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Project Title:

A Comparison of Antibiotic Susceptibility Patterns for Staphylococcus aureus in Organic and Conventional Dairy Herds

FINAL PROJECT REPORT

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Project Summary

The purpose of this project was to compare antibiotic susceptibilities of *Staphylococcus aureus* isolates from 22 certified organic and 16 conventional (antibiotic using) dairy herds located throughout New York State and Vermont.

Isolates from the organic and conventional herds, when compared on a qualitative basis of sensitive, intermediate and resistant susceptibilities, showed statistically significant differences for ampicillin, penicillin and tetracycline. More isolates from the conventional herds were resistant to these three antibiotics than isolates from the organic herds. In addition, when compared on the basis of quantitative zone diameters, an even larger difference between isolates from organic herds and those from conventional herds was noted. Isolates from organic herds were more susceptible (in the sense of larger growth inhibition zones in millimeters) than the isolates from conventional herds to most of the antibiotics that we tested.

Introduction

Mastitis is considered to be the most costly disease affecting the dairy industry with annual losses in the United States exceeding $2 billion (Philpot, 1984). Losses stem from milk discard, drug costs, veterinary care, increased labor, and premature- culling. *Staphylococcus aureus* is one of the most important pathogens causing intramammary infections in dairy cattle (Gonzalez et al.,1988) and continues to be one of the major causes of mastitis in dairy herds worldwide (Barkema et al. 1998, Gonzalez et al. 1988, Nickerson et al. 1999, Osteras et al. 1999, Sol et al. 1997, Zecconi and Picini 1998). This microorganism causes both clinical and subcllnlcal mastitis, and appears to be present in both well managed herds and herds with a general management and hygiene problem (Barkema et al. 1998 Zecconi, A., and R. Piccini 1998). To reduce the impact of *Staph aureus* on farms, it is important to both reduce the incidence of infections and shorten the duration of existing infections. Shortening the duration of infection occurs through either successful treatment or culling of the infected animal. Treatment with antibiotics is either done during lactation or at dry off. Use of antibiotics for dry cow treatment is a routine part of dairy herd health-management schemes (Gonzalez 1988, Schukken 1993). On farms with *Staph aureus* mastitis problems, (cl)oxacillin is the drug of choice for dry cow treatment (Sol and Sampimon 1995, Browning et al. 1990, Cummins and McCaskey 1987) and currently, cloxacillin is widely used throughout the United States and Europe. Selective pressure created by use of antibiotics offers resistant strains a survival advantage (Chambers 1997). Thus, dairy farms constitute an environment where *Staph aureus* is highly prevalent, where antimicrobials (such as cloxacillin) are routinely used, and where selective pressure favors the survival of resistant strains.

Seventeen million pounds of antibiotics are used in animals each year in the United States (Animal Health Institute 2001). Approximately 90% of the antibiotics used in agriculture are given as growth-promotants and prophylactic agents (e.g. dry cow therapy) (Khachatourians 1998). Many of the antibiotics used on farms are given by farm workers that may not be familiar with principles of antibiotic therapy and may not adhere to recommended therapeutic regimens.

There is concern that antibiotics that are used in animals have created a reservoir of resistant bacteria that may be transferred to humans via various food product (Bates et al. 1994). In Europe, use of avoparcin has been used as a feed additive in many species as a growth promotant (Witte 1997). In separate studies by Aarestrup (1995) and Witte (2000), vancomycin resistant enterococci (VRE) were isolated from manure from pig and poultry farms using avoparcin in the feed, -whereas on farms where avoparcin was not used, VRE were not detected. Many of the antibacterial resistance genes are on plasmids that may transfer themselves to other genera and species of bacteria (Tenover and McGowan 1996). In vitro transfer of VR from enterococci to staphylococci (in *particular, Staphylococcus aureus)* has already occurred in the laboratory (Noble et al. 1992).

Routine, non-therapeutic use of antibiotics and growth-promotants in animals raised on certified organic farms is discouraged. Antibiotics are used in rare cases for the welfare of the animal to treat a specific disease. Withdrawal times following this use are extensive (California Certified Organic Farmers 2000). This is in contrast to conventional farms where antibiotics are used as growth promotants (milk replacers), routine therapeutics (mastitis and other diseases) and prophylaxis (dry cow treatment) -

In a previous small scale project done at our laboratory at Quality Milk Promotion Services, differences in the antibiotic susceptibility patterns of *StaphyIococcus aureus* isolates obtained from an organic dairy herd were compared with the patterns obtained from isolates from a conventional dairy herd. Statistically significant differences were noted with the isolates from the organic farm exhibiting a higher degree of susceptibility than those from the conventional farm.

Busato, et al.(2000) looked at udder health and risk factors for subclinical mastitis on dairy farms in Switzerland. They observed no difference in antibiotic resistance between conventional and organic farms, however, only a few samples were analyzed for antibiotic susceptibility. They also acknowledged that antibiotics were allowed on these organic farms for therapy of clinical mastitis. There are very few studies that touch on antibiotic resistance on organic farms in a valid comparison with conventional herds.

Consumer interest in the health and safety of their food is ever growing and is creating a high value market for food free of possible exposure to pesticides, hormones and antibiotics. To meet these increased interests, organic farming has become one of the fastest growing segments of US agriculture (Economic Research Service 2000). Total organic dairy sales were the most rapidly growing segment of the US dairy industry last year at $200 million dollars. The additional benefit of producing organic milk (with less antibiotic resistance) would create a stronger competitive edge for organic products with the health conscious public.

Objectives

Our objective was to compare the antibiotic susceptibility patterns of all *Staphylococcus aureus* isolates obtained from composite milk samples from all cows from 22 organic dairy herds with to a similar number of isolates from 16 conventional dairy herds.

Materials and Methods

Twenty-five certified organic herds, located throughout New York State were contacted to assess interest in this project. Twenty-four agreed to participate and have bulk tank samples taken to screen for the presence of *Staphylococcus aureus* in their herd. Seventeen herds of these twenty-four herds qualified for the project on the basis of a bulk tank sample culturing positive for *Staph aureus.*

Each of the seventeen farms were visited by Quality Milk Promotion Service (QMPS) personnel for a full herd survey. During a herd survey, composite milk samples were aseptically taken from each lactating cow according to NMC guidelines for sampling. Samples were cooled immediately and transported to a QMPS laboratory for culture and pathogen identification. Also during the herd survey a full inspection and testing of the mechanical milking system was performed and producers were questioned about their management and treatment practices. A total of 113 *Staph aureus* isolates were collected from the seventeen New York herds. Also available were thirty-one *Staph aureus* isolates from five certified organic herds located in Vermont. No management practice information was available from these five herds but all were certified organic.

Staphylococcus aureus isolates, from sixteen conventional (antibiotic-using) herds were available at QMPS. These sixteen herds represented herds of similar size and geographic distribution as the New York organic herds. A total of 117 isolates were available from these 16 herds. During the herd surveys on these farms a full inspection and testing of the mechanical milking systems were performed and producers were questioned about their management and antibiotic use practices. Descriptive statistics for both the organic and conventional herds are available in Table 1.

To isolate mastitis agents, approximately 0.0l ml of each composite sample was streaked onto one half of a trypticase soy agar plates (BBL, Becton Dickinson, Cookeysville, MD) containing 5% sheep blood and 0.1% esculin. Plates were incubated at 37°C for 48 hours. After observation of colony morphology and hemolytic patterns, isolates were examined further by Gram stain and catalase testing. *Staphylococcus aureus* isolates were identified based on a positive tube test for free coagulase and hemolytic pattern. Isolates identified as *Staphylococcus aureus* were subcultured and were stored on microbeads (Microbank, Pro-Lab Diagnostics) at -80°C.

Antibiotic susceptibility testing was performed by the agar disk diffusion method on Mueller-Hinton agar with 5% calf serum and results were interpreted according to NCCLS standards (NCCLS 1997).

Table 1. Descriptive statistics of the 22 organic and 16 conventional herds

Parameter	Organic	Conventional	Difference
# of herds	22	16	
Herd size	65.8	70.2	p = 0.682
Production Level (lbs/cow/lactation)	14600	15300	p = 0.666
Mean bulk milk somatic cell count (SCC)*	273000	559300	p < 0.000
Total # isolates	144	117	

*Somatic cell count is the number of leukocytes/ml of milk and is a measure of milk quality.

Prior to antibiotic susceptibility testing, all isolates were revived by placing a single microbead in Todd-Hewitt broth and incubating for 6 hours at 37°C. Ten microliters were then streaked on TSA plates for colony isolation and incubated for overnight at 37°C. Two 24-hour old colonies were transferred from TSA to Todd Hewitt broth using a sterile loop. The culture was incubated at 37°C until it reached a turbidity of 0.5 McFarland standard. The culture suspension was swabbed onto a 150mm petri dish containing Mueller-Hinton agar and antibiotic disks (BBL Sensi-diSCS TM) were applied. Plates were incubated at 37°C for 18 hours. Diameters of growth inhibition were measured in millimeters and recorded.

Antibiotics were chosen based on their activity against Gram positive cocci and included ampicillin (10µg), cephalothin (30µg), erythromycin (15µg), novobiocin (30µg), oxacillin (1µg), penicillin (10 IU), penicillin-novobiocin (10 IU /30µg), pirlimycin (2µg), tetracycline (30µg) and vancomycin (30µg).

For statistical comparison, results were placed into two categories : sensitive and resistant. The resistant category includes those isolates that had a result of either intermediate or resistant susceptibility.

The strength of association between antibiotic use (conventional or organic) and proportion susceptible or resistant for each antibiotic were evaluated by Chi-square analysis. Where expected counts in the 2 x 2 contingency table were less than 5, a Fisher's exact test was applied (SAS proc freq v. 8.2) Zone diameters for all antibiotics were compared for organic and conventional herds using One-way ANOVA testing (SAS proc GLM, v. 8.2). Results for chi-square analysis, Fisher's exact test, and Analysis of Variance were determined as significant at $p \leq .05$.

Project Results

Overall antimicrobial susceptibility, as judged by the S-I-R classification system, of *Staph aureus* isolates from the conventional herds were congruent with results previously reported. The exception to this was sensitivity to erythromycin; the percent sensitive observed in our isolates was lower than previously reported. (Watts and Salmon 1997, Franklin 1999, Gentilini et al. 2000, Vecht et al. 1989). Percent sensitivity for isolates from both organic and conventional herds for cephalothin, cloxacillin, novobiocin, penicillin-novoblocin, pirlimycin, and vancomycin were all high at approximately 100%. Fewer isolates from the conventional herds were observed in the sensitive range for the remaining antibiotics than from the organic herds: ampicillin (61.5% vs. 80.5%), erythromycin (49.5% vs. 55.5%), penicillin (65.8% vs. 79.9%) and tetracycline (87.2% vs. 99.3%). All results are presented in tabular form in Appendix I.

For some antibiotics, *Staph aureus* isolates from the organic herds exhibited a higher percentage falling into the sensitive range than those isolates from the conventional herds. Results for cephalothin, cloxacillin , erythromycin, novobiocin, penicillin-novobiocin, pirlimycin and vancomycin were not different for both groups. Antibiotics for which a statistically significant higher degree of sensitivity was observed in the organic herds were ampicillin, penicillin, and tetracycline. These results are summarized in Table 2.

Table 2. Comparison of Sensitive and Resistant Results for Organic and Conventional Herds

	Conventional (#of isolates)		**Organic** (#of isolates)		**Significant Difference**
Antibiotic	***Sensitive***	***Resistant***	***Sensitive***	***Resistant***	
ampicillin	72	45	116	28	p = 0.0007
cephalothin	117	0	144	0	No difference
cloxacillin	117	0	144	0	No difference
erythromycin	58	59	80	64	p = 0.3365
novobiocin	116	1	144	0	No difference
penicillin	77	40	115	29	p = 0.0106
penicillin-novobiocin	117	0	144	0	No difference
pirlimycin	117	0	144	0	No difference
tetracycline	102	15	143	1	p = .00003
vancomycin	117	0	144	0	No difference

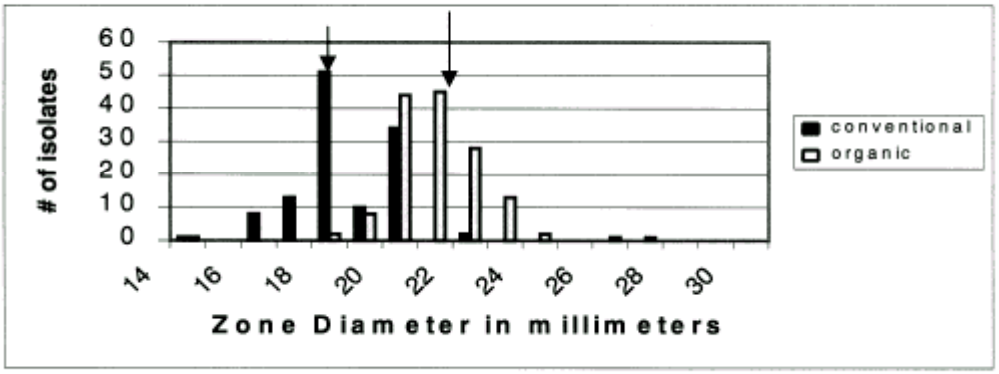
When results were compared on the basis of zone of growth inhibition in millimeters, significant differences in zone diameter were observed for ampicillin, cephalothin, novobiocin, cloxacillin, penicillin, penicillin-novobiocin, pirlimycin, and tetracycline. There were no significant differences in zone diameter for either erythromycin or vancomycin. Results are summarized in the Table 3.

Table 3. Comparison of zone diameter from disk diffusion test between *S. aureus* isolates from conventional and organic herds.

Antibiotic	Mean Zone Conventional	Mean Zone Organic	Range Conventional	Range Organic	Significant Difference
ampicillin	27.81	32.00	16-42 (median=30)	18-40 (median=35)	p <.0001
cephalothin	32.71	34.81	24-42 (median=32)	26-44 (median=35)	p <.0001
cloxacillin	20.70	22.90	13-28 (median=20)	17-28 (median=23)	p <.0001
erythromycin	22.86	22.76	0-26 (median=22)	0-33 (median=23)	p=0.7850
novobiocin	24.67	26.36	16-34 (median=24)	18-37 (median=25)	p<.0001
penicillin	28.81	32.85	7-46 (median=32)	17-44 (median=37)	p<.0001
tetracycline	22.44	23.69	9-34 (median=24)	0-34 (median=23)	p =0.0175
penicillin-novobiocin	30.52	33.20	20-44 (median=30)	23-46 (median=36)	p =0.0001
pirlimycin	18.44	21.04	14-22 (median=18)	0-26 (median=21)	p <.0001
vancomycin	17.13	16.91	14-20 (median=17)	14-19 (median=17)	p =0.096

All isolates may fall within the 'sensitive' category for a given antibiotic, but within that qualitative category there appear to be two different populations. *Staphylococcus aureus* isolates from conventional antibiotic-using herds appear to exhibit a reduced level of antibiotic susceptibility when compared to organic herds. (See figure 1 below and figures 2-11 in Appendix II).

Figure 1. Zone Diameters for pirlimycin
(Arrows indicate the median values for conventional and organic herds)



Discussion

Antibiotic susceptibility testing has two major functions in both human and veterinary medicine. Primarily, it can be used to guide the choice of antimicrobial treatment for the patient. Secondarily, it may be used at a surveillance tool to monitor antimicrobial resistance.

The Kirby-Bauer agar disk diffusion places test results into the qualitative categories of sensitive, intermediate, and resistant and may fail to detect subtle changes in antimicrobial resistance. As noted in the results from this study, most isolates tested expressed antibiotic susceptibilities well within the 'sensitive' range. Significant differences in these qualitative categories between conventional and organic herds were noted for ampicillin, penicillin, and tetracycline. When these results were analyzed on a more quantitative basis using zone diameters, isolates from the organic herds and conventional herds fell into two significantly different populations (see Figure 1). Isolates from the conventional herds showed smaller zones of growth inhibition for the antibiotics tested than the isolates from the organic herds.

The development of antibiotic resistance in bacteria is mediated by both selective pressure due to antibiotic use and the presence of resistance genes. A close relationship exists between the rate of development of resistance development and the quantities of antibiotics used (Lopez-Lozano et al. 2000). Shortly after penicillin was introduced in 1944, resistant strains of bacteria emerged (Livermore 2000). By 1948, greater than 50% of the *Staph aureus* strains isolated from hospitals were resistant to penicillin (Barber and Rozwadowska-Dowzenko 1948).

Resistance to antibiotics is by four major mechanisms: alterations in the target site of the antibiotic (such as changes in penicillin binding proteins), drug degradation and enzymatic inactivation of the antibiotic (e.g., penicillinases), changes in cell wall permeability that prevent access to antibiotics, and increases in the activity of efflux pumps in the cell wall which prevent accumulation of antibiotic within the cell (Wise 1999, Hawkey 2000).

Resistance mechanisms may arise either through mutations in bacterial chromosomal genes that code for antibiotic susceptibility or through the transfer of extrachromosomal elements such as plasmids, integrons, and transposons (Yhachtourians 1998, Mazel and Davies 1999, Kruse and Sorum 1994). The elements may be transferred between bacteria by conjugation (cell to cell contact), transduction (bacterlophage introduction) or transformation (uptake of naked DNA) (Davies 1997, Tenover and McGowan 1996, Levy 1994). Conjugation is considered the most important means of transfer (Davies 1994).

If antibiotic use is the major selective pressure encouraging the development of antibiotic resistant, then reducing antibiotic use should result in decreased antibiotic resistance. A decrease in tetracycline resistance in *Salmonella* spp. isolated from man and pigs was observed after tetracycline was banned as a growth promotant in feed in 1974 (Van Leeuwen et al. 1979) Similarly, when avoparcin was banned as a feed additive for poultry in Denmark in 1995, the prevalence of vancomycin resistant enterococci isolated from broilers has decreased from 80% to 5% (Aarestrup et al. 1998).

Bovine mastitis is the single most common cause for antimicrobial use in dairy cattle. 77% of US herds practice blanket dry cow therapy where each cow is infused intramammarily with antibiotic at dry off (National Research Council, 1999). Amoxicillin and pirlimycin were the most common lactating treatments used in our conventional herds; twelve of the sixteen treated all clinical cases with antibiotics. Blanket dry cow therapy was practiced by 100% of our conventional herds; a commercial penicillin-novobiocin intramammary preparation was the most common treatment used.

All of the organic herds in our study had been certified as organic for at least three years; most had farmed organically much longer than that. The most common certifying organization for our herds was the Northeast Organic Farming Association of New York. Use of antimicrobial therapy is restricted to health care emergencies, where no alternative treatment exists. This precludes the use of blanket dry cow therapy on these farms. In fact, all farms recorded no antimicrobial therapies for greater than three years; all relied upon alternative (i.e., homeopathic) remedies. Their decisions to avoid antimicrobial therapy were based on the extended withholding times for milk and meat required by the certifying agencies and personal philosophy. In contrast with our conventional herds, there is little selective pressure by antimicrobials on the population of *Staph aureus* isolated from our organic herds.

β-lactams are some of the most clinically important and widely used antibiotics in mastitis therapy. Their mode of action against Gram-positive bacteria such as *Staphylococcus aureus* is via interaction with three high molecular weight and one low molecular weight penicillin-binding proteins. Specific functions of these penicillin binding proteins are not clear but they are appear to play roles in the synthesis of cell wall peptidoglycan and cell growth (Hakenbeck and Coyette 1998, Berger- Bachil 1995).

Shortly after the introduction of β-lactam antibiotics, resistant strains of *Staph aureus* carrying enzymes which hydrolyze β-lactams were identified (Hakenbeck and Coyette 1998, Livermore 2000, Jeljaszewicz et al. 2000). Over 200 different types of beta-lactamases have been described (Hawkey 2000) The gene (blaZ) encoding for these enzymes may be located either on plasmids or chromosomally and may be transferred horizontally via plasmids (Zhang et al. 2001, Vesterholm-Nielsen et al. 1999, Lyon and Skurray 1987). The expression of blaZ is regulated by two genes: blaI which represses blaZ gene transcription and blaR1 which plays a role in induction of blaZ in the presence of β-lactam antibiotics (Chambers 1997, Hackbarth and Chambers 1993).

β-lactamase resistant penicillins, such as methicillin and cephalosporins, were subsequently introduced; however, *Staph aureus* strains (methicillin resistant *Staph aureus* or MRSA) which are resistant to multiple antibiotics were soon identified (Lyon and Skurray 1987). Methicillin resistance is a result of acquisition of foreign DNA, the mec determinant, which incorporates itself in a specific chromosomal site Geljaszewicz et al. 2000). The mecA gene, located on this determinant, codes for the alternative penicillin binding protein 2a (PBP2a), a protein with a low affinity for beta- lactam antibiotics. Since PBP2a is not inhibited by beta lactams, *Staph aureus* can continue to synthesize peptidoglycan for a structurally sound cell wall.

In addition to mecA, additional genes are associated with the regulation of methicillin resistance and are located on this determinant: mecI and mecR1. Their functions are similar to that of blaI and blaR1. Mecl represses the transcription of mecA and mecR1 is needed for induction (Jeljaszewicz et al. 2000, Chambers 1997, Berger- Bachii 1995). The bla regulatory system may also be found in methicillin resistant *Staph aureus* but will exhibit less strict control of expression that the mec regulatory system (Hackbarth and Chambers 1993). Additional factors such as fem (factor essential for methicillin resistance) genes, llm (lipophilic protein) genes, and aux (auxiliary) genes also affect expression of methicillin resistance (Berger- Bachii 1995).

Borderline resistance to β-lactams was initially theorized to be due to extrinsic mechanisms leading to the overproduction of β-lactamases (McDougal and Thornsberry, 1986). However, borderline resistance is likely to be more complicated than this. Intrinsic mechanisms leading to modification of penicillin-binding proteins (such as mecA coding for PBP2a) or alterations in normal PBPs may also be involved (Massidda et al. 1996). Additionally, more general mechanisms of resistance such as decreased membrane permeability or membrane-associated active efflux also exist (Nikaido 1994). Combinations of two or more of these mechanisms may explain some of the variability in antibiotic susceptibility within the 'susceptible' class seen in this study.

Mechanisms of resistance in *Staphylococcus aureus* for antibiotics other than β-lactams have been less extensively studied. β-lactam antibiotics are the most -widely used but erythromycin, pirlimycin, and tetracycline all have roles in treatment protocols for dairy cattle.

Erythromycin (a macrolide antibiotic) is another widely used intramammary antibiotic and is available as both lactating and dry cow therapies. Multiple resistance mechanisms have been described. Some *Staphylococcus aureus* possess erythromycin-resistant methylase (ermA, ermB and ermC) genes (Pechere 2001) that code for an alteration in ribosomal RNA which prevents macrolide binding, resulting in a high level of resistance (Lai and Weisblum 1971). In a study done by Khan et al. (2000), the ermA gene, located on a transposon, appeared to predominate; it was found in 91% of *Staphylococcus* spp. isolated from bovine milk samples. Macrolide efflux mechanisms have also been identified that prevent accumulation of antibiotic within the bacterial cell. The msrA genes coding for efflux pumps are located on transferable plasmids and confer a low-level resistance when compared with erm genes (Pechere 2001).

Similarly, the most prevalent mechanism of resistance for lincosamides, of which pirlimycin is a member, is also enzymatic alteration of ribosomal RNA (Davies 1994).

Tetracycline is primarily used as a parenteral, not an intramammary, therapy. Three mechanisms of resistance to tetracycline have been described: protection of ribosomal RNA, increased activity of efflux pumps, and inactivation of the antibiotic by enzymes (Schnappinger and Hillen 1996). Resistance genes coding for these mechanisms are located on transposons, conjugative plasmids or conjugative transposons. One class of conjugative transposon can transfer itself from streptococci to a variety of other gram positive and negative bacteria (Speer et al. 1992). The predominant gene in staphylococci, which codes for active efflux of tetracycline is TetA(K) and is located on a plasmid (Skurray and Firth 1997).

In the human medicine, vancomycin has become the drug of choice for methicillin resistant *Staph* aureus. Intermediate resistance to vancomycinin Staphaureus is rare but was first reported in Japan and was associated with treatment failure (Hiramatsu et al. 1997). Other reports of vancomycinresistant *Staph aureus* have since followed. The genetic mechanism for vancomycin resistance in *Staph aureus* is not well understood but is phenotypically associated with accelerated cell wall synthesis, leading to a thickened cell wall. Theoretically, this thickened cell wall is capable of trapping large amounts of vancomycin and protecting target site (Geisel et al. 2001). In our laboratory, each *Staph aureus* isolate undergoing susceptibility testing is also screened for vancomycin resistance. None to date have been detected.

In our study, we observed significant differences between organic and conventional herds in diameters of zones of growth inhibition for all beta lactam antibiotics tested (ampicillin, cephalothin, cloxacillin, penicillin, and penicillin-novobiocin). Oxacillin is used to phenotypically identify methicillin- resistant staphylococci. None of the isolates in our current study were determined to be methicillin resistant based on this method.

Several hypotheses of important biological and managerial interest arise from our current report. Differences in mean zone sizes may be accounted for in several ways. Selective pressure from the use of lactating and dry cow therapy may induce variable expression of penicillinases and β-lactamases in the isolates from our conventional herds. Similar variability in the expression of the resistance mechanisms resulting from antibiotic use for both pirlimycin and tetracycline may also account for difference in zone size between organic and conventional herds for those two antibiotics. Additionally, there may be different strains populating organic and conventional herds, which may exhibit different susceptibility patterns; no genotypic strain identification has been performed. A number of these hypotheses can be tested with the current collection of *Staph aureus* isolates using genomic tools.

The disk diffusion method of determining antimicrobial sensitivity although widely used and economically attractive has its limitations. It is used mainly in a qualitative manner placing isolates in either a sensitive, intermediate, or resistant category. NCCLS interpretative criteria for most antibiotics have been set using human pathogens and pharmokinetics. Only pirlimycin and penicillin-novobiocin have interpretive criteria that have been validated for bovine mastitis pathogens (Owens et al. 1997, Thornsberry et al. 1993). Categorizing results for *Staphylococcus aureus* isolated from bovine milk as simply sensitive, intermediate, or resistant may lead to erroneous conclusions. We chose to also compare results on the basis of zone diameter in millimeters for a more quantitative interpretation.

In essence, our study design resulted in a cross-sectional evaluation of antibiotic susceptibility patterns at one point in time. A longitudinal follow-up of organic and conventional herds would be of value to understand the dynamics of developing antibiotic resistance or the return of antibiotic sensitivity in endemic bacteria.

Conclusions

Staphylococcus aureus isolates in this study show good susceptibility to most of the antimicrobial agents that are commercially available for the treatment of bovine mastitis. For our conventional herds, there was a significantly larger number of resistant isolates for ampicillin, penicillin, and tetracycline suggesting that antibiotic use in these herds may be related to increased resistance. Similarly, when isolates from the conventional herds were compared to those from organic herds based on the diameter of the zone of growth inhibition, isolates from conventional herds were less susceptible than organic isolates.

Because of this association between decreased antibiotic use and an increase in susceptibility, antibiotic use in our dairy herds should be reevaluated. Guidelines for rational and limited antibiotic antibiotic use should be set in place and communicated to both the veterinarian and dairy producer so that development of antibiotic resistance can be prevented or reduced.

Further studies examining the mechanisms of resistance at work in both conventional and organic mastitis pathogens are warranted. Genotypic identification of *Staph aureus* strains would further elucidate whether differences in antimicrobial susceptibility are due to differences in the populations *of Staph aureus* within a herd or whether identical strains are exhibiting different susceptibilities. It would be of great additional value to follow mastitis pathogens and their susceptibilities over time in herds undergoing transition from conventional farming to organic to determine if there is a return to increased susceptibility in the same populations of *Staph aureus* strains.

Outreach

Preliminary data has already been reported to an organic dairy farm focus group here at Cornell. This focus group consists of organic dairy producers, researchers interested in organic farming methods, veterinarians, and board members of organic certifying agencies. A formal report will be presented to this group, NOFA-NY, and the dairy producers participating in this project upon completion.

Our intent is also to submit this report to both a peer-reviewed journal for publication and lay publications. An oral presentation or poster will also be presented at a major veterinary conference (e.g., American Association of Bovine Practitioners or National Mastitis Council) in the next year.

References

Aarestrup, F.M. 1995. Occurrence of glycopeptide resistance *among Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb. Drug Res. 1:255.

Aarestrup, F.M., F. Bager, M. Madsen, N. E. Jensen, A. Meyling, and H.C. Wegener. 1998. Surveillance of antimicrobial resistance in bacteria isolated from food animals to antimicrobial growth promoters and related therapeutic agents in Denmark. APMIS 106:606.

Animal Health Insititute. 2001. Survey of Antibiotic Use in Animals and Humans in 1998. http:// www.ahi.org/News%20Room/Press%20Release/2001/February/usage.htm

Barber, M. and M. Rozwadowska-Dowzenko. 1948. Infection by penicillin resistant staphylococci. Lancet. 11:641.

Barkema, H.W., Y.H. Schukken, TJ.G.M. Lam, M.L. Beiboer, H. Wilmink, G. Benedictus, and A. Brand. 1998. Incidence of clinical mastitis in dairy herds grouped in three categories by bulk milk somatic cell count. J. Dairy Scl. 81:41 1.

Bates, J., J.Z. Jordens, and D.T. Griffiths. 1994. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infections in man. J. Antimicrob. Chemother. 34(4):507

Berger-Bachii, B. 1995. Factors affecting methicillin resistance in *Staphylococcus aureus. Int.* J. Antimicrob. Agents. 6:13.

Browning, J.W., G.A. Mein, M. Barton, T.J. Nicholls, and P. Brightling. 1990. Effects of antibiotic therapy at drying off on mastitis in the dry period and early lactation. Austr. Vet. J. 67:440.

Busato, A., P. Traschel, M. Schallibaum, and J.W. Blum. 2000. Udder health and risk factors for subclinical mastitis in organic dairy farms in Switzerland. Prev. Vet. Med. 44:205.

California Certified Organic Farmers. 2000. Certification standards. www.ccof.org/handbook

Chambers, H.F. 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clin. Microb. Rev. 10:781.

Cummins, K.A., and T.A. McCaskey. 1987. Multiple infusions of cloxacillin for treatment of mastitis during the dry period. J. Dairy Sci. 70:2658.

Davies, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. Science. 264:375.

Davies, J. 1997. Origins, acquisition, and dissemination of antibiotic resistance determinants. Ciba Foundation Symposium 207. Antibiotic Resistance: origins, evolution, selection, and spread. 15.

Economic Research Service- United States Department of Agriculture. 2000. U.S. Organic Agriculture. ers.usda.gov/whatsnew/issues/organic

Franklin, A. 1999. Current status of antibiotic resistance in animal production. Acta Vet. Scand. 92:23.

Gelsel, R., F.J. Schmitz, A.C. Fluit, and H. Labischinski. 2001. Emergence, mechanism and clinical implications of reduced glycopeptide susceptibility in *Staphylococcus aureus.* Eur. J. Clin. Microbiol. Infect. Dis. 20:685.

Gentilini E., G. Denamiel, P. Llorente, S. Godaly, M.Rebuelto, O. DeGregorio. 2000. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from bovine mastitis in Argentina. J Dairy Sci. 83(6):1224.

Gonzalez, R. N., D. E. Jasper, T. B. Farver, R. B. Bushnell, and C. E. Franti. 1988. Prevalence of udder infections and mastitis in 50 California dairy herds. J. Am. Vet. Med.Assoc. 193:323.

Hackbarth, C.J. and H.F. Chambers. 1993. BlaI and blarl regulate β-lactamase and PBP2a production in methicillin-resistant *Stahbylococcus aureus.* Antimicrob. Agents Chemother. 37:1149.

Hakenbeck, R. and J. Coyette. 1998. Resistant penicillin-binding proteins. Cell. Mol. Life. 54:332.

Hawkey, P.M. 2000. Mechanisms of resistance to antibiotics. Intensive Care Med. 26 Suppl 1:S9

Hiramatsu, K., H. Hanaki, T. Ini, K. Yabuta, T. Oguri, and F.C. Tenover. 1997. Methicillin resistant *Staphylococcus aureus* clinical strain with reduced susceptibility to vancomycin. J. Antimicrob. Chemother. 40:135.

Jeljaszewicz, J., G. Mlynarczyk, and D.L. Monnet. 2000. Antibiotic resistance in Gram-positive cocci. Intl. J. Antimicrob. Agents. 16:473.

Khachatourians, G.G. 1998. Agricultural use of antibiotics and the evolution and transfer of anti biotic resistant bacteria. CMAJ. 159:1130.

Khan, S.A., M.S. Nawez, A.A.Khan, and C.E. Cerniglia. 2000. Transfer of erythromycin resistance from poultry to human clinical strains of *Staphylococcus aureus.* J. Clin. Microbiol. 38:1832.

Kruse, H. and K. Sorum. 1994. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural environments. Appl Environ. Microbiol. 60:4015.

Lai, C.J. and B. Weisblum. 1971. Altered methylation of ribosomal RNA in an erythromycin resistant strain of *Staphylococcus aureus.* Proc. Natl. Acad. Scl. 68:856.

Levy, S.B., 1994. Balancing the drug-resistance equation. Trends Microbiol. 10:341.

Livermore, D.M. 2000. Antibiotic resistance in staphylococci; resistance mechanisms. Intl. J. Antimicrob. Agents. 16:S3.

Lopez-Lozano, J.M., D.L. Monnet, A. Yague, A. Burgos, N. Gonzalo, P. Campillos, and M. Saez. 2000. Modelling and forecasting antimicrobial resistance and its dynamic relationship to antimicrobial use: a time series analysis. Intl. J. Antimicrob. Agents. 14:21.

Lyon, B.R. and R.A. Skurray. 1987. Antimicrobial resistance of *Staphylococcus aureus:* genetic basis. Microbiol. Rev. 51:88.

Massidda, O., M.P. Montanari, M. Mingola, and P.E. Varaldo. 1996. Borderline methicillin susceptible *Staphylococcus aureus* strains have more in common than reduced susceptibility to penicillinase-reistant penicillins. Antimicrob. Agents Chemother. 40:2769.

Mazel, D. and J. Davies. 1999. Antibiotic resistance in microbes. Cell. Mol. Life. 56:742.

McDougal, L.K. and C. Thornsberry. 1986. J. Clin. Microbiol. 23(5):832.

National Research Council. 1999. Food animal production and drug use. The Use of Drugs in Food Animals: Benefits and Risks. Natl. Acad. Press. 44.

NCCLS. 1997. Performance standards for antimicrobial susceptibility testing. 6th ed. Approved Standards.

Nickerson, S.C., W.E. Owens, L.K. Fox, C.C. Scheifinger, T.R. Shryock, and T.E. Spike. 1999. Comparison of tilmicosin and cephapirin as therapeutics for *Staphylococcus aureus* mastitis at dry-off. J. Dairy Sci. 82:696.

Nikaido, H. 1994. Prevention of drug access to bacterial targets: permeability barriers and active efflux. Science. 264:382.

Noble, W.C., Z. Virani, and R.G.A. Cree. 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus.* FEMS Microbiol. Lett. 93:195.

Osteras O., V.L. Edge, and S.W. Martin. 1999. Determinants of success or failure in the elimination of major mastitis pathogens in selective dry cow therapy. J. Dairy Sci. 82:1221.

Owens, W.E., C.H. Ray, J.L. Watts, and R.J. Yancey. 1997. Comparison of success of antibiotic therapy during lactation and results of antibiotic susceptibility tests for bovine mastitis. J. Dairy Sci. 80:313

Pechere, J.C. 2001. Macrolide resistance mechanism in Gram-positive cocci. Intl. J. Antimicrob. Agents. 18:S25.

Philpot, W.N. 1984. Economics of mastitis control. Vet. Clin. North Am. 6:233.

Schnappinger D. and W. Hillen. 1996. Tetracyclines: antibiotic action, uptake, and resistance mechanisms. Arch Microbiol. 165(6):359.

Schukken, Y. H., J. van Vliet, D. van de Geer, and F. J. Grommers. 1993. A randomized blind trial on dry cow antibiotic infusion in a low somatic cell count herds. J. Dairy Sci. 76:2925.

Skurray, R.A. and N. Firth. 1997. Molecular evolution of multiply-antibiotic-resistant staphylococci. Ciba Foundation Symposium 207. Antibiotic Resistance: origins, evolution, selection, and spread. 167.

Sol, J. and O.C. Sampimon. 1995. Dry cow treatment with 600 mg dynomilled cloxacillin or 250 mg cephalonium: comparison of cure rate, new intramammary infection rate and somatic cell count. In: National Mastitis Council Annual Meeting Proceedings, Arlington, VA, pp. 146.

Sol, J., O.C. Sampimon, Jj. Snoep, and Y.H. Schukken. 1997. Factors associated with bacteriological cure during lactation after therapy for subclinical mastitis caused by *Staphylococcus aureus. J.* Dairy Sci. 80:2803.

Speer, B.S., N. B. Shoemaker, and A.A. Salyers. 1992. Bacterial resistance to tetracycline: mechanisms, transfer, and clinical significance. Clin. Microbiol. Rev. 5:387.

Tenover, F. C. and J. E. McGowan. 1996. Reasons for the emergence of antibiotic resistance. Am. J. Med. Sci. 311:9.

Thornsberry, C., J.K. Marler, J.L. Watts, and R.J. Yancey. 1993. Activity of pirlimycin against pathogens from cows with mastitis and recommendations for disk diffusion tests. Antimicrob. Agents Chemother. 37:1122.

Van Leeuwen, Wj., J. van Embden, and P. Guinee. 1979. Decrease of drug resistance in *Salmonella* in the Netherlands. Antimicrob. Agents Chemother. 16:237.

Vecht U., H.J. Wisselink, H.M. Vette. 1989. Sensitivity pattern of *Staphylococcus aureus* isolated from quarter milk from cattle Tijdschr Diergeneeskd. 114(5):260.

Vesterholm-Nielsen, M., M. Larsen, J. Olsen, and F.M. Aarestrup. 1999. Occurrence of the blaZ gene in penicillin resistant *Staphylococcus aureus* isolated from bovine mastitis in Denmark. Acta Vet. Scand. 40:279.

Watts. J.L. and S.A. Salmon. 1997. Activity of selected antimicrobial agents agains strains of *Staphylococcus aureus* isolated from bovine intramammary infections that produce β-lactamase. J. Dairy Sci. 80:788.

Wise, R. 1999. A review of the mechanisms of action and resistance of antimicrobial agents. Can. Resp. J. 6 Suppl:20A..

Witte, W. 1997. Impact of antibiotic use in animal feeding on resistance of bacterial pathogens in humans. Antibiotic resistance: origins, evolution, selection and spread. Ciba Foundation Symposium 201. 61.

Witte, W. 2000. Ecological impact of antibiotic use in animals on different complex microflora: environment. Int. J. Antimicrob. Agents. 14:32.

Zecconi, A., and R. Piccini. 1998. *Staphylococcus. aureus:* a problem for Italian dairy herds. Bull. Int. Dairy Fed. 330:25.

Zhang, H.Z., C.J. Hackbarth, K.M. Chansky, and H.F. Chambers. 2001. A proteolytic transmembrane signaling pathway and resistance to β-lactams in staphylococci. Science. 291:1962.

Appendix II

Figures 2-11. Arrow along horizontal axis denotes the beginning of the sensitive range according to NCCLS standards. Diameters to the right of the arrow are progressively more sensitive.

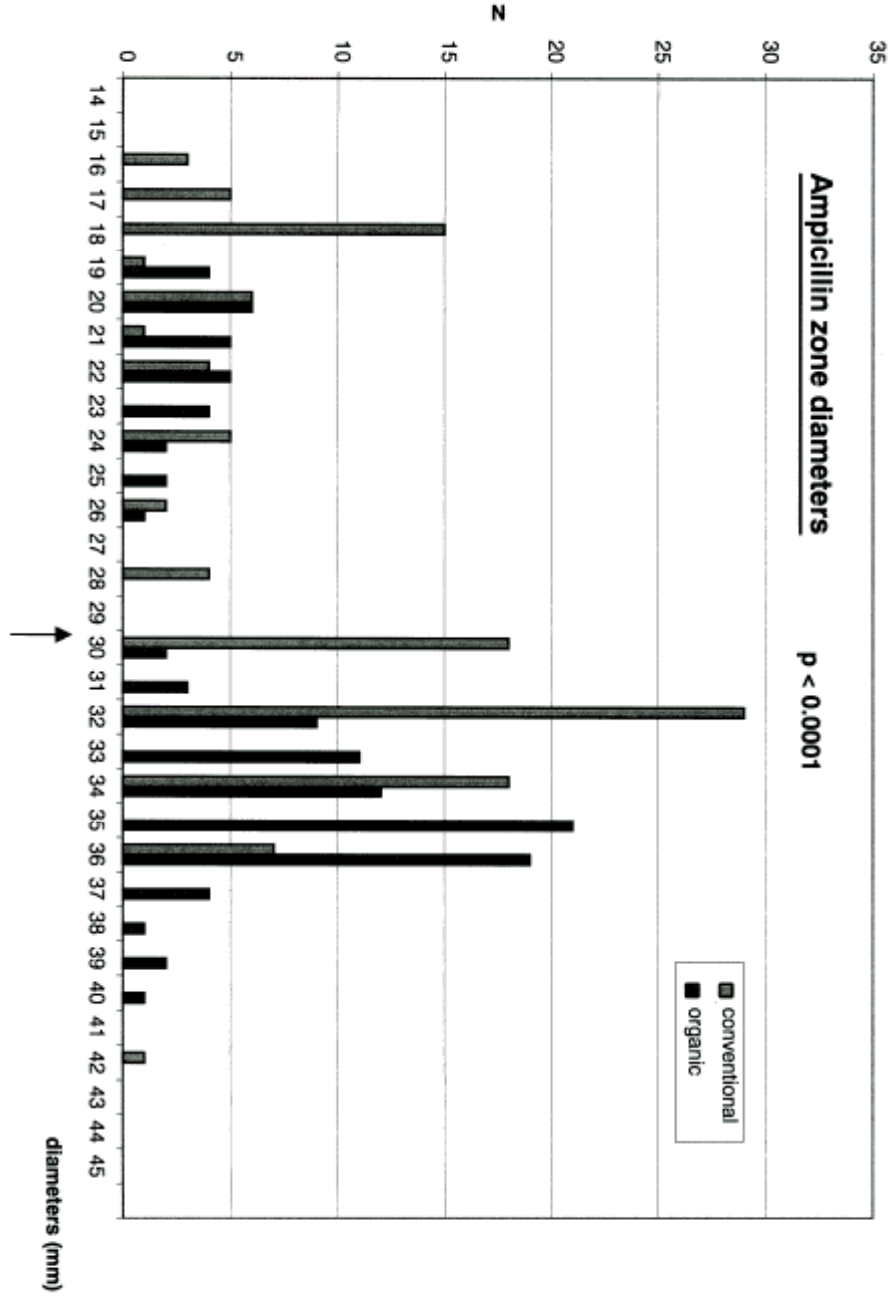


Figure 2

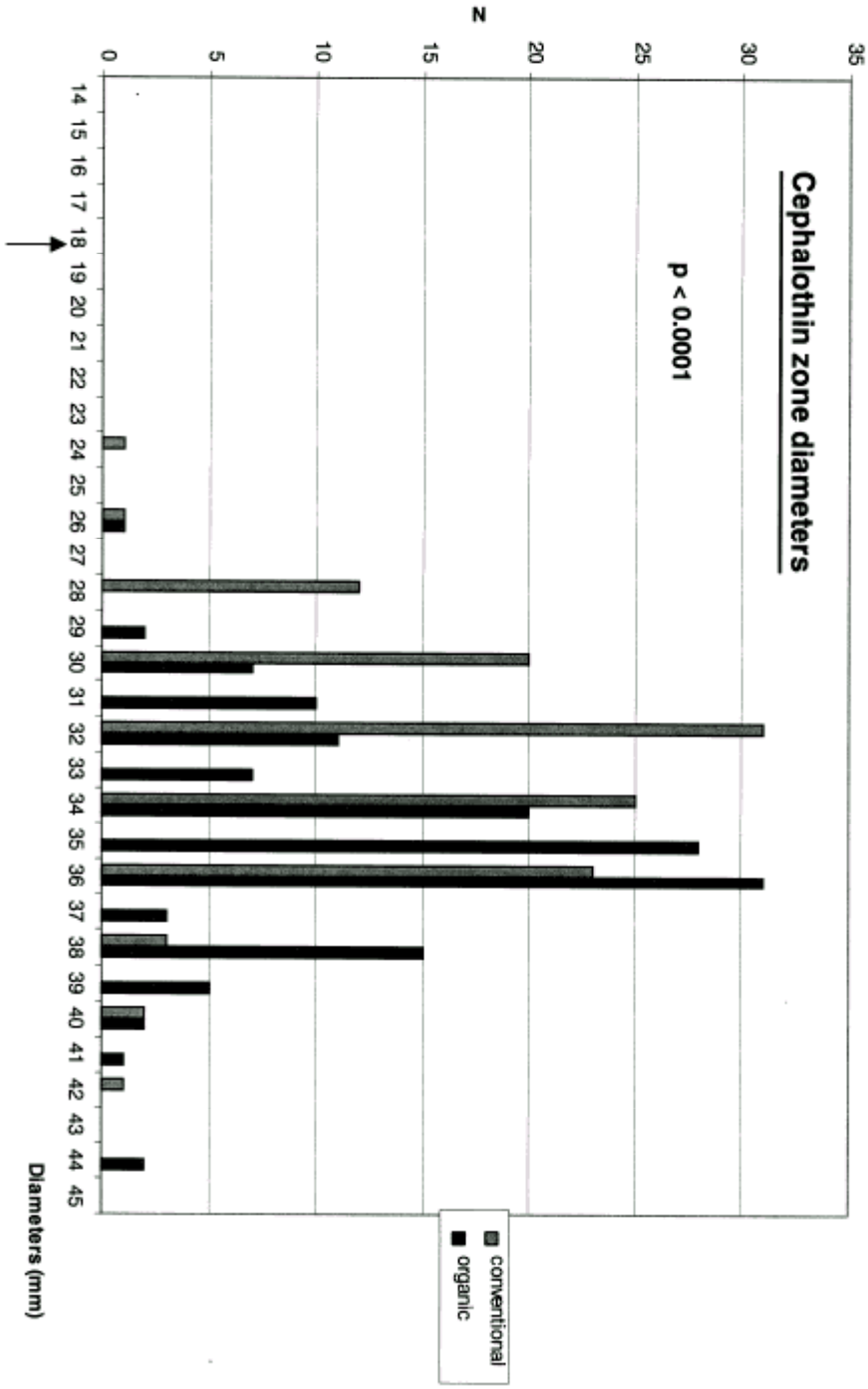

Cephalothin zone diameters
p < 0.0001
N
0
5
10
15
20
25
30
35
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Diameters (mm)
conventional
organic

Figure 3

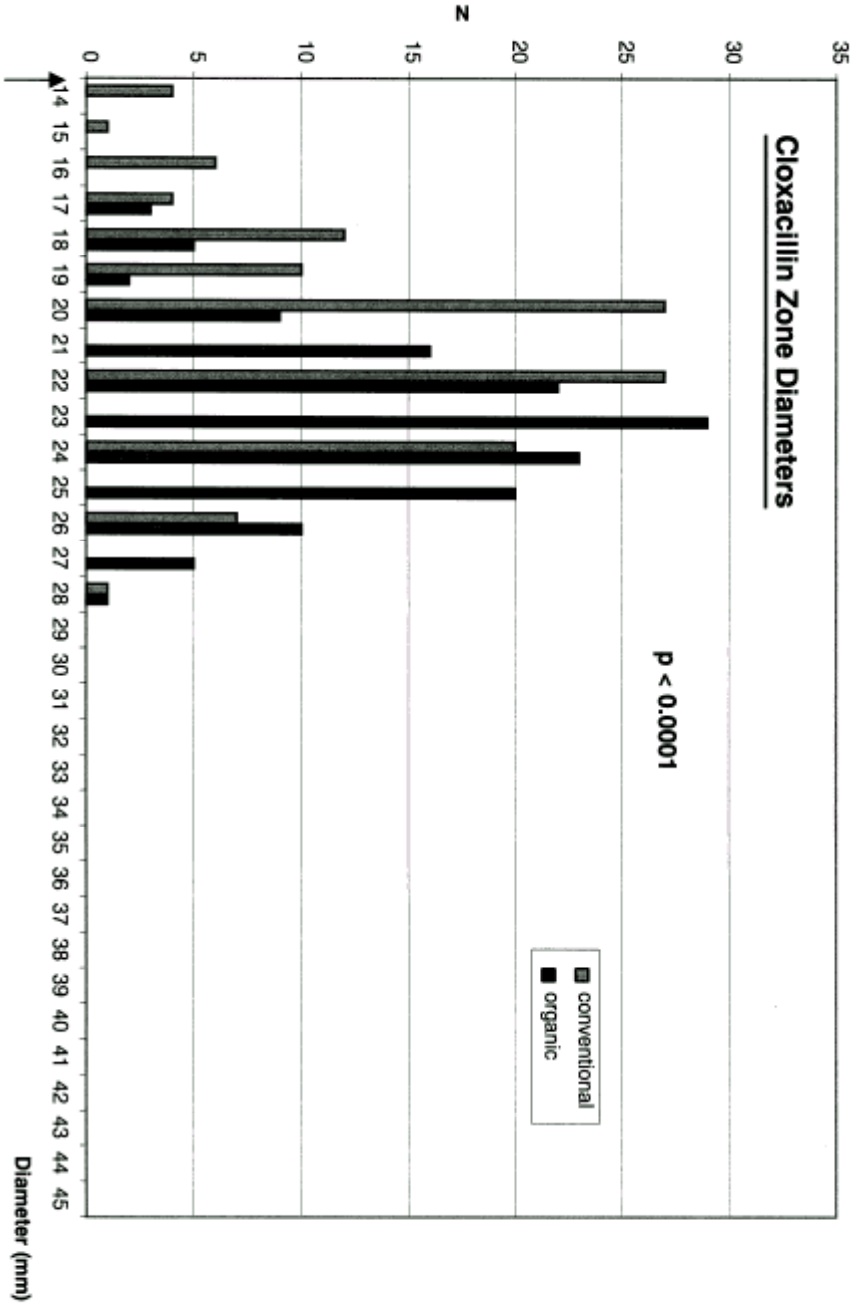

Cloxacillin Zone Diameters
N
0
5
10
15
20
25
30
35
p < 0.0001
conventional
organic
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Diameter (mm)

Figure 4

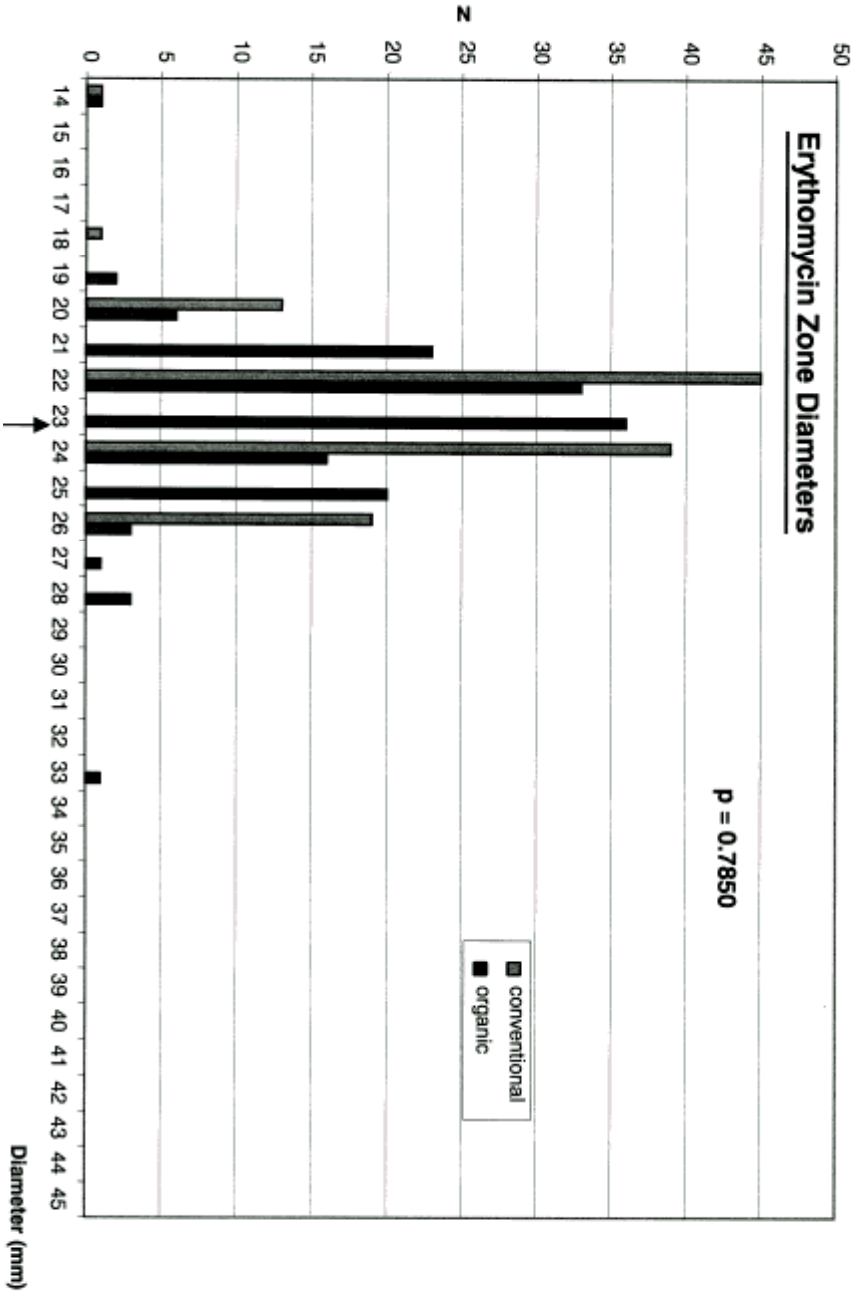

Erythomycin Zone Diameters
p = 0.7850
N
conventional
organic
Diameter (mm)

Figure 5

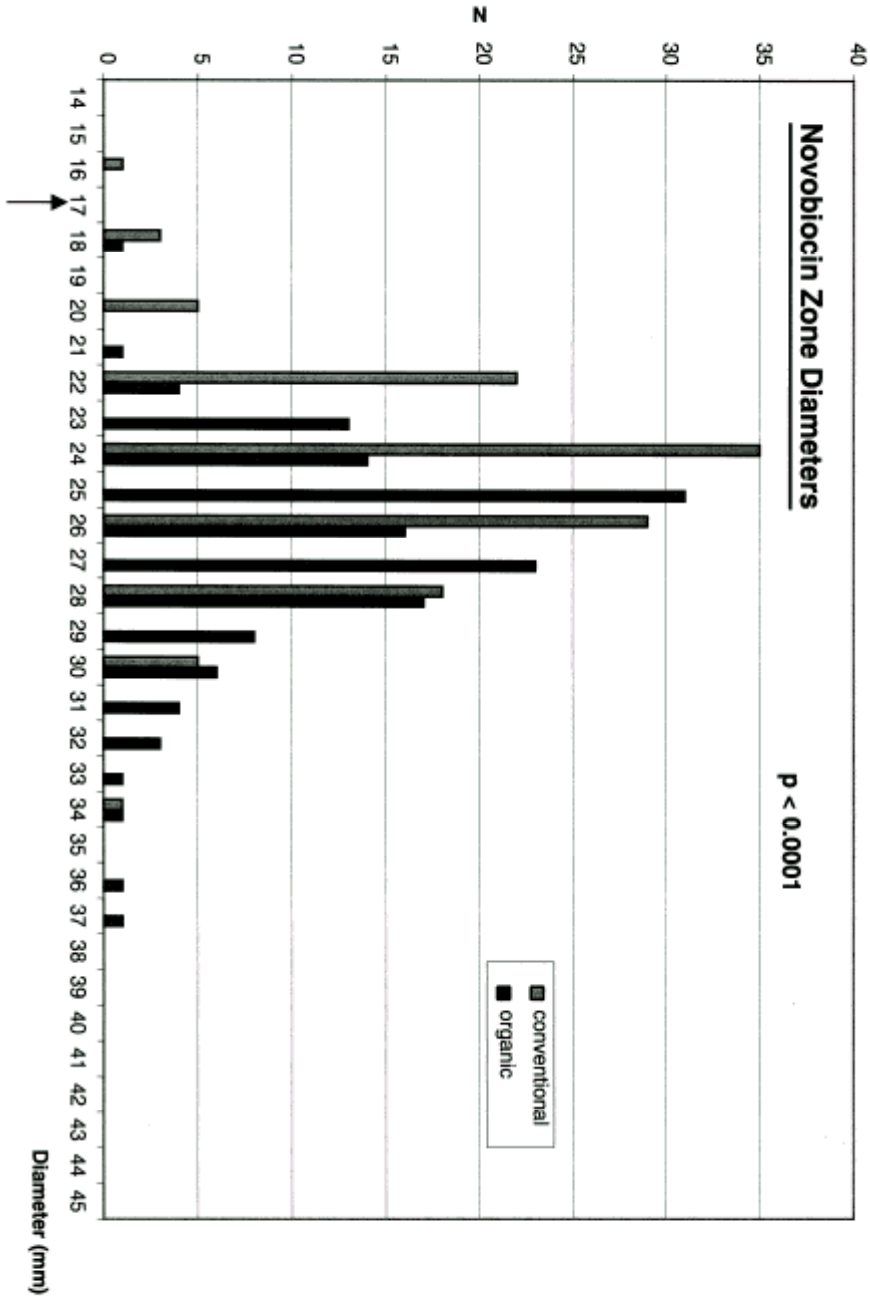

Novobiocin Zone Diameters
p < 0.0001
N
conventional
organic
Diameter (mm)

Figure 6

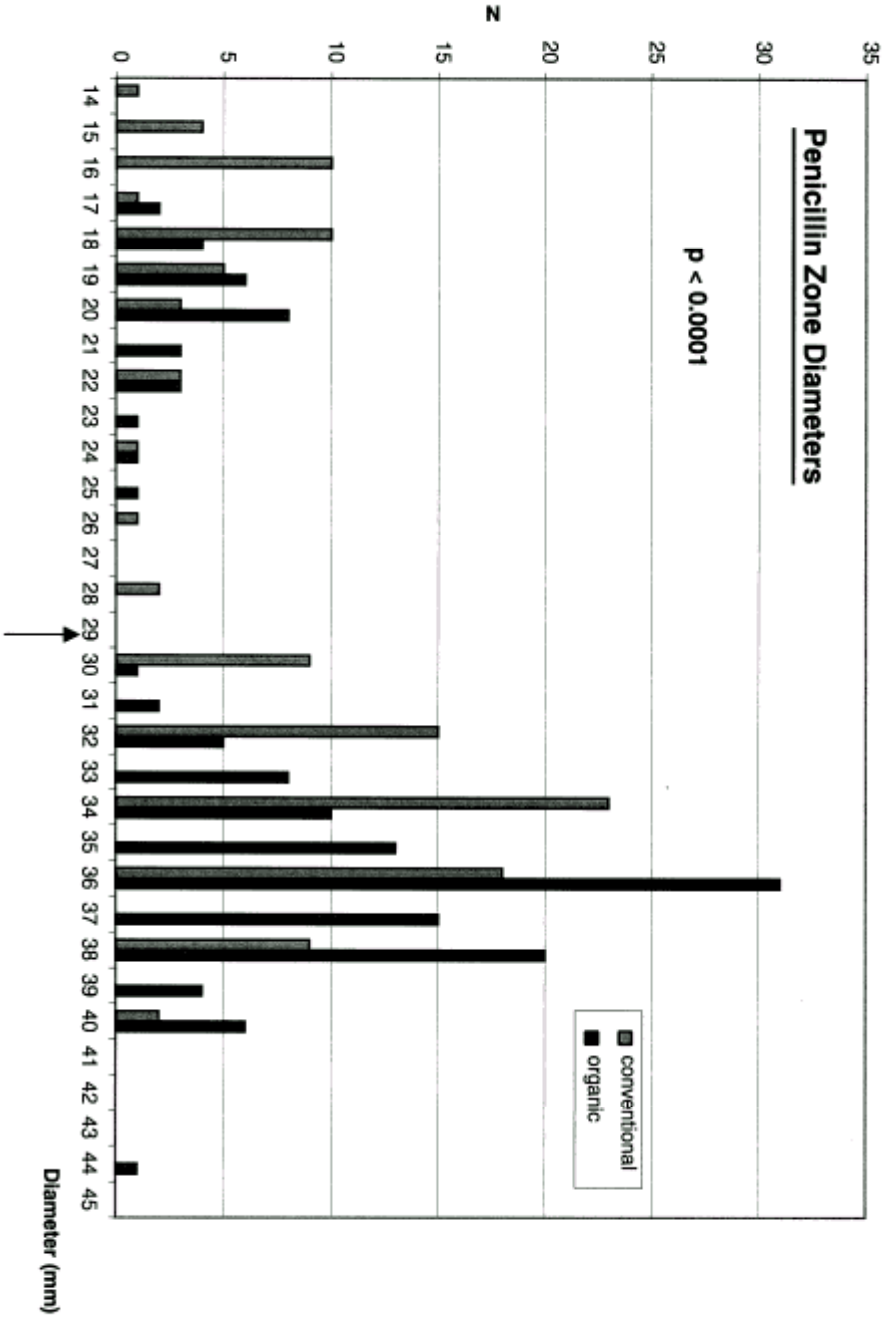

Penicillin Zone Diameters
p < 0.0001
N
0
5
10
15
20
25
30
35
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Diameter (mm)
conventional
organic

Figure 7

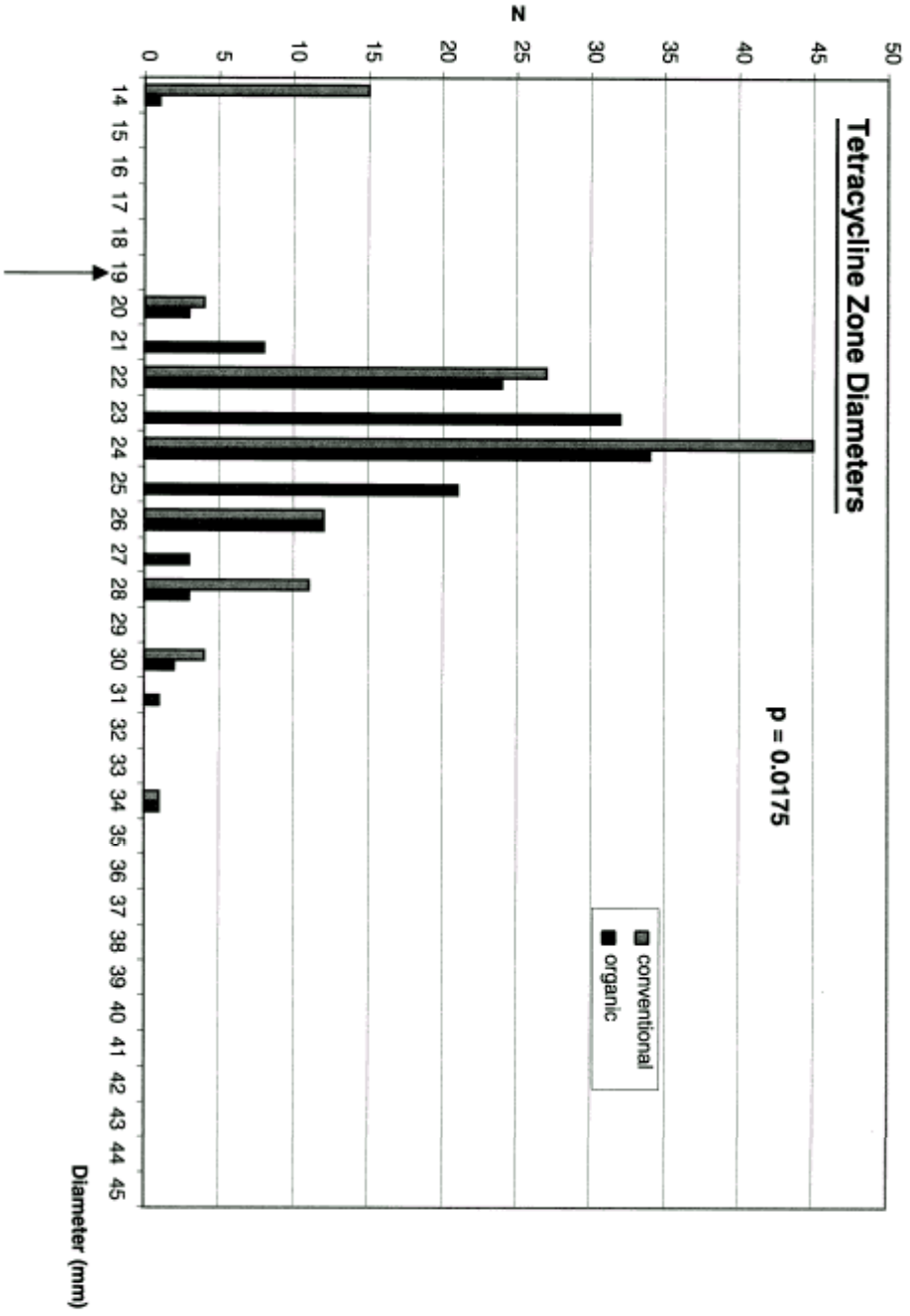

Tetracycline Zone Diameters
p = 0.0175
N
conventional
organic
Diameter (mm)

Figure 8

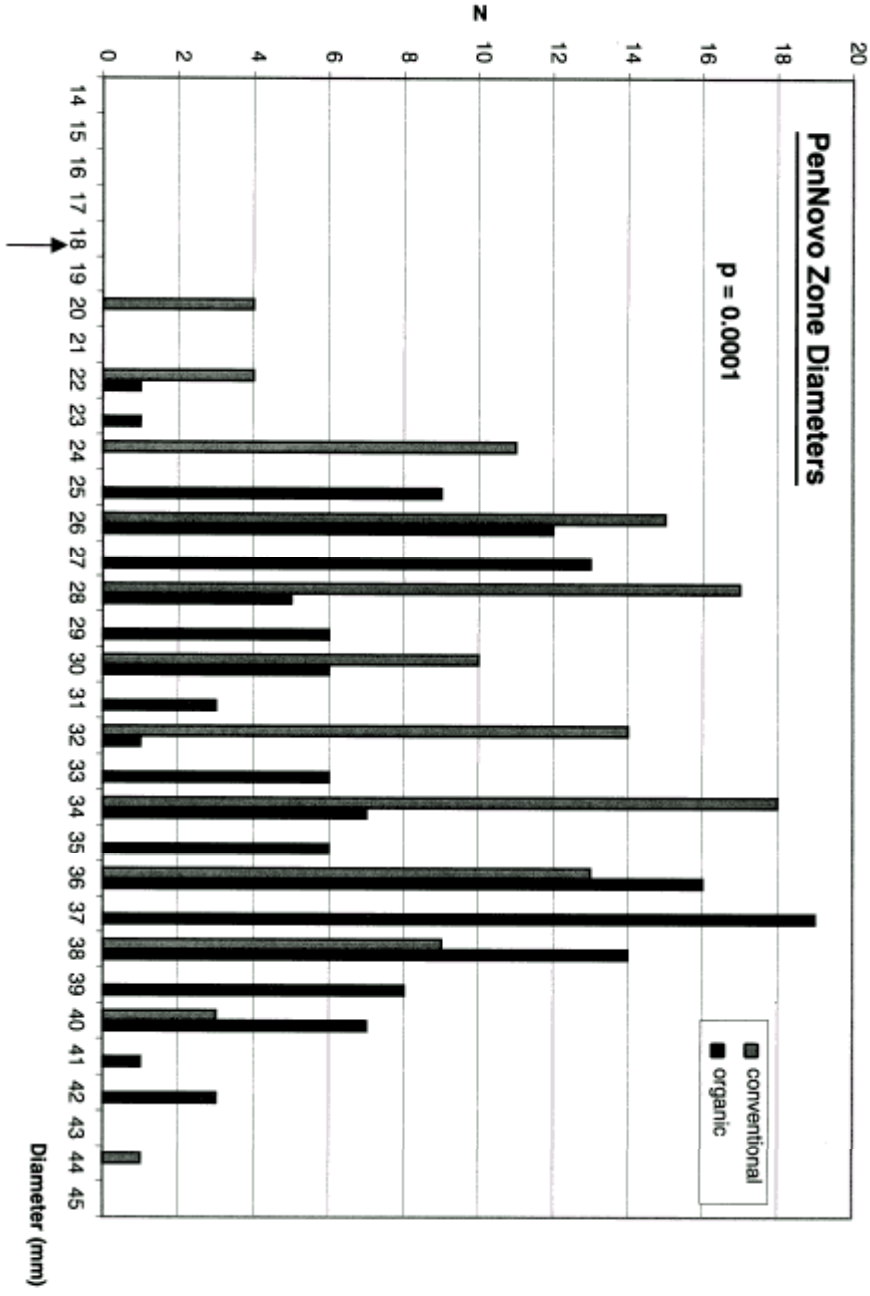

PenNovo Zone Diameters
p = 0.0001
N
conventional
organic
Diameter (mm)

Figure 9

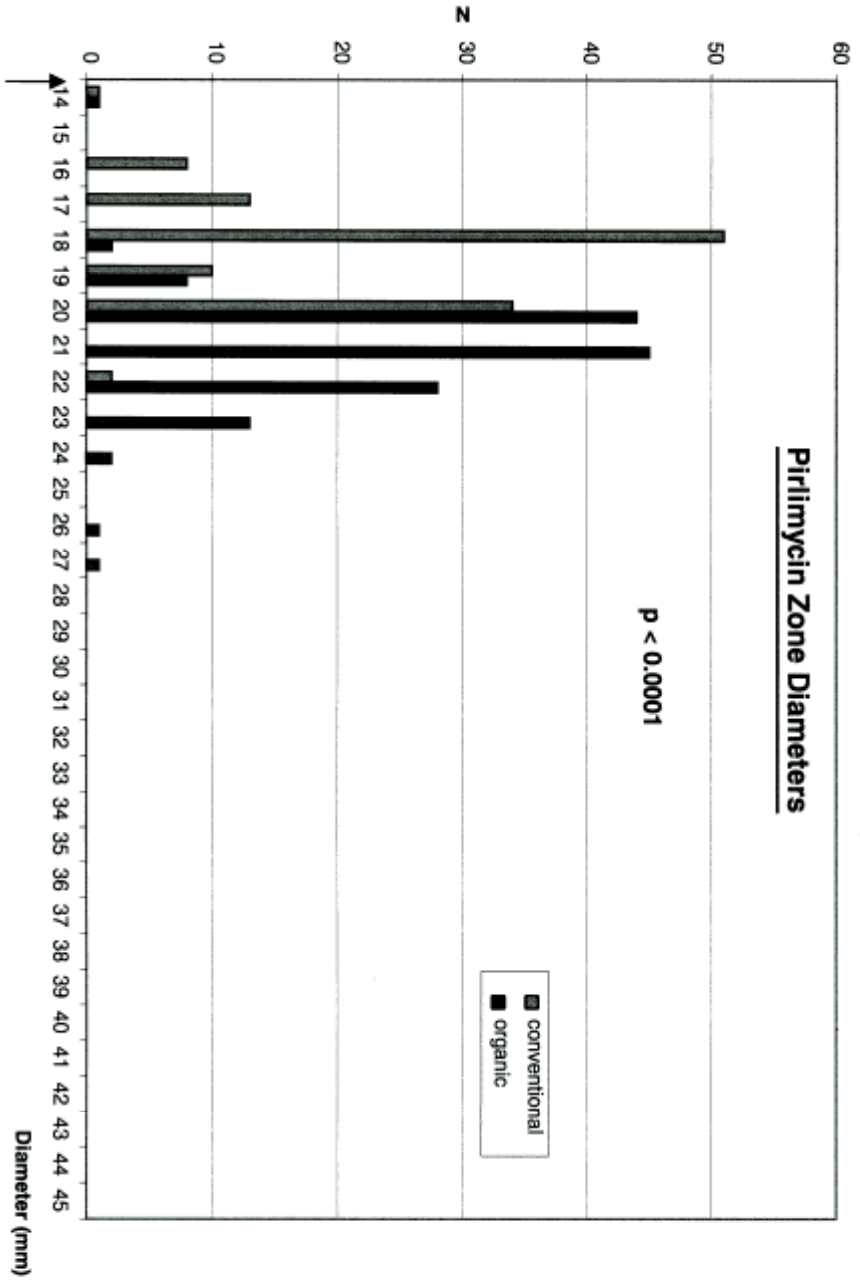

Pirlimycin Zone Diameters
p < 0.0001
N
0
10
20
30
40
50
60
conventional
organic
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Diameter (mm)

Figure 10

ix

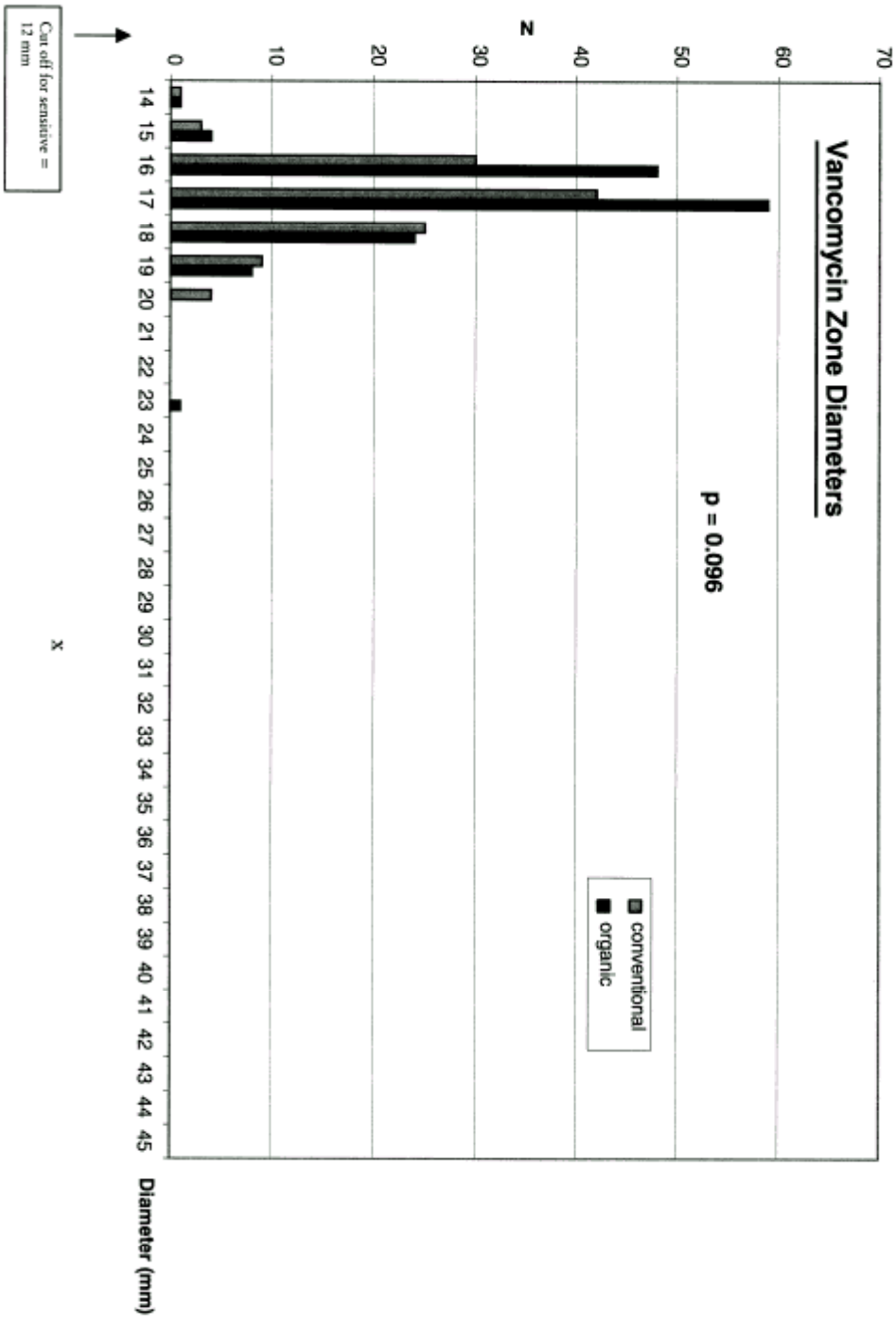

Vancomycin Zone Diameters
p = 0.096
N
0
10
20
30
40
50
60
70
conventional
organic
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
Diameter (mm)
Cut off for sensitive = 12 mm
x

Figure 11