MIC's for Selected Antimicrobials Against *Escherichia coli.* Recovered From Various Animal Species in Zimbabwe Compared With Isolates From Australia and the United States

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Abstract

The minimal inhibitory concentrations (MIC) of selected antimicrobials were determined for isolates of *Escherichia coli* obtained from various animal species in Australia, U.S.A. and Zimbabwe. The MIC medians were compared between locations by antimicrobic using two nonparametric tests, the Median and Kruskal-Wallis test. Typically, isolates from Zimbabwe were more sensitive ($p \leq 0.01$) to several of the antimicrobials than were isolates from Australia and the U.S.A. There were few distinct or consistent differences between isolates from Australia and the U.S.A.

Introduction

There are numerous reports linking antimicrobial usage with changes in susceptibility of bacteria. Increased resistance with respect to antimicrobial usage in human hospitals has been shown for example, for *E. coli* (Atkinson, 1986, Courcol *et al.*, 1989) *Pseudomonas* (Courcol *et al.*, 1989) and *Salmonella* (Voogd *et al.*, 1977 Ryder *et al.*, 1980). Studies in animals similarly indicate the occurrence of marked changes in susceptibility with prolonged use of antimicrobials for a variety of bacteria, including *E. coli* (Siegel *et al.*, 1974 Smith, 1980), *Actinobacillus* (Vailancourt *et al.*, 1988), *Pasteurella* (Fales *et al.*, 1981) and gram-negative bacteria in general (Gellin *et al.*, 1989). These changes over time, ranging from 5 to 15 years, are of importance in therapy of infectious diseases and because of concerns regarding possible linkage between use of antimicrobials in animals and resistance in man have resulted in recommendations to curtail the use of such drugs in animals (Report, 1969).

Similar comparisons between geographical locations for specific bacteria are more difficult to demonstrate for a number of reasons including variations in methodology (Atkinson, 1986). Another factor limiting comparisons is the use of agar diffusion methods (Bauer *et al.*, 1966) because such qualitative tests require relatively large changes in susceptibility for detection of differences.

With the advent and availability of commercially prepared trays containing antimicrobial dilutions, the use of minimal inhibitory concentration (MIC) antimicrobic susceptibility testing is becoming more widely accepted in veterinary medicine (Reeves *et al.*, 1980; Franklin and Wierup, 1982; Fales and Burrows, 1983). Facilitated by the development of computerized interpretation of MIC results (Wertz and Swartzberg, 1981) such testing is performed routinely at many veterinary diagnostic laboratories as a guide to antimicrobic selection and dosage. An additional benefit to the increasing availability of quantitative susceptibility results lies in their use for assessment of changes in sensitivity or development of resistance. This is particularly important in developing countries where antimicrobial usage may be expected to increase markedly in future years. The opportunity to compare sensitivity profiles for various bacterial pathogens be-

came available to the senior author (GEB) during sabbatic leave visits to Zimbabwe and Australia in 1986/1987. The present study was undertaken to provide a comparison of antimicrobic sensitivity between countries with divergent drug availability and use patterns.

Materials and Methods

Bacterial isolates

Specimens for antimicrobial testing in Zimbabwe were obtained from routine submissions to the Veterinary Research Laboratory, Harare, from July 1986 through April 1987. In Australia, bacterial isolates were obtained from several sources including the Department of Animal Health, University of Sydney, Camden, New South Wales and the Central Veterinary Laboratory, State Department of Agriculture, Glenfield, New South Wales. The isolates were obtained from submissions received during 1985 through July 1987. Specimens for the U.S.A. were obtained from routine submissions to the Oklahoma Animal Disease Diagnostic Laboratory, College of Veterinary Medicine, Stillwater, Oklahoma, from July 1986 through December 1987.

Antimicrobial testing

Determination of the MIC for each isolate was carried out using a commercial microtitration method (Sensititre, Gibco Laboratories, Lawrence, Massachusetts, U.S.A.) as previous described (Fales and Burrows, 1983) The microtitration trays contained the following array of antimicrobials in a range of doubling μg/ml concentrations; ampicillin (.25-32), cephalothin (.5-64). erythromycin (.125-16), gentamicin (.25-16), kanamycin (.5-32), oxytetracycline (.25-32), penicillin G (.125-16), spectinomycin (.75-96), sulphachlorpyridazine (12.5-400), sulphadimethoxine (12.5-400), trimethoprim-sulphamethoxazole (.25/4.75-32/608), and tylosin (.31-40).

Isolates were inoculated to brain-heart infusion broth and allowed to grow to achieve a turbidity of a 0.5 nephelometer tube (McFarland Scale). Ten μl of inoculum was transferred to 12 ml of Mueller-Hinton broth and the 96 well microtitration tray inoculated with an 8 channel pipette delivering 50 μl to each well. The trays were sealed with an adhesive plastic cover and incubated at 35C for 18 hours. The MIC was determined visually as the lowest concentration that prevented macroscopically detectable growth. The MIC of sulphonamides was recorded when 80% of the growth, with respect to controls, was inhibited.

Quality control testing

The trays were tested periodically with positive controls, using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 to insure that the antimicrobial activity of the trays remained within the limits of the test organisms. In addition, 2 test wells are available on each tray to evaluate bacterial growth as a negative control.

Statistical analysis

The distribution of MIC values for each location by organism and by antimicrobial combination appears to be highly skewed; usually in the same direction. For this reason, the center for each of the above distributions is represented by the median rather than the mean. Location comparisons for each organism by antimicrobial were carried out using nonparametric procedures: Kruskal-Wallis test and the Median Test (Conover, 1980) These nonparametric procedures are equivalent to one-way analysis followed by a means separation that is carried out routinely for bell-shaped distribution data. The Kruskal-Wallis test, the more powerful of the two, tests the null hypothesis that all three locations have MIC distribution functions that are identical. These location comparisons were carried out for each organism by antimicrobials for each animal species and for a composite of all species. Probability values less than 0.01 were considered significant (0.01 used rather than 0.05 because of the limitations in sample size).

Results

The MIC data for an array of antimicrobials are presented using two methods, with respect to different locations. In Tables 1&2, the data are presented as range and concentrations inhibiting 50% (MIC_{50}) and 90% (MIC_{90}) of the isolates. These results are based upon the type of routine submissions which would form the basis for recommendations forthcoming from laboratory diagnosticians. In this instance, there are consistent examples of increased sensitivity of bacterial isolates from Zimbabwe as compared to those from the U.S. and Australia. The distribution of MIC values for each location by antimicrobial combination appears to be highly skewed; usually in the same direction. For this reason, the center for each of the above distributions is represented by the median rather than the mean. The data are presented in Table 3 as median MIC's and interquartile range (IQR) to facilitate statistical analysis. Violation of the assumptions for the one-way ANOVA for comparisons of the MIC values between geographical locations is evident for these kinds of data especially with respect to normality and variance homogeneity. Thus two nonparametric tests, the Median test and the Kruskal-Wallis test, available in the NPAR1WAY procedure of SAS[b] are used to compare locations for each organism and each antimicrobial irrespective of animal species. In order for populations to be considered different,

Table 1. Comparison of MICs for various antimicrobials for *Escherichia coli,* isolated from cattle in Australia, the USA and Zimbabwe.

		Country		
Antimicrobial		Zimbabwe (60*)	Australia (13)	USA(27)
Ampicillin	Range	1->32	4->32	1->32
	MIC_{50}	4	8	>32
	MIC_{90}	>32	>32	>32
Cephalothin	Range	4->64	8->64	8->64
	MIC_{50}	16	64	32
	MIC_{90}	>64	>64	>64
Gentamicin	Range	≤.25-8	.5-4	.5>16
	MIC_{50}	.5	2	2
	MIC_{90}	2	4	16
Kanamycin	Range	≤.5->32	4->32	1->32
	MIC_{50}	4	8	>32
	MIC_{90}	16	16	>32
Oxytetracycline	Range	1->32	4->32	1->32
	MIC_{50}	4	32	>32
	MIC_{90}	>32	>32	>32
Penicillin G	Range	4->16	16->16	8->16
	MIC_{50}	>16	>16	>16
	MIC_{90}	>16	>16	>16
Spectinomycin	Range	12->96	12->96	12->96
	MIC_{50}	24	48	96
	MIC_{90}	>96	>96	>96
Sulphachlorpyridazine	Range	≤12.5->400	≤12.5->400	≤12.5->400
	MIC_{50}	100	>400	>400
	MIC_{90}	>400	>400	>400
Sulphadimethoxine	Range	≤12.5->400	25->400	25->400
	MIC_{50}	100	>400	>400
	MIC_{90}	>400	>400	>400
Trimethoprim/ Sulphamethoxasole	Range	≤.25-32 4.75-304	≤.25-2 4.75-38	≤.25->32 4.75 304
	MIC_{50}	.25	.5	.25
	MIC_{90}	1	2	>32

*Number of isolates tested.

Table 2. Comparison of MICs for various antimicrobials for *Escherichia coli,* isolated from animals in Australia, the USA and Zimbabwe.

		Country		
Antimicrobial		Zimbabwe(97)*	Australia(33)	USA(51)
Ampicillin	Range	1->32	1->32	1->32
	MIC_{50}	4	4	>32
	MIC_{90}	>32	>32	>32
Cephalothin	Range	4->64	4->64	4->64
	MIC_{50}	16	32	32
	MIC_{90}	>64	>64	>64
Gentamicin	Range	≤.25-8	.5-4	≤.25->16
	MIC_{50}	.5	2	1
	MIC_{90}	2	4	16
Kanamycin	Range	≤.5->32	2->32	1->32
	MIC_{50}	4	8	16
	MIC_{90}	16	16	>32
Oxytetracycline	Range	.5->32	4->32	1->32
	MIC_{50}	8	>32	>32
	MIC_{90}	>32	>32	>32
Penicillin G	Range	2->16	4->16	4->16
	MIC_{50}	>16	>16	>16
	MIC_{90}	>16	>16	>16
Spectinomycin	Range	6->96	12->96	12->96
	MIC_{50}	24	24	48
	MIC_{90}	>96	>96	>96
Sulphachlorpyridazine	Range	≤12.5->400	≤12.5->400	≤12.5->400
	MIC_{50}	200	>400	>400
	MIC_{90}	>400	>400	>400
Sulphadimethoxine	Range	≤12.5->400	≤12.5->400	25->400
	MIC_{50}	400	>400	>400
	MIC_{90}	>400	>400	>400
Trimethoprim/ Sulphamethoxazole	Range	≤.25->32 4.75-304	≤.25->32 4.75-304	≤.25->32
	MIC_{50}	≤.25	≤.25	≤.25
	MIC_{90}	1	2	32

*Number of isolates tested.

Table 3. Median, interquartile range (IQR) and P values for various antimicrobials against *Escherichia coli,* isolated from cattle in Australia, the USA and Zimbabwe.

	Country			P Values	
Antimicrobial and Isolate	Australia 33*	USA 51*	Zimbabwe 97*	KW Test	Median Test
Ampicillin	4(60)	64(60)	4(62)	.0027	.0005
Cephalothin	32(112)	32(48)	16(16)	.0125	.0174
Gentamicin	2(3)	1(7.5)	.5(.5)	.0003	.0001
Kanamycin	8(4)	64(60)	4(2)	.0001	.0001
Oxytetracycline	32(56)	64(0)	8(60)	.0003	.9999
Penicillin G	32(0)	32(0)	32(0)	.2916	.9999
Spectinomycin	24(72)	48(168)	24(36)	.0001	.0007
Sulphachlorpyridazine	800(700)	800(400)	200(387.5)	.0001	.0001
Sulphfadimethoxine	800(400)	800(0)	400(750)	.0001	.9999
Trimethoprim/ Sulphamethoxazole	.25(1.75)	.25(7.75)	.25(.25)	.1312	.2990
Tylosin	80(0)	80(0)	80(0)	.1040	.9999
Erythromycin	32(0)	32(0)	32(0)	.0398	.9999

K-W, Kruskal-Wallis Test.
Median, Median 1-way analysis.
*Number of isolates tested
**(IQR)

both tests should give statistically significant results. There is statistical evidence ($P \leq 0.01$) that the medians for distribution of MIC values in the three locations are different. The more powerful Kruskal-Wallis test (tests whether population distribution functions are identical for all three locations using ranking of observations) results indicate at least one of the populations has a higher MIC value than at least one of the other two. The Median test (tests whether populations representing three locations have the same median or two have different medians) results indicate that at least two populations have different medians. These differences are apparent with ampicillin, gentamicin, kanamycin, spectinomycin and sulfachlorpyridazine. It is of interest to note that for those situations where, due to mechanism of action or other limiting factors, little likelihood of antimicrobial effect exists, there were no differences. This is exemplified with erythromycin, penicillin and tylosin.

Further evaluation can be attained using the IQR

which gives the distance between the lower and upper quartiles (i.e. IQR = Q3-Q1). An approximate 95% confidence interval for the median can be calculated based on: Median ± 1.58[IQR/ n], where n is the number of samples involved in the calculation. The quantity added and subtracted in the formula above is called a notch. Two distributions whose notches do not overlap can be considered to differ significantly in the "central values" at the 5% significance level.

Discussion

While isolates from Zimbabwe are typically most sensitive, there are no consistent patterns in comparison of isolates from Australia and the U.S. although more often than not, isolates from the U.S. were the least sensitive, having the highest MIC's. These results while subject to the limitations imposed by the small sample sizes, seem to reflect differences among isolates from areas with differing antimicrobial use patterns. If, in Zimbabwe there has been limited use of many antimicrobials because of availability and/or economic considerations, then unless the limited use resulted in emergence of extensive multiple resistance patterns, as has been suggested with previous studies with fecal isolates, isolates from Zimbabwe should be more sensitive to antimicrobials in general as compared with countries where such drugs are more extensively employed (Gellin *et al*, 1989; Report, 1969).

If antimicrobial usage creates a strong selective influence to increase the number and pattern of resistant organisms (Linton, 1977) then one would suspect that in countries where there is limited use of the drug, the number of bacteria being susceptible to a particular antimicrobial would be higher. Such differences have been shown in comparisons between localities in the United States with markedly different antimicrobial usage (Gellin *et al.*, 1988; Siegal *et al.*, 1974). However, in comparisons of susceptibility of human bacterial pathogens between countries, these differences are not readily apparent (Atkinson, 1986). Although some differences have been observed, the reliance on Bauer-Kirby culture sensitivity results makes it difficult to clearly identify differences. This is also apparent from studies of animal pathogens from Zambia and Zimbabwe, countries where antimicrobial use in animals is likely to be somewhat limited by availability and/or economics (Burrows *et al.*, 1986; Falade *et al* 1989). There appears to be considerable resistance development among patients in human hospitals in large population centers in the tropics where large amounts of antimicrobials are used and where the population density favors spread of resistant organisms (Slack, 1989). It is doubtful if either of these two conditions exist among livestock populations in the same countries. Once a significant level of resistance develops it may not be readily reversed by decreased antimicrobial use possibly as a result of the population density favoring continued transfer of resistance among large animals (Langlois *et al*, 1983).

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