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Comparison of Antibiotic Susceptibility Patterns of Selected Bacterial Species from Bovine, Agricultural and Human Sources

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Abstract

Objective: This study evaluates the antimicrobial susceptibility of bacteria from bovine mastitis, human and agricultural sources and compares the incidence of antimicrobial resistance in bacteria from these divergent sources.

Samples: Bovine bacterial isolates were obtained from clinical milks samples. Human isolates were obtained from a local municipal hospital, and agricultural isolates were obtained from water, and soil samples from agricultural land.

Procedures: All organisms were identified using standard microbiological procedures. *Enterobacteriaceae* were identified using the API 20E system. Staphylococcus species were identified using the API STAPH-TRAC System. Disk diffusion antimicrobial susceptibility testing, and susceptible or resistant determinations were performed following the guidelines established by the Clinical Laboratory Standards Institute. A total of 138 bovine isolates, 84 human isolates, and 82 agricultural isolates were evaluated.

Results: *Staphylococcus aureus* isolates from humans were more resistant to 11 of the 17 antibiotics tested than *S. aureus* isolated from cases of bovine mastitis. *Staphylococcus aureus* isolates from bovine mastitis were less or equally resistant to all of the antibiotics tested than *S. aureus* isolates from humans with the exception of tetracycline. *Staphylococcus* species had similar results with 12 of 17 human isolates more resistant than agricultural or bovine isolates. *Staphylococcus* species from agricultural sources had a higher percentage resistance for clindamycin and oxacillin. *Escherichia coli* isolates from humans were more resistant to 5 of the 14 tested antibiotics than isolates from bovine mastitis or the agricultural environment. *Escherichia coli* isolates from bovine mastitis were more resistant than agricultural and human isolates for tetracycline only. Both *Klebsiella pneumoniae* and *Enterobacter* species from humans were more resistant to most antibiotics tested than isolated from the agricultural environment or cows.

Conclusions: Antimicrobial resistance to antibiotics is a major human and veterinary issue. Use of antibiotics in agriculture has been implicated in the increasing incidence of resistance observed; however, there is disagreement among researchers on the role of agricultural practices in this increase. Results indicate that bacterial isolates from bovine mastitis and the agricultural environment are similar in resistance incidence to tested antibiotics and are both less resistant to most commonly used antibiotics than human isolates. Continued monitoring of bacteria is warranted to determine relation between agricultural activities and increases in resistance.

Keywords: Antibiotic susceptibility; Bacterial species; Bovine

Introduction

Bacterial resistance to antibiotics is a major concern for both human health and production agriculture. Resistant bacteria are on the increase and have become a significant human health issue. Researchers agree that overuse and misuse of antibiotics in humans play a major role in the increase of resistant bacteria [1-4]. There is less certainty in the scientific community concerning the impact of antibiotic use in animals on bacterial resistance to antibiotics [5,6]. Considerable effort has been expended in recent years to determine the extent of agriculture's role and what measures can be taken and are needed to mitigate the problem. The FDA has published guidelines to help prevent antimicrobial resistance that may result from antibiotic use in animals [1,6,7]. These guidelines provide a scientific process for assessing the likelihood that an antibiotic used to treat an animal might cause antimicrobial resistance problems in humans.

Antibiotic resistant bacteria from animals or in the environment can colonize humans by occupational exposure, via the food chain or from waste water runoff. The rate of this colonization, or how often and how easily animal strains adapt to and become human strains is unknown. Researchers in Japan evaluating *Staphylococcus aureus* strains from bovine mastitis and humans found no common pulsotypes among isolates from humans and bulk tank milk suggesting no epidemiological association [8]. Animal strains of *Escherichia coli* and other enteric pathogens have been implicated in food borne illness, and studies have shown that some multidrug-resistant *E. coli* and *Salmonella* species may have animal origins [9-12]. Most resistance in humans is generated by overuse of antibiotics to treat disease not by agricultural practices [13-15]. The idea that banning antibiotic use in animals represents a viable solution is unlikely. Avoparcin, a related compound to vancomycin, has been widely used as a growth promoter in poultry, and has been linked to vancomycin resistance in enterococci. However, the highest incidence of vancomycin resistant enterococci (VRE) is in the United States, and avoparcin has never been approved for use here. Thus the high incidence of VRE in this country seems to be more related to human use of vancomycin [4]. Recently, quinupristin/dalfopristin, has been introduced for human therapeutic use after 25 years of agricultural use of virginiamycin, a related compound. Despite widespread resistance in animal isolates of Enterococcus to virginiamycin there was rare resistance in humans suggesting no crossover from animals to humans in this case [16]. There is a need for continual monitoring of human, animal, and environmental bacterial species to determine the degree of antimicrobial resistance, which bacterial species and antibiotics are involved and the possible contribution of agriculture.

This study evaluates the antimicrobial susceptibility of bacteria from human, bovine mastitis and agricultural sources and compares the

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incidence of antimicrobial resistance in bacteria from these divergent sources.

Materials and Methods

The Mastitis Research Laboratory at the Hill Farm Research Station processes milk samples from across the state of Louisiana and the United States to identify bacterial causes of bovine mastitis. In addition, the laboratory is involved in water quality and environmental studies that provide bacterial species from the agricultural environment including poultry and dairy waste. A close relationship with the local hospital clinical laboratory provides access to human bacterial pathogens from a wide range of clinical specimens. This cross section of available organisms affords a unique opportunity to monitor antimicrobial susceptibility of a variety of bacterial species from human, bovine and agricultural sources.

Bovine bacterial isolates were obtained from clinical milks samples submitted to the Mastitis Research Laboratory at the Hill Farm Research Station. Clinical samples represented 58 dairies from 12 states. Human isolates were obtained from the microbiology laboratory at a local 45 bed community hospital, and agricultural isolates were obtained from soil and water collected at the Hill Farm Research Station. All organisms were identified using standard microbiological procedures as outlined by NMC [17]. Enterobacteriaceae were identified using the API 20E System, and the staphylococci were identified using the API STAPH-TRAC System. This system has been extensively analyzed and proven to be an excellent method for identification of bacterial isolates from human, veterinary and agricultural sources [18]. In addition, this biochemical method provides a numerical code that allows differentiation of strains and helps avoid duplication of strains within collection of organisms. Bacterial isolates were stored frozen at -20 degrees C in trypticase soy broth with 20% glycerin. Prior to susceptibility testing bacterial isolates was sub cultured to trypticase soy agar plate supplemented with 5% bovine blood and incubated for 18 to 24 hrs.

Disk diffusion antimicrobial susceptibility testing was performed following the guidelines established by the Clinical Laboratory Standards Institute [19]. All tests were performed on Mueller-Hinton II agar. Zones were measured in millimeters and determinations of susceptibility or resistance were made using CLSI zone interpretive recommendations for humans except for the veterinary antiboitics pirlimycin, penicillin/novobiocin and cefitiofur where bovine recommendations were used. Statistical analysis compared mean zone diameters in millimeters for each antibiotic from human, environmental and bovine isolates using ANOVA. Comparisons were made between the same antibiotics for the same organisms across the different sample sources. For example, the mean zone diameters to oxacillin for *S. aureus* from humans were compared to the mean zone diameters to oxacillin for *S. aureus* from bovine mastitis and so forth.

Results

Disc diffusion susceptibility results indicate that for most of the antibiotics tested a higher percentage of human isolates were resistant than either bovine or agricultural isolates. Table 1 shows percent resistance for organisms tested in this study. *Staphylococcus aureus* isolates from humans were more resistant than bovine isolates for 11 of the 17 antibiotics tested. No *S. aureus* isolates were obtained from the agricultural samples. Results of a statistical comparison of mean disc diameters for the organisms tested are in Table 2. All of the zone diameters from *S. aureus* isolate from cows and humans to beta lactam antibiotics except linezolid were different. No statistical zone differences for *S. aureus* from humans and cows were noted to gentamicin, linezolid, tetracycline, tigecycline, and vancomycin. No methcillin (oxacillin) resistant *S. aureus* were noted in bovine isolates compared to 63% of human isolates. Staphylococcus species had similar results with 12 of 17 antibiotics having greater percentage resistance and having statistically different mean zone diameters among human, agricultural and bovine isolates. In most cases the zone diameters from human isolates were smaller than both the bovine and agricultural isolates but the bovine and agricultural isolates were not statistically different from each other. This trend was reversed for clindamycin and oxacillin with *Staphylococcus* species from agricultural sources having a higher percentage resistance.

Antibiotic	*Staph. aureus*		*Escherichia coli*			*Klebsiella pneumoniae*			*Enterobacter species*			*Staphylococcus species*		
	Human (22)	**Bovine (45)**	**Human (35)**	**Bovine (42)**	**Agri. (15)**	**Human (6)**	**Bovine (18)**	**Agri. (15)**	**Human (8)**	**Bovine (7)**	**Agri. (48)**	**Human (11)**	**Bovine (17)**	**Agri. (12)**
ampicillin	*	*	74.3	19	25	100	100	100	100	57	61.7	*	*	*
azithromycin	90.9	0	*	*	*	*	*	*	*	*	*	90.9	0	0
aztreonam	*	*	0	4.8	0	50	5.6	0	71.4	0	2.1	*	*	*
cephalothin	18.2	0	71.4	64.3	75	83.3	16.7	6.7	100	42.9	68.8	27.3	0	0
ceftazidime	*	*	2.9	4.7	0	33.3	5.6	0	71.4	0	2.1	*	*	*
clindamycin	36.4	2.2	*	*	*	*	*	*	*	*	*	54.5	29.4	83.3
ceftiofur	77.3	0	8.6	4.8	0	50	11.1	6.7	80	14.3	2.1	81.8	0	0
erythromycin	99.5	11.1	*	*	*	*	*	*	*	*	*	90.9	11.8	16.7

gentamicin	9	0	20	0	0	0	0	0	12.5	0	0	9.1	0	0
imipenem	*	*	0	0	0	16.7	0	0	0	0	0	*	*	*
levofloxacin	63.6	0	51.4	0	0	16.7	0	0	50	0	2.1	54.5	0	0
linezolid	0	0	*	*	*	*	*	*	*	*	*	0	0	0
oxacillin	63	0	*	*	*	*	*	*	*	*	*	81.8	11.7	83.8
penicillin	63	0	100	100	100	100	100	100	100	100	100	90.9	29.4	69.2
penicillin/ novo	0	0	97	97	100	100	94.7	100	100	100	100	0	0	0
pirlimycin	27.3	2.2	*	*	*	*	*	*	*	*	*	27.3	11.8	0
piperacillin/ tazo	63.6	0	0	2.4	0	83.3	5.6	6.7	25	14.3	0	36.4	0	0
SXT	4.5	0	40	2.4	0	33.3	11.1	0	50	0	0	0	27.3	0
tigecycline	0	0	0	2.4	0	50	38.9	20	28.6	14.3	2.1	0	0	8.3
tetracycline	0	6.7	31.4	42.9	12.5	83.3	27.8	46. 7	62.5	14.3	10. 4	27.3	17.6	0
vancomycin	0	0	*	*	*	*	*	*	*	*	*	0	0	0

Table 1: Comparison of percent resistance of selected bacterial species from human, bovine, and agricultural sources *Indicates antibiotics that were either gram positive or gram negative specific.

Antibiotic	*Staph. aureus*		*Escherichia coli*			*Klebsiella pneumoniae*			*Enterobacter species*			*Staphylococcus species*		
	Human (22)	**Bovine (45)**	**Human (35)**	**Bovine (42)**	**Agri. (15)**	**Human (6)**	**Bovine (18)**	**Agri. (15)**	**Human (8)**	**Bovine (7)**	**Agri. (48)**	**Human (11)**	**Bovine (17)**	**Agri. (12)**
ampicillin	*	*	10.8 a	17.3 b	17.4 b	6.7	9	9.7	6.9 a	12.7 b	13.9 b	*	*	*
azithromycin	8.4 a	23 b	*	*	*	*	*	*	*	*	*	8.7 a	22.2 b	21.5 b
aztreonam	*	*	33	28.5	29	20.8 a	29.1 b	28.2 b	15.6 a	29.4 b	29.1 b	*	*	*
cephalothin	22.3 a	35.8 b	15.6	16.3	16.1	12.8 a	19.3 b	20.2 b	8.1 a	14.9 b	12.8 b	24.8 a	35.5 b	32.8 b
ceftazidime	*	*	28.6	25.7	27.1	18.7 a	25.5 b	24.5 b	13.6 a	26.6 b	26.7 b	*	*	*
clindamycin	20.9 a	24.9 b	*	*	*	*	*	*	*	*	*	19.1 b	22 b	17 a
ceftiofur	14.5 a	30.8 b	22.8	24.1	23.8	17.2	22.9	22.5	16.8 a	23.4 b	24.6 b	16.5 a	28.3 b	25.1 b
erythromycin	10.2 a	24.8 b	*	*	*	*	*	*	*	*	*	9.6 a	24.1 b	25 b
gentamicin	19.8	21.9	19.6	20.1	19.9	20.2	19.6	21.5	19.8	19.4	23.1	24.7	24	23.2
imipenem	*	*	29.8	27	25.7	21.2	25.1	25.1	25.9	26.7	25.6	*	*	*
levofloxacin	16.7 a	28.2 b	20.1 a	29.2 b	28.8 b	22.2 a	27 b	26.9 b	17.9 a	29.1 b	29.6 b	17.3 a	27.9 b	24.8 b

linezolid	28	27.3	*	*	*	*	*	*	*	*	*	33.2 a	28.3 b	27.3 b
oxacillin	10.5 a	22.2 b	*	*	*	*	*	*	*	*	*	9.9 a	21.1 b	16 b
penicillin	9.9 a	31.7 b	6.9	8.1	7.2	6	6.4	6.4	8.4	7	7.4	13.9 a	32.5 b	26.1 b
penicillin/ novo	30 a	36.9 b	11.3	12.1	11.4	12.3	12.4	11.3	11.2	8.4	10.5	31.4	36.6	27
pirlimycin	15.9 a	21.5 b	*	*	*	*	*	*	*	*	*	18	20.5	20.2
piperacillin/ tazo	15.9 a	31.6 b	27.3	24.8	25	17.7a	23.7b	22.9 b	21.8 a	24.6 b	26 b	23	32	28.8
SXT	15.9 a	27.4 b	18.5 a	25 b	25.3 b	17.3 a	22 b	23.8 b	17.9 a	27.1 b	27.5 b	18.7 a	25.2 b	27.7 b
tigecycline	15.9	21.8	22	22.1	22	19	19.8	20.1	20.1 a	20.9 b	22.3 b	26.9	21.1	21.5
tetracycline	15.9	24.8	17	16.7	18.2	15	17.8	19.1	16.1	18.6	21.3	21.9	22.1	24.7
vancomycin	15.9	17	*	*	*	*	*	*	*	*	*	19.8	16.9	17.3

Table 2: Statistical evaluation of mean zone diameters (in millimeters) of selected bacterial species from human, bovine and agricultural sources a,b - Values within an organism with different letters are different for that antibiotic,(P<.01) *indicates antibiotics that were either gram positive or gram negative specific.

When differences existed among the gram negative organisms tested, (*Escherichia coli*, *Klebsiella pneumoniae* and Enterobacter species) isolates from humans were all more resistant than isolates from the agricultural environment and from cows except for tetracycline against *E. coli*. Mean zone diameters for *Escherichia coli* isolates from humans were statistically smaller than isolates from cows and the agricultural environment for ampicillin, levofloxacin and SXT. As was the case with *S. aureus*, zone diameters from most agricultural and bovine isolates were different from human isolates but not from each other. Both *K. pneumoniae* and *Enterobacter* species from humans were more resistant to aztreonam, ceftazidime cephalothin, levofloxacin, tetracycline, and SXT than isolates from the agricultural environment and cows. In addition, *Enterobacter* species from humans were also more resistant to ampicillin ceftiofur, tigecycline and piperacillin/tazobactam.

Discussion

Results from this study using interpretive standards of resistance established by CLSI revealed a much higher percentage resistance to tested antibiotics in isolates from humans than did similar organisms from cattle and the agricultural environment. Also, mean disc zone diameters from the organisms tested from humans were significantly smaller for many of the antibiotics tested than similar organisms from cows or the agricultural environment.

Antibiotics commonly used in cattle to treat *S. aureus* mastitis and other gram positive infections include ceftiofur, penicillin/novobiocin, pirlimycin, cephalothin and tetracycline. Interestingly, there was minimal resistance (in most cases 0%) to these antibiotics in *S. aureus* isolates from cattle or the agricultural environment, while resistance was high in isolates from humans. This suggests that the high resistance to these antibiotics in organisms from humans is not originating from use of these antibiotics in cattle. Overall, there was little indication that antibiotic use in bovine mastitis is resulting in increased resistance in organisms isolated from cattle. This supports similar results reported by Erskine [20]. Results for tetracycline against *E. coli* and *S. aureus* showed increased resistance in bovine isolates compared to human isolates; however, the mean zone diameters were not significantly different. There is substantial data to suggest that most antibiotic resistance in humans is the result of overuse or misuse of antibiotics to treat human disease not by agricultural practices [9,13,16]. These data would indicate that the suggestion of banning antibiotic use in animals to reduce the development of antibiotic resistant bacteria represents a viable solution is unlikely. As stated previously, Avoparcin, a compound related to vancomycin, has been widely used in other countries as a growth promoter in poultry, and has been linked to vancomycin resistance in Enterococcus species. However, the highest incidence of VRE is in the United States, and avoparcin has never been approved for use here. The high incidence of VRE in this country seems to be more related to human use of vancomycin [15]. Quinupristin/dalfopristin has been introduced for human therapeutic use after 25 years of agricultural use of virginiamycin, a related compound. Despite widespread resistance in animal isolates of *Enterococcus faecium* to virginiamycin there was rare resistance in isolates from humans suggesting no crossover from animals to humans in this case [16].

A number of publications have been generated in recent years with guidance and recommendations from government agencies, scientific committees, and research scientists for dealing with development of antimicrobial resistance in bacteria present in the food chain. These studies and reports agree that the increased use of antibiotics increases the risk of emergence of organisms that are resistant to antibiotics and the use of antibiotic in food animals will likely result in development of resistant organisms in these animals. The incidence and spread of

disease as a result of this use is historically very low but data are seriously lacking in most areas. All of these studies and reports have in common the call for increased surveillance [2-4,13-15,21-27]. There is a need for continual monitoring of human, animal, and environmental bacterial species to determine the degree of antimicrobial resistance, which bacterial species and antibiotics are involved, and the possible contribution of agriculture.

Additional studies are needed to monitor resistance in cattle, humans and the agricultural environment to determine the impact of agricultural practices on antimicrobial resistance and the potential impact on human and animal health.

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