., the second most frequently isolated taxon in this study, is also one of the common causative agents of mastitis in cows (Oikonomou et al., 2012). Three species (*S. agalactiae, S. dysgalactiae,* and *S. uberis*) have been described as the most frequently isolated (Baseggio et al., 1997). As for antibiotic resistance, there are reports in China (Gao et al., 2012), Egypt (El-Jakee et al., 2013), France (Guérin-Faublée et al., 2002), Germany (Minst et al., 2012), New Zealand (McDougall et al., 2013), Switzerland (Corti et al., 2003) and others, although none of these have reported the high frequencies of resistance to enrofloxacin, ciprofloxacin, oxacillin, doxycycline, neomycin, trimethoprim + sulfamethoxazole, and erythromycin seen in our study.

*Corynebacterium* spp. and *Escherichia coli* were less frequent in our study. Nevertheless, the resistance to oxacillin, doxycycline, trimethoprim + sulfamethoxazole, tetracycline, erythromycin, and penicillin, and the sensitivity to enrofloxacin in both taxa was evident.

The high frequencies of antibiotic resistance observed in this study, in contrast to the low frequencies observed in other countries like Finland (Pitkälä et al., 2004), France (Botrel et al., 2010), Portugal (Rato et al., 2013) may be due to cultural differences in mastitis management practices. The improper and uncontrolled use of antibiotics has probably contributed to the observed resistance of mastitiscausing bacteria. Dairy farmers in Honduras, face a rising challenge to successfully treat mastitis. Furthermore, the massive and routine use of antibiotics and the proliferation of resistant bacteria strains could lead to unknown impacts on dairy product consumers. Bando et al. (2009), found over 41% prevalence of antibiotic residues in pasteurized milk samples in Paraná, Brazil and this could be a reality in other countries where mastitis caused by antibiotic-resistant bacteria is common.

#### CONCLUSIONS

The more frequently isolated taxa were *Staphylococcus* spp., followed by *Streptococcus* spp., *Corynebacterium* spp., and *Escherichia* spp. We found resistance cases to practically all the tested antibiotics (even the broad-spectrum ones), however, resistance patterns were not common throughout the evaluated period. More research needs to be done to determine the mastitis-causing bacteria in Honduras to the species level and to better understand the ecological relationship between bacterial species and dairy cows.

### ACKOWLEGMENTS

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