Journal of Dairy Research (2008) **75** 491–496. © *Proprietors of Journal of Dairy Research 2008*
doi:10.1017/S0022029908003567 *Printed in the United Kingdom*

Pasteurized whole milk confers reduced susceptibilities to the antimicrobial agents trimethoprim, gatifloxacin, cefotaxime and tetracycline via the *marRAB* locus in *Escherichia coli*

Yang Peng, Ricardo L Hernandez, Robert R Crow, Suzanna E Jones, Sara A Mathews, Ayanna M Arnold, Eliseo F Castillo, Jennifer M Moseley and Manuel F Varela*

Department of Biology, Eastern New Mexico University, Portales, New Mexico 88130 USA

Received 22 June 2006, revised form 5 May 2008; accepted for publication 12 June 2008

We inoculated pasteurized whole milk with *Escherichia coli* strains GC4468 (intact *marRAB* locus), JHC1096 (Δ *marRAB*), or AG112 (Δ *marR*), and incubated each overnight at 37 °C. All strains were then recovered from the milk cultures, and susceptibilities to antimicrobial agents were determined by the E-test strip method (CLSI). Cells of strain GC4468, prior to culturing in milk, were susceptible to trimethoprim, gatifloxacin, cefotaxime and tetracycline. After culturing GC4468 in pasteurized milk, however, the minimal inhibitory concentrations (MICs) increased 1·4-fold for trimethoprim ($P \leqslant 0{\cdot}05$), 1·5-fold for gatifloxacin ($P \leqslant 0{\cdot}05$), 2·0-fold for cefotaxime ($P = 0{\cdot}008$), and 1·4-fold for tetracycline ($P \geqslant 0{\cdot}05$). After culturing GC4468 on milk count agar the MICs were enhanced 3·4-fold for trimethoprim ($P \leqslant 0{\cdot}05$), 10-fold for gatifloxacin ($P = 0{\cdot}001$), 7·1-fold for cefotaxime ($P = 0{\cdot}011$), and 40·5-fold for tetracycline ($P = 0{\cdot}074$), but exhibiting tetracycline resistance with a mean MIC of 74·7 ± 18·47 μg/ml (CLSI). The MICs of the antimicrobial agents for JHC1096 cells after culturing in pasteurized whole milk were indistinguishable ($P \geqslant 0{\cdot}05$) from baseline MICs measured before culturing in the same type of milk. Thus, *Esch. coli* cells harbouring the *marRAB* locus exhibit reduced susceptibilities to multiple antimicrobial agents after culturing in pasteurized whole milk.

Keywords: Antimicrobial agent, bacteria, milk, *marRAB* locus, susceptibility.

Infectious diseases caused by bacteria that are resistant to antimicrobial agents are a serious public health concern (Neu, 1992 Barbosa & Levy, 2000b; Levy & Marshall, 2004). Members of the Enterobacteriaceae family of bacteria are causative agents of infectious disease, and their resistance to antimicrobial agents compromises chemotherapeutic efforts (Paterson, 2002). Antimicrobial agents are used in agriculture for the treatment of infection, prophylaxis and growth promotion (Levy, 2002; Levy & Marshall, 2004).

Bacterial resistance to antimicrobial agents may be of clinical significance due to dissemination of pathogenic bacteria through a population of food animals (McDermott et al. 2002; Angulo et al. 2004; Silbergeld et al. 2008). In isolated instances, outbreaks of food-borne infectious disease from contaminated milk and occurrences of milk-derived isolates of members from the Enterobacteriaceae family of bacteria have been studied (Gillespie et al. 2003). Although relatively well-studied within dairy cattle (Hershberger et al. 2005), bacteria that are resistant to antimicrobial agents in other dairy farm environments, such as soil (Burgos et al. 2005), water (Biyela et al. 2004) and milk (Makovec & Ruegg, 2003) are less well-characterized. Dissemination mechanisms for drug resistant bacterial pathogens within dairy farms are also poorly understood (Hershberger et al. 2005).

The *marRAB* locus is a well studied genetic element in *Escherichia coli* and mediates bacterial resistance to multiple antimicrobial agents such as β-lactams, chloramphenicol, quinolones, and tetracycline (Randall & Woodward, 2002). Several organic compounds are known to modulate resistance to antimicrobial agents via the *marRAB* locus (Alekshun & Levy, 1999). Previously, milk-containing foods were shown to modulate the *marRAB* locus (Rickard et al. 2004); the effects of milk, however, were not directly examined. Thus, we tested the hypothesis that milk confers reduced susceptibilities to antimicrobial agents in *Esch.*

*For correspondence; e-mail: Manuel.Varela@enmu.edu

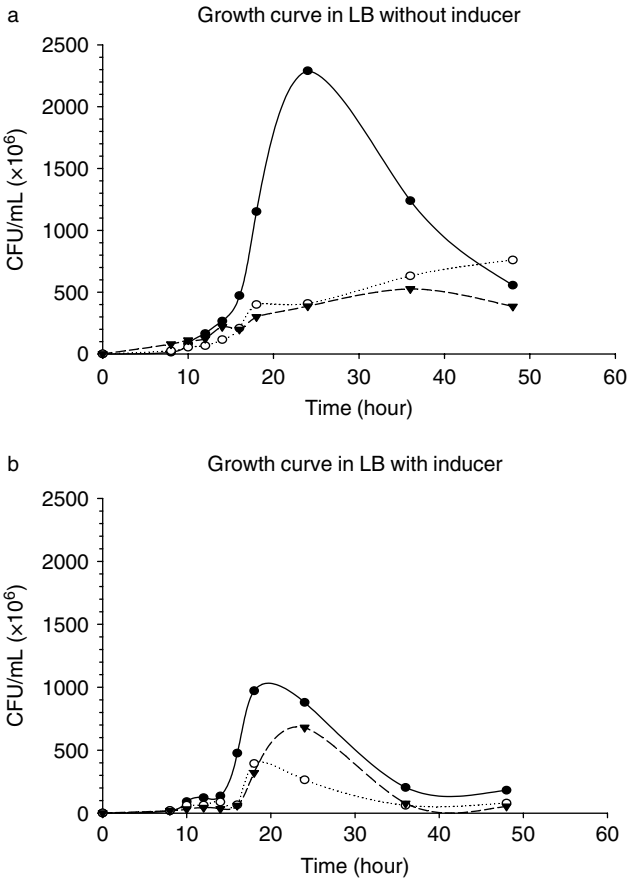

Fig. 1. Effects of salicylate on bacterial growth (CFU) in LB broth.

Esh. coli cells of strains AG112 (filled circles), JHC1096 (empty circles) and GC4468 (filled triangles) were incubated in LB broth without inducer (i.e., no salicylate), panel A, or in LB broth with inducer (5 mM-salicylate), panel B. See methods for details

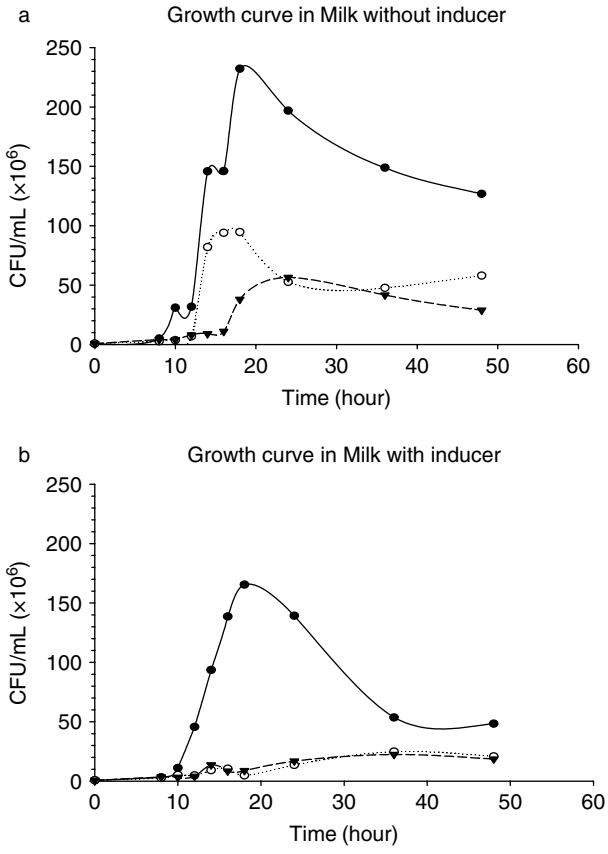

Fig. 2. Effects of salicylate on bacterial growth (CFU) in milk.

Esch. coli cells of strains AG112 (filled circles), JHC1096 (empty circles) and GC4468 (filled triangles) were incubated in pasteurized milk without inducer (i.e., no salicylate), panel A, or in pasteurized milk containing inducer (5 mM-salicylate), panel B. See methods for details

coli cells harboring the *marRAB* locus. The objective of this study was to examine the relationship between the culturing of *Esch. coli* in milk and the subsequent development of resistance to antimicrobial agents. Here, we found that upon culturing in pasteurized milk, antimicrobial agent-sensitive *Esch. coli* becomes less susceptible to multiple antimicrobial agents, a phenotype conferred by the *marRAB* locus.

Materials and Methods

Bacterial strains

Esch. coli K-12 bearing a deletion of the *marRAB* operon (strain JHC1096), was a generous gift from Dr. Jean Greenberg (Harvard University, MA; Greenberg et al. 1991). The *Esch. coli* strain harbouring a deletion of *marR* (strain AG112) (George & Levy, 1983; Moken et al. 1997), and a wild-type strain harbouring an intact *marRAB* locus (strain GC4468) (Carlioz & Touati, 1986; Oethinger et al. 1998) were generous gifts from the laboratory of Dr. Stuart Levy (Tufts University, MA).

Growth determination in Luria-Bertani broth and milk

Cells JHC1096, AG112, and GC4468 were grown overnight at 37 °C while shaking in Luria-Bertani (LB) broth (Difco), which is routinely used for maintenance and propagation of *Esch. coli* (Luria et al. 1960). Then 20 µl of culture was added to 20 ml fresh LB broth and grown to the mid-log phase of growth ($O.D._{600}$=0·35). Next, 10 µl of the mid-log culture was used to inoculate 10 ml of fresh LB broth or commercially available pasteurized whole milk. This procedure was repeated as above except that the LB broth and the pasteurized whole milk each contained 5 mM-Salicylate. The cultures were incubated while shaking at 37 °C for the times indicated in Figs. 1 & 2. Samples (10 µl) of the cultures were removed at the time points indicated, plated onto MacConkey agar, and incubated for 12 h. Colony forming units (CFU/ml) were then determined. The means plotted were the results of 4 independent experiments, each in triplicate.

https://doi.org/10.1017/S0022029908003567 Published online by Cambridge University Press

Antimicrobial susceptibility testing

Prior to antimicrobial susceptibility testing via the E-Test assay using current CLSI guidelines (2006), *Esch. coli* cells JHC1096, AG112, and GC4468 were treated with LB broth (Difco) with and without 5 mM-salicylate, commercially available pasteurized whole milk, or milk count agar (OXIOD) in the following manner: *Esch. coli* strains JHC1096, AG112 and GC4468 were inoculated in LB Broth (Difco), incubated overnight at 37 °C with aeration and then used to inoculate refresher cultures in LB broth (Difco) for growth to the mid-log phase ($O.D._{600} \approx 0{\cdot}35$). The mid-log cells were used for incubation in LB broth (Difco) (with and without 5 mM-salicylate) at 37 °C with aeration for 8 h or for incubation in commercially available pasteurized whole milk at 30 °C with aeration for 8 h. An additional 10 ml pasteurized whole milk was inoculated with 10 µl sterile distilled water as a negative control. For the treatment in LB broth (Difco) with or without 5 mM-salicylate, a total of 50 µl of each culture was plated onto LB agar medium (Difco) plates and incubated overnight at 37 °C. For the treatment in pasteurized whole milk, a total of 50 µl of each culture was plated onto either LB agar (Difco) or milk count agar (OXIOD) and incubated at 30 °C for 24 h.

For the antimicrobial agent susceptibility measurements, three single colonies of each strain were randomly picked from each of the plates, resuspended in 1 ml per colony of 0·85% NaCl and each immediately spread uniformly onto cation-adjusted Muller-Hinton II Agar (Difco) plates or Milk Count Agar. A portion of the 0·85% NaCl resuspension (0·1 ml) was plated onto LB agar, and cells were identified by BD BBL Crystal Enteric/Nonfermentor Identification Kits (Becton Dickinson Microbiology Systems, Cockeysville, MD) according to the manufacturer specifications. E-Test strips (AB Biodisk) were placed on the surface as per the manufacturer's instructions, and the plates were incubated at 37 °C for 16 h. The minimal inhibitory concentrations (MICs) were read and interpreted according to current CLSI guidelines (2006). The means ± SD (ranges) were the result of three independent experiments, each in triplicate, $n=3$. Break points for susceptibility interpretations were MICs $\leqslant 8$ µg/ml for trimethoprim, $\leqslant 2$ µg/ml for gatifloxacin, $\leqslant 8$ µg/ml for cefotaxime, and $\leqslant 4$ µg/ml for tetracycline. The MIC break point for resistance to tetracycline was $\geqslant 16$ µg/ml.

Statistics

All data represent the mean plus or minus the standard deviation and range of at least three independent experiments ($n=3$) conducted under standardized conditions. The nonparametric Holm-Sidak method was used to determine the significance of mean MIC data compared with mean baseline MICs within each strain. Results were considered significant at $P \leqslant 0{\cdot}05$.

Results and Discussion

Baseline determinations of antimicrobial susceptibilities

Prior to culturing *Esch. coli* strains GC4468 (intact *marRAB* locus), JHC1096 (*marRAB* deleted) AG112 (*marR* deleted) in pasteurized whole milk or milk count agar, the minimal inhibitory concentrations (MICs) were determined by the standard E-test method (2006). All three *Esch. coli* strains were susceptible to the antimicrobial agents trimethoprim, gatifloxacin, cefotaxime and tetracycline (Table 1). These antimicrobial agents represent distinct drug classes and have different mechanisms of actions (McDermott et al. 2003). These agents also have significance in clinical therapy, such as trimethoprim (Juckett, 1999; Casewell et al. 2003), gatifloxacin (Lutsar et al. 1999), and cefotaxime (Wittmann et al. 1997) or significance in agriculture for the purposes of growth promotion or prophylaxis in dairy calves, such as tetracycline (Constable & Morin, 2002).

Antimicrobial susceptibilities after culturing in pasteurized milk and milk count agar

We found that after culturing *Esch. coli* GC4468 (intact *marRAB* locus) in pasteurized milk, the antimicrobial agent MICs increased by 1·4-fold for trimethoprim ($P=0{\cdot}05$), by 1·5-fold for gatifloxacin ($P \leqslant 0{\cdot}05$), and by 2·0-fold for cefotaxime ($P=0{\cdot}008$), as shown in Table 1. The 1·4-fold increase in mean MIC for tetracycline was not significantly different in GC4468 ($P \geqslant 0{\cdot}05$). The mean MIC in AG112 (*marR* deleted) increased ($P=0{\cdot}003$) by 2·8-fold for trimethoprim, but not for gatifloxacin, cefotaxime, and tetracycline ($P=0{\cdot}10$). The MICs for the same antimicrobial agents in *Esch. coli* AG112 were not significantly different ($P \geqslant 0{\cdot}05$) compared with the MIC values obtained prior to culturing in milk and milk count agar, implying that the *marRAB* locus in AG112 was already intrinsically induced, as expected, and that another inducer (milk) would not further modulate susceptibility. The MICs of the antimicrobial agents tested in this study (ranged between 0·01 and 0·017 µg/ml) for cells of JHC1096 (*marRAB* locus deleted) after culturing in pasteurized whole milk were indistinguishable ($P \geqslant 0{\cdot}05$) from the MICs determined prior to culturing (Table 1) in the same type of milk.

As shown in Table 1, we found that after culturing *Esch. coli* GC4468 on milk count agar, the antimicrobial agent MICs increased by 3·4-fold for trimethoprim ($P=0{\cdot}036$), by 10-fold for gatifloxacin ($P=0{\cdot}001$), by 7·1-fold for cefotaxime ($P=0{\cdot}011$), and by 40·5-fold for tetracycline ($P=0{\cdot}074$) compared with the mean MICs derived prior to culturing on milk count agar. Interestingly, GC4468 cells on milk count agar with a mean MIC 74·67 µg/ml are considered resistant to tetracycline, if not susceptible. As expected, cells of AG112 (*marR* deleted) were not less susceptible to the antimicrobial agents tested compared with baseline MICs ($P \geqslant 0{\cdot}05$), regardless of whether milk or milk count agar was used, further implying that within induced cells, susceptibilities are not further reduced. For

https://doi.org/10.1017/S0022029908003567 Published online by Cambridge University Press

Table 1. Minimal inhibitory concentrations of antimicrobial agents for *Esch. coli* in pasteurized milk, milk count agar or salicylate

MIC values (CLSI) are presented as the mean ± SD (range), $n=3$. See methods for details

		Antimicrobial Agent MICs (μg/ml)			
Esch. coli Strains	Variables	Trimethoprim	Gatifloxacin	Cefotaxime	Tetracycline
GC4468 (wild-type *marRAB*)	Baseline MIC	0·33 ± 0·046 (0·3–0·38)	0·01 ± 0·001 (0·01–0·012)	0·053 ± 0·003 (0·05–0·055)	1·83 ± 0·288 (1·5–2·0)
	Milk	0·46 ± 0·069* (0·38–0·5)	0·0147 ± 0·0023* (0·012–0·016)	0·104 ± 0·018*** (0·094–0·125)	2·5 ± 0·5 (2·5–3·0)
	Milk Count Agar	1·12 ± 0·44* (0·75–1·6)	0·10 ± 0·019** (0·09–0·125)	0·38 ± 0·125* (0·25–0·5)	74·67 ± 18·47 (64–96)
	Salicylate	0·5 ± 0* (0·5–0·5)	0·021 ± 0·0017 (0·02–0·023)	0·104 ± 0·018*** (0·094–0·125)	2·17 ± 0·288 (2·0–2·5)
JHC1096 (deleted *marRAB*)	Baseline MIC	0·25 ± 0 (0·25–0·25)	0·0126 ± 0·0006 (0·012–0·013)	0·064 ± 0 (0·064–0·064)	1·67 ± 0·288 (1·5–2·0)
	Milk	0·35 ± 0·046 (0·3–0·38)	0·017 ± 0·0023 (0·016–0·02)	0·061 ± 0·005 (0·055–0·064)	2 ± 0 (2·0–2·0)
	Milk Count Agar	0·42 ± 0·069 (0·38–0·5)	0·063 ± 0·0057*** (0·06–0·07)	0·0447 ± 0·004 (0·04–0·047)	1·42 ± 0·144 (1·25–1·5)
	Salicylate	0·25 ± 0·128 (0·125–0·38)	0·011 ± 0·0017 (0·008–0·012)	0·042 ± 0·0087 (0·032–0·047)	1·67 ± 0·288 (1·5–2·0)
AG112 (deleted *marR*)	Baseline MIC	0·34 ± 0·04 (0·3–0·38)	0·017 ± 0·002 (0·016–0·02)	0·0997 ± 0·023 (0·08–0·0125)	213·33 ± 36·95 (192–256)
	Milk	0·916 ± 0·144*** (0·75–1·0)	0·037 ± 0·0086 (0·032–0·047)	0·147 ± 0·0375 (0·125–0·19)	256 ± 0 (256–256)
	Milk Count Agar	0·75 ± 0 (0·75–0·75)	0·069 ± 0·009 (0·064–0·08)	0·25 ± 0 (0·25–0·25)	256 ± 0 (256–256)
	Salicylate	0·75 ± 0 (0·75–0·75)	0·037 ± 0·0086 (0·032–0·047)	0·115 ± 0·018 (0·094–0·125)	256 ± 0 (256–256)

*$P \leqslant 0{\cdot}05$, **$P \leqslant 0{\cdot}01$ and ***$P \leqslant 0{\cdot}001$ compared to baseline MICs for the same *Esch. coli* strain

instance, AG112 cells were considered tetracycline resistant regardless of the variable tested (Table 1), as would be expected if the *marRAB* locus was induced. The MICs of the antimicrobial agents for JHC1096 cells (ranging between 0·01–0·017 μg/ml) after culturing in pasteurized whole milk or milk count agar were indistinguishable ($P \geqslant 0{\cdot}05$) from the MICs determined prior to culturing (Table 1) in the same milk-type, except for an observed 5-fold increase in the MIC of gatifloxacin ($P < 0{\cdot}05$) for JHC1096 cultured on milk count agar (but not pasteurized milk) compared with the baseline MIC in gatifloxacin. Prior to culturing in milk or milk agar, *Esch. coli* strains GC4468 and JHC1096 were susceptible to all antimicrobial agents tested. The reductions in antimicrobial agent susceptibilities in milk were more pronounced in GC4468 after sub-culturing on milk count agar compared with sub-culturing in pasteurized milk. We conclude that culturing in pasteurized milk or milk agar, *Esch. coli* cells harboring an intact *marRAB* locus become less susceptible to multiple antimicrobial agents.

Measurement of antimicrobial susceptibilities with salicylate

As a positive control experiment for the induction of the *marRAB* locus, the antimicrobial agent MICs were determined in 5 mM-salicylic acid, a well-known *marRAB* locus inducer (Alekshun & Levy, 1999). As shown in Table 1, in the presence of salicylate, the MICs increased for trimethoprim ($P = 0{\cdot}01$) and cefotaxime ($P = 0{\cdot}008$) in GC4468 cells. AG112 cells (*marR* deleted) with or without salicylate had tetracycline MICs of 213 and 256 μg/ml, respectively, while JHC1096 cells (*marRAB* locus deleted) remained susceptible to tetracycline and the other antimicrobial agents tested, consistent with the known *marRAB* locus resistance properties (Alekshun & Levy, 1999).

The observation that the antimicrobial agent susceptibilities were unchanged in the *Esch. coli* strain that lacks MarR and which constitutively expresses MarA, a multiple antibiotic resistance activator (Hachler et al. 1991; Barbosa & Levy, 2000a), confirms the role of the *marRAB* locus in conferring reduced susceptibilities to antimicrobial agents by milk in GC468 cells, which possess an intact *marRAB* locus. The component within milk that modulates the *marRAB* locus remains to be identified. Although chromosomal elements are directly involved in the reduced antimicrobial susceptibilities observed here, we are nonetheless not able to definitively rule out the possibility that extra-chromosomal elements (i.e., plasmids, etc.) may be involved in naturally-derived environmental or agriculture bacterial isolates (Burgos et al.

https://doi.org/10.1017/S0022029908003567 Published online by Cambridge University Press

2005). Future studies are necessary to distinguish between these alternatives. Along these lines, we are presently examining the antimicrobial agent susceptibility profiles for previously published *Esch. coli* isolates from dairy soil (Burgos et al. 2005) and from dairy water for a new panel of antimicrobial agents.

Growth in LB medium and milk

The growth of *Esch. coli* strain AG112 cells reached higher growth plateaus when compared with those of GC4468 and JHC1096 strains in both LB broth and in milk (see Figs 1 & 2). All three strains, however, reach higher growth plateaux in LB broth than in milk. Our investigation of the relationship of *Esch. coli* growth indicates that the total growth yield in LB broth is better than that in liquid milk while the antimicrobial agent susceptibilities are reduced, which is consistent with the contention that milk itself, and not growth in milk, is the factor conferring the reduced susceptibilities that we observe here.

In summary, pasteurized milk or milk agar media confers reduced susceptibilities to multiple antimicrobial agents in *Esch. coli*. Our studies provide evidence to support the contention that the mode of acquisition of reduced susceptibilities to antimicrobial agents by *Esch. coli* in pasteurized milk is due to modulation of the *marRAB* locus. We examined *Esch. coli*, which is a model bacterium and member of the Enterobacteriaceae family of bacteria, because of its relevance to physiology, its biomedical role as a causative agent of infectious disease and its antimicrobial agent resistance properties, which in turn compromise therapy (Sanders & Sanders, 1992; Baquero et al. 1998; Alekshun & Levy, 2006). *Esch. coli* microorganisms have profound significance as a tool for molecular biology, microbial genetics, and as indicators of environmental sewage contamination, plus pathogenic *Esch. coli* variants have considerable clinical importance (Neidhardt, 1996). Because of their demonstrated transferability of antimicrobial agent resistance determinants among bacteria of distinct species (Neu, 1984; Nord, 1993; Leverstein-van Hall et al. 2003), human consumption of raw milk contaminated with multidrug resistant microorganisms may contribute to the dissemination of multidrug resistant bacteria in agriculture and could be a source of potentially untreatable zoonotic infectious disease (Tollefson et al. 1999; McEwen & Fedorka-Cray, 2002; Angulo et al. 2004). Lastly, because the *marRAB* locus is present in other members of the Enterobacteriaceae family of bacteria, such as *Enterobacter*, *Klebsiella*, *Shigella*, and *Salmonella* (Cohen et al. 1993), the findings presented here have relevance to these medically important microorganisms and their relationships with milk.

This publication was made possible by National Institutes of Health grants 1 R15 GM070562-01 and P20 RR016480, the latter of which is from the NM-INBRE program of the National Center for Research Resources, an internal research grant awarded by Eastern New Mexico University, and a contribution from Calton Research Associates in honor of George and Clytie Calton. E.F.C. was supported by NIH grants RR-16480, R25-GM56626 Bridges fellowship and by NSF grants NM-AGEP and HRD-0086701. R.R.C. was supported by an American Society for Microbiology Undergraduate Research Fellowship. We thank Dr. Sanath Kumar, Jamey F. Licata and Johnny Sena for expert technical assistance. We thank Dr. Jeffrey K. Griffith (University of New Mexico, Albuquerque, NM) and Dr. Tomofusa Tsuchiya (Okayama University, Okayama, Japan) for critical reading of the manuscript before publication.

References

Alekshun MN & Levy SB 1999 Alteration of the repressor activity of MarR, the negative regulator of the *Escherichia coli marRAB* locus, by multiple chemicals in vitro. *Journal of Bacteriology* **181**(15) 4669–4672.

Alekshun MN & Levy SB 2006 Commensals upon us. *Biochemical Pharmacology* **71**(7) 893–900

Angulo FJ, Baker NL, Olsen SJ, Anderson A & Barrett TJ 2004 Antimicrobial use in agriculture: controlling the transfer of antimicrobial resistance to humans. *Seminars in Pediatric Infectious Diseases* **15**(2) 78–85

Baquero F, Negri MC, Morosini MI & Blazquez J 1998 Antibiotic-selective environments. *Clinical Infectious Diseases* **27** (Suppl. 1) S5–11

Barbosa TM & Levy SB 2000a Differential expression of over 60 chromosomal genes in *Escherichia coli* by constitutive expression of MarA. *Journal of Bacteriology* **182**(12) 3467–3474

Barbosa TM & Levy SB 2000b The impact of antibiotic use on resistance development and persistence. *Drug Resistance Update* **3**(5) 303–311

Biyela PT, Lin J & Bezuidenhout CC 2004 The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. *Water Science Technology* **50**(1) 45–50

Burgos JM, Ellington BA & Varela MF 2005 Presence of multidrug-resistant enteric bacteria in dairy farm topsoil. *Journal of Dairy Science* **88**(4) 1391–1398

Carlioz A & Touati D 1986 Isolation of superoxide dismutase mutants in *Escherichia coli*: is superoxide dismutase necessary for aerobic life? *EMBO Journal* **5**(3) 623–630

Casewell M, Friis C, Marco E, McMullin P & Phillips I 2003 The European ban on growth-promoting antibiotics and emerging consequences for human and animal health. *Journal of Antimicrobial Chemotherapy* **52**(2) 159–161

Clinical and Laboratory Standards Institute 2006 Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard – Seventh Edition (M7-A7). Wayne PA

Cohen SP, Yan W & Levy SB 1993 A multidrug resistance regulatory chromosomal locus is widespread among enteric bacteria. *Journal of Infectious Diseases* **168**(2) 484–488

Constable PD & Morin DE 2002 Use of antimicrobial susceptibility testing of bacterial pathogens isolated from the milk of dairy cows with clinical mastitis to predict response to treatment with cephapirin and oxytetracycline. *Journal of the American Veterinary Medical Association* **221**(1) 103–108

George AM & Levy SB 1983 Amplifiable resistance to tetracycline, chloramphenicol, and other antibiotics in *Escherichia coli*: involvement of a non-plasmid-determined efflux of tetracycline. *Journal of Bacteriology* **155**(2) 531–540

Gillespie IA, Adak GK, O'Brien SJ & Bolton FJ 2003 Milkborne general outbreaks of infectious intestinal disease, England and Wales, 1992–2000. *Epidemiology and Infection* **130**(3) 461–468

Greenberg JT, Chou JH, Monach PA & Demple B 1991 Activation of oxidative stress genes by mutations at the *soxQ/cfxB/marA* locus of *Escherichia coli*. *Journal of Bacteriology* **173**(14) 4433–4439

Hachler H, Cohen SP & Levy SB 1991 *marA,* a regulated locus which controls expression of chromosomal multiple antibiotic resistance in *Escherichia coli*. *Journal of Bacteriology* **173**(17) 5532–5538

Hershberger E, Oprea SF, Donabedian SM, Perri M, Bozigar P, Bartlett P & Zervos MJ 2005 Epidemiology of antimicrobial resistance in enterococci of animal origin. *Journal of Antimicrobial Chemotherapy* **55**(1) 127–130

Juckett G 1999 Prevention and treatment of traveler's diarrhea. *American Family Physician* **60**(1) 119–124, 135–136

Leverstein-van Hall MA, Blok HEM, Donders ART, Paauw A, Fluit AC & Verhoef J 2003 Multidrug resistance among Enterobacteriaceae is strongly associated with the presence of integrons and is independent of species or isolate origin. *Journal of Infectious Diseases* **187**(2) 251–259

Levy SB 2002 The 2000 Garrod lecture. Factors impacting on the problem of antibiotic resistance. *Journal of Antimicrobial Chemotherapy* **49**(1) 25–30

Levy SB & Marshall B 2004 Antibacterial resistance worldwide: causes, challenges and responses. *Nature Medicine* **10** (Suppl. 12) S122–129

Luria SE, Adams JN & Ting RC 1960 Transduction of lactose-utilizing ability among strains of *E. coli* and *S. dysenteriae* and the properties of the transducing phage particles. *Virology* **12** 348–390

Lutsar I, Friedland IR, Jafri HS, Wubbel L, Ng W, Ghaffar F & McCracken Jr GH 1999 Efficacy of Gatifloxacin in Experimental *Escherichia coli* Meningitis. *Antimicrobial Agents and Chemotherapy* **43**(7) 1805–1807

Makovec JA & Ruegg PL 2003 Antimicrobial resistance of bacteria isolated from dairy cow milk samples submitted for bacterial culture: 8,905 samples (1994–2001). *Journal of the American Veterinary Medical Association* **222**(11) 1582–1589

McDermott PF, Walker RD & White DG 2003 Antimicrobials: modes of action and mechanisms of resistance. *International Journal of Toxicology* **22**(2) 135–143

McDermott PF, Zhao S, Wagner DD, Simjee S, Walker RD & White DG 2002 The food safety perspective of antibiotic resistance. *Animal Biotechnology* **13**(1) 71–84

McEwen SA & Fedorka-Cray PJ 2002 Antimicrobial use and resistance in animals. *Clinical Infectious Diseases* **34** (Suppl. 3) S93–S106

Moken MC, McMurry LM & Levy SB 1997 Selection of multiple-antibiotic-resistant (*mar*) mutants of *Escherichia coli* by using the disinfectant pine oil: roles of the *mar* and *acrAB* loci. *Antimicrobial Agents and Chemotherapy* **41**(12) 2770–2772

Neidhardt FC 1996 *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology. Washington, D.C.: ASM Press

Neu HC 1984 Current mechanisms of resistance to antimicrobial agents in microorganisms causing infection in the patient at risk for infection. *American Journal of Medicine* **76**(5A) 11–27

Neu HC 1992 The crisis in antibiotic resistance. *Science* **257**(5073) 1064–1073

Nord CE 1993 The effect of antimicrobial agents on the ecology of the human intestinal microflora. *Veterinary Microbiology* **35**(3–4) 193–197

Oethinger M, Podglajen I, Kern WV & Levy SB 1998 Overexpression of the *marA* or *soxS* regulatory gene in clinical topoisomerase mutants of *Escherichia coli*. *Antimicrobial Agents and Chemotherapy* **42**(8) 2089–2094

Paterson DL 2002 Serious infections caused by enteric gram-negative bacilli–mechanisms of antibiotic resistance and implications for therapy of gram-negative sepsis in the transplanted patient. *Seminars in Respiratory Infections* **17**(4) 260–264

Randall LP & Woodward MJ 2002 The multiple antibiotic resistance (mar) locus and its significance. *Research in Veterinary Science* **72**(2) 87–93

Rickard AH, Lindsay S, Lockwood GB & Gilbert P 2004 Induction of the *mar* operon by miscellaneous groceries. *Journal of Applied Microbiology* **97**(5) 1063–1068

Sanders CC & Sanders Jr WE 1992 beta-Lactam resistance in gram-negative bacteria: global trends and clinical impact. *Clinical Infectious Diseases* **15**(5) 824–839

Silbergeld EK, Graham J & Price LB 2008 Industrial food animal production, antimicrobial resistance, and human health. *Annual Review of Public Health* **29** 151–169

Tollefson L, Fedorka-Cray PJ & Angulo FJ 1999 Public health aspects of antibiotic resistance monitoring in the USA. *Acta Veterinaria Scandinavica* (Suppl.) **92** 67–75

Wittmann D, Jones R, Malledant J & Privitera G 1997 Cefotaxime in the treatment of prophylaxis of surgical infections. *Journal of Chemotherapy* **9** (Suppl. 2) 19–33