Analysis of Long Term (1990-2009) *in Vitro* Susceptibility to Antibacterial Drugs of the Most Prevalent Animal Bacterial Pathogens Isolated in Israel

Part 1: Trends and Fluctuations

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ABSTRACT

The emergence of bacterial strains resistant to antibacterial drugs has become one of the most significant problems in human and veterinary medicine. To examine the extent of the problem in bacteria of veterinary importance in Israel, the results of the *in vitro* susceptibility tests of *Salmonella enterica* serogroup B, *Escherichia coli*, *Proteus spp*., *Pseudomonas aeruginosa*, *Pasteurella multocida* and *Mannheimia haemolytica*, isolated from animals between 1990 and 2009, were assessed and statistically analyzed. The results showed that out of 52 bacterium/drug combinations tested, no statistically significant changes or increase in susceptibility were observed in 59.6% and 26.9% of the combinations, respectively. A decrease in susceptibility was found in 13.5% of the combinations, most significantly for the fluoroquinolones.

Keywords: Long term, animal, bacterial pathogens, antibacterial, susceptibility

INTRODUCTION

Recent reports of increased prevalence of bacteria resistant to various antibacterial drugs as well as the phenomenon of drug multi-resistance are one of the most disconcerting trends of contemporary bacteriology. This trend has been attributed primarily to the exposure of bacteria to a wide variety of antibacterial drugs at subtherapeutic concentrations in hospitals, their abuse in the community and, in the veterinary field, their use as growth promoters and in the prophylaxis of animal diseases (1). In spite of the importance of the problem, epidemiological data related to resistant bacterial strains still remains scant (2). Such data is of fundamental importance in understanding the various factors contributing to the problem. This survey aims to analyze data gathered in Israel in the years 1990-2009 from *in vitro* susceptibility tests by the disc diffusion method, of bacteria isolated at the Department of Bacteriology at the Kimron Veterinary Institute from dogs, cats, large and small ruminants and horses.

A special case in this report is that of the prevalence of *S. enterica* sgr. B isolates resistant to gentamicin. This drug was introduced in Israel for farm animal therapy in 1980. In the following years the quantity of gentamicin used by the veterinarians of the "Haklait", an organization providing clinical services to a large majority of Israeli cattle, and the prevalence of bovine *S.* Typhimurium isolates resistant to the drug were recorded and the correlation between the two determined.

MATERIALS AND METHODS

Strains

All the strains were isolated from samples originating from sick animals, submitted to the Department of Bacteriology, Kimron Veterinary Institute. Only recognized animal patho-

gens were included in the survey, on condition that at least 30 isolates were examined each year. Based on these criteria the following bacteria were examined:

1. *Salmonella enterica* serogroup (sgr) B, isolated primarily from enteric infections of ruminants. The survey includes isolates only from 1990 to 2004. (From 2005 isolates declined below the cut-off of 30 isolates per year and therefore could not be included). Serotype identification was performed at the Central Laboratories for *Enterobacteriaceae* of the Israeli Ministry of Health.
2. *Escherichia coli*, *Proteus spp*. and *Pseudomonas aeruginosa*, isolated primarily from various infections of domestic carnivores.
3. *Pasteurella multocida* is one of the most important etiological agents of respiratory and other infections in various animals. The micro-organisms included in this survey were isolated from both ruminant and domestic carnivores in approximately equal numbers.
4. *Mannheimia haemolytica*, isolated from respiratory infections of ruminants.

The strains were identified by standard methods (3).

Isolates from mastitis cases and avian species were not included in the survey, as they are dealt with in other laboratories.

Susceptibility

Fifty two bacterium/drug combinations were included in the survey (Table 1).

Table 1: Bacterium/drug combinations included in the survey

Drug	*M. haemolytica/ P. multocida*	*E. coli/Proteus spp./ S. enterica* sgr. B	*P. aeruginosa*
Sulfamethoxazole Trimethoprim	+	+	
Ampicillin	+	+	
Amoxycillin/ Clavulanate	+	+	
Cephalothin	+	+	
Cefotaxime	+	+	
Gentamicin	+	+	+
Amikacin		+	+
Fluoroquinolones	+	+	+
Tetracyclines	+		
Chloramphenicol/ Florfenicol	+	+	
Polymyxin B		+	+

In vitro susceptibility testing was performed by the CLSI (Clinical and Laboratory Standards Institute, formerly NCLS) standard disk diffusion method. Inhibition zones were measured with a digital caliber and recorded quantitatively (diameter). Interpretation was made according to the most recent CLSI criteria (4).

Statistical analysis

Eventual trends of susceptible isolate rates during the survey period were analyzed with the LINEST() function of MS Excel®. This function calculates the statistics for a line

Table 2: Percent susceptible *Proteus spp*. isolates

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
N	65	68	59	56	62	71	76	83	92	97	117	110	127	116	81	87	102	94	61	95
Sxt	46.2	42.6	40.7	39.3	41.9	42.3	40.8	37.3	28.3	48.5	48.7	48.2	47.2	41.4	41.5	57.5	52.9	56.4	55.7	54.7
Cep			20.3	21.4	27.4	29.6	28.9	12.0	29.3	33.0	53.0	32.7	29.1	34.5	34.2	38.4	52.9	65.6	68.9	73.7
Amp	44.6	41.2	28.8	48.2	46.8	33.8	34.2	36.1	34.8	43.2	51.7	43.1	52.4	44.8	37.8	48.3	54.9	60.2	50.8	49.5
Amc			25.4	46.4	54.8	54.9	47.4	48.2	57.6	57.9	68.4	56.0	41.7	40.5	32.1	41.9	56.9	84.0	78.7	79.0
Gen	81.5	82.4	79.7	83.9	88.7	91.5	85.5	81.9	83.7	75.3	88.9	84.5	81.1	83.6	75.6	85.1	84.3	89.4	82.0	84.2
Flq	86.2	97.1	91.5	91.1	82.3	88.7	82.9	74.7	79.8	90.7	94.0	80.9	87.4	83.6	80.3	80.5	83.3	90.4	63.9	60.0
Ctx	58.5	63.2	66.1	60.7	54.8	66.2	50.0	50.6	54.3	70.8	75.0	70.6	71.6	81.7	73.2	82.8	81.4	88.3	91.8	90.5
PB	9.2	8.8	13.6	12.5	21.0	23.9	17.1	14.5	15.2	13.5	25.0	19.0	13.4	9.6	12.2	11.5	11.0	3.2	6.6	4.2
Clm	20.0	13.2	25.4	33.9	46.8	50.7	40.8	30.1	29.7	35.1	41.9	25.4	21.3	18.7	17.3	75.0	89.2	90.4	85.7	94.7
Amk	71.4	67.6	59.3	69.1	88.7	91.5	82.9	70.0	89.1	90.7	92.2	85.4	81.1	75.0	76.5	85.1	82.3	95.7	90.2	85.3

N: Number of isolates, Sxt: Sulphamethoxazole-Trimethoprim, Cep: Cephalothin, Amp: Ampicillin, Amc: Amoxicillin-clavulanic acid, Gen; Gentamicin, Flq: Floroquinolones, Ctx: Cefotaxime, PB: Polymyxin B, Clm: Chloramphenicol, Amk: Amikacin

by using the "least squares" method. One of the parameters it calculates is the F statistic used to determine whether the observed relationship between the dependent and independent variables occurs by chance by consulting *F* percentile distribution tables. Changes were considered significant at p values lower than 0.05.

RESULTS

The results of the survey are presented in Tables 2-7 as the percentage of susceptible isolates. The bacterium/drug combinations with statistically significant trends are presented in Table 8.

Table 3: Percent susceptible *E. coli* isolates

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
N	272	225	225	206	315	370	366	330	281	364	344	335	368	454	389	367	408	420	278	228
Sxt	44.1	39.6	42.2	42.7	47.3	46.2	54.6	57.0	59.4	59.9	51.5	56.1	48.6	54.9	52.7	55.5	49.5	51.3	55.4	50.0
Cep			16.9	13.6	15.6	26.5	17.2	20.9	28.1	20.9	32.3	9.0	7.1	8.6	7.7	8.0	11.3	8.6	2.9	5.3
Amp	11.4	18.7	27.6	24.8	19.0	19.2	18.0	23.9	29.9	38.7	37.5	34.1	27.5	30.8	21.1	27.0	24.0	25.0	12.0	15.0
Amc			40.0	44.7	43.8	54.3	40.2	50.6	61.9	68.4	71.5	53.4	28.8	20.9	16.5	17.2	22.0	49.3	48.2	56.1
Gen	67.6	75.1	73.3	80.6	76.8	79.5	75.7	79.7	83.6	75.8	73.8	77.6	80.2	78.4	71.7	73.0	70.4	75.3	73.7	74.5
Flq	86.4	91.1	91.1	83.0	82.1	81.1	84.7	77.9	82.7	72.5	76.2	77.0	74.5	75.3	74.2	70.8	64.5	72.4	59.7	56.3
Ctx	58.8	67.1	62.7	67.0	78.1	82.2	65.0	72.7	82.2	79.5	82.3	83.6	77.5	85.2	81.9	78.9	81.9	82.2	63.3	76.1
PB	51.1	54.2	73.3	75.2	87.3	92.2	87.4	82.4	87.9	81.0	86.3	76.6	77.1	84.4	73.8	74.9	80.9	73.3	48.6	73.0
Clm	44.9	40.9	40.9	52.4	53.7	61.6	65.0	74.8	76.4	72.0	72.7	73.0	69.0	72.0	75.3	77.6	77.0	76.7	73.4	75.8
Amk	53.4	53.3	41.5	56.8	78.1	85.9	76.2	73.9	89.0	92.3	90.7	78.4	80.7	68.8	73.6	73.0	84.4	88.0	78.1	90.3

N: Number of isolates, Sxt: Sulphamethoxazole-Trimethoprim, Cep: Cephalothin, Amp: Ampicillin, Amc: Amoxicillin-clavulanic acid, Gen; Gentamicin, Flq: Floroquinolones, Ctx: Cefotaxime, PB: Polymyxin B, Clm: Chloramphenicol, Amk: Amikacin

Table 4: Percent susceptible *S. enterica* serogroup B. isolates

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
N	89	115	91	106	94	128	160	131	77	54	72	77	83	44	40	12	30
Sxt	39.3	34.8	37.4	58.5	71.3	74.2	82.5	72.5	85.7	79.6	83.3	77.9	77.1	79.6	65.0	91.7	66.7
Cep			72.5	59.4	78.7	87.5	68.1	71.8	84.4	64.8	88.9	71.4	61.7	43.2	27.5	53.9	43.3
Amp	29.2	33.9	40.7	45.3	37.2	34.4	36.9	45.0	27.3	37.0	38.9	50.0	56.6	51.2	32.5	41.7	50.0
Amc			61.5	57.5	61.7	51.6	53.1	53.4	31.2	58.8	44.4	57.1	57.8	45.5	35.0	33.3	50.0
Gen	52.8	47.8	67.0	79.2	86.2	85.9	93.1	95.4	92.2	79.6	90.3	92.2	97.6	84.1	65.0	100	86.7
Flq	89.9	93.9	96.7	86.8	67.0	98.4	96.3	91.6	91.9	92.6	98.6	98.7	96.4	95.5	75.0	91.7	96.7
Ctx	59.6	73.9	69.2	69.8	83.0	78.9	70.6	65.6	80.5	100	79.2	81.8	73.5	77.3	65.0	75.0	63.3
PB	74.2	66.1	81.3	77.4	89.4	89.8	89.4	86.3	93.5	79.6	94.4	76.6	81.9	72.7	65.0	81.8	72.4
Clm	32.6	35.7	39.6	50.9	44.7	36.7	45.0	51.1	32.5	40.7	37.5	58.4	83.1	72.4	60.0	75.0	82.8
Amk	72.6	76.5	82.4	76.4	95.7	97.7	90.0	87.5	96.1	90.7	94.4	88.3	86.6	83.7	80.0	81.8	82.8

N: Number of isolates, Sxt: Sulphamethoxazole-Trimethoprim, Cep: Cephalothin, Amp: Ampicillin, Amc: Amoxicillin-clavulanic acid, Gen; Gentamicin, Flq: Floroquinolones, Ctx: Cefotaxime, PB: Polymyxin B, Clm: Chloramphenicol, Amk: Amikacin

Table 5: Percent susceptible *Pasteurella multocida* isolates

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
N	100	56	64	77	99	147	122	138	122	108	102	91	141	111	98	108	137	108	32	44
Sxt	70.0	87.5	79.7	87.0	85.9	81.6	86.9	82.6	77.0	83.3	93.1	90.4	89.4	86.7	93.8	89.8	88.3	89.8	93.8	87.2
Cep	85.0	96.4	90.6	92.2	93.9	87.8	89.3	87.7	69.7	87.0	94.1	90.4	92.9	87.6	86.7	81.5	92.7	92.5	84.4	89.4
Amp	88.0	92.9	92.2	93.5	92.9	91.8	90.2	89.1	86.9	92.6	88.2	88.4	94.3	92.0	93.9	87.0	90.5	92.6	65.6	91.5
Amc			92.2	92.2	94.9	97.3	94.3	91.3	95.1	88.9	96.1	87.5	92.2	85.6	85.7	77.1	86.9	94.4	93.8	95.7
Gen	73.0	94.6	95.3	87.0	96.0	95.2	92.6	79.7	89.3	82.4	89.2	76.0	83.7	69.4	75.3	78.0	83.2	91.6	81.3	87.2
Flq			93.8	90.9	92.9	88.4	86.9	78.3	100	78.5	92.2	93.3	95.0	93.8	94.9	94.5	91.2	96.3	96.9	95.7
Ctx			89.1	83.1	82.8	87.8	81.1	76.1	100	79.6	83.3	84.6	89.4	80.1	90.8	73.4	84.7	89.8	81.3	87.2
Tet	72.0	83.9	64.1	71.4	47.5	52.4	60.7	52.9	68.0	60.2	81.2	79.8	75.9	68.1	78.4	68.8	72.4	80.2	78.1	76.6
Clm	89.0	92.9	90.6	93.5	92.9	92.5	90.2	91.3	87.7	89.8	96.1	97.1	93.6	92.9	92.8	96.2	97.1	97.2	100	93.6

N: Number of isolates, Sxt: Sulphamethoxazole-Trimethoprim, Cep: Cephalothin, Amp: Ampicillin, Amc: Amoxicillin-clavulanic acid, Gen; Gentamicin, Flq: Floroquinolones, Ctx: Cefotaxime, Tet: Tetracyclines, Clm: Chloramphenicol

Table 6: Percent susceptible *Mannheimia haemolytica* isolates

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
N	106	75	63	91	98	102	64	73	65	63	50	49	52	54	57	59	59	73	49	42
Sxt	90.6	88.0	93.7	80.2	100.0	90.2	84.4	78.1	68.8	88.9	76.0	94.6	92.3	90.7	94.6	89.8	94.9	89.0	81.6	92.9
Cep	95.3	98.7	96.8	91.2	78.6	98.0	98.4	91.8	92.3	93.7	94.0	98.2	94.2	98.2	92.9	96.6	94.9	97.2	87.8	85.7
Amp	93.4	89.3	93.7	78.0	94.9	86.3	76.6	78.1	75.4	81.0	86.0	96.4	90.4	94.4	96.5	87.9	86.4	93.2	61.2	83.3
Amc			96.8	98.9	74.5	98.0	95.3	93.2	93.8	93.5	96.0	100.0	94.2	90.7	94.7	91.6	94.9	97.3	100.0	97.6
Gen	80.2	85.3	90.5	83.5	99.0	96.1	98.4	87.7	95.2	81.0	86.0	87.5	78.9	70.4	73.7	71.2	89.8	81.9	71.4	78.6
Flq			93.7	92.3	92.9	90.2	93.8	90.4	87.7	87.3	84.0	92.9	86.5	90.7	98.2	98.3	94.9	98.6	87.8	85.7
Ctx			90.5	92.3	95.9	95.1	92.2	91.8	86.2	85.7	94.0	98.2	88.5	92.6	98.2	86.4	94.9	94.5	87.5	97.6
Tet	82.1	69.3	81.0	64.8	54.1	55.9	54.7	42.5	53.8	60.3	59.2	66.1	65.4	55.6	61.4	61.0	50.9	54.8	61.2	71.4
Clm	95.3	94.7	93.7	91.2	94.9	93.1	90.6	94.5	93.8	90.3	88.0	91.1	90.4	98.1	80.4	98.2	98.3	98.6	95.9	97.6

N: Number of isolates, **Sxt**: Sulphamethoxazole-Trimethoprim, **Cep**: Cephalothin, **Amp**: Ampicillin, **Amc**: Amoxicillin-clavulanic acid, **Gen**; Gentamicin, **Flq**: Floroquinolones, **Ctx**: Cefotaxime, **Tet**: Tetracyclines, **Clm**: Chloramphenicol

Table 7: Percent susceptible *Pseudomonas aeruginosa* isolates

Year	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
N	58	75	45	54	64	57	77	82	83	127	116	149	117	131	104	111	147	110	74	114
Sxt	37.9	49.3	55.6	83.3	79.7	89.5	87.0	76.8	91.6	63.8	78.4	68.5	60.7	57.7	50.0	76.0	82.4	89.1	79.7	75.4
Flq	96.6	92.0	100	98.1	96.9	91.2	85.7	75.6	83.1	83.5	94.0	83.2	84.6	92.4	77.9	87.0	83.8	83.5	18.9	10.5
Gen	81.0	82.7	84.4	85.2	95.3	94.7	88.3	91.5	93.9	87.4	84.3	86.6	87.2	87.8	87.3	97.0	95.2	92.7	78.4	93.9
PB	70.9	71.6	75.6	85.2	81.3	86.0	88.3	82.7	95.2	84.0	91.4	85.2	78.6	72.5	75.0	94.0	90.5	90.9	85.1	86.0

N: Number of isolates, **Sxt**: Sulphamethoxazole-Trimethoprim, **Gen**; Gentamicin, **Flq**: Floroquinolones, **PB**: Polymyxin B

Table 8: Bacterium/drug combinations that showed a statistically significant change in susceptibility during the survey.

Drug	*Salmonella* sgr. B	*E. coli*	*Proteus spp.*	*P. aeruginosa*	*P. multocida*	*M. heamolytica*
	Increase in susceptibility (statistical significance)					
Cefotaxime		P<0.05	P<0.01			
Amikacin		P<0.01	P<0.05			
Sulphamethoxazole/Trimethoprim		P<0.05	P<0.01		P<0.01	
Ampicillin	P<0.05		P<0.01			
Chloramphenicol	P<0.01	P<0.01	P<0.01		P<0.01	
Cephalothin			P<0.01			
	Decrease in susceptibility (statistical significance)					
Fluoroquinolone		P<0.01	P<0.01	P<0.01		
Polymyxin B	P<0.05					
Cephalothin	P<0.05	P<0.05				
Gentamicin						P<0.05

The majority (59.6%) of the bacterium/drug combinations examined showed no significant differences in the susceptibility values from the beginning to the end of the survey. Two patterns were observed in this group: a) the trend did not change throughout the period of the survey or b) values followed a curve, returning at the end of the survey period to values observed at the beginning. Among the remaining combinations, the majority showed an increase in susceptibility, which was statistically highly significant (p<0.01) in 10 cases (19.2%) or at a lesser significance (p<0.05) in 4 cases (7.7%). A decrease in susceptibility was observed in 7 combinations, of which 3 (5.8%) were associated with fluoroquinolones and showed a high statistical significance. The most noteworthy results are detailed below:

Salmonella enterica sgr. B (Figure 1)

While statistically insignificant, the susceptibility to sulfamethoxazole-trimethoprim and gentamicin increased between 1992 and 1997 and remained essentially unchanged, however, with periodical fluctuations. The overall trend for chloramphenicol susceptibility showed a highly statistically significant increase ($p<0.01$). However this increase was not homogeneous: susceptibility was stable till 2000, increased rapidly until 2002 and returned to stability (with fluctuations) thereafter.

No correlation ($r=0.08$) was found between the quantity (in kilograms) of gentamicin used by the field clinicians of the "Haklait" and the prevalence of bovine *S.* Typhimurium resistance to the drug (Figure 2a).

However, moving the curve of the resistant strains forward in time (Figure 2b), improved the correlation gradually to $r=0.42$. The curves seemed to follow the same pattern till 1994, with a correlation coefficient of 0.88. In the following years, however, the curves diverged (Figure 2b).

Escherichia coli (Figure 3)

The susceptibility of this microorganism to chloramphenicol throughout the survey period increased significantly ($p<0.01$). However, this increase was relatively steep between 1992 and 1998 and was followed by a period of stability. Analyzing the susceptibility rate of *E. coli* to amoxicillin-clavulanate throughout the survey resulted in no significant change. In fact the value in 1992 (the year in which the drug was first tested) was 40% and in 2009 about 56%. However, the relevant curve showed that the rate increased between 1990 and 2000, declining steeply until 2004 and increasing almost as steeply until 2007 and then more moderately for the following 2 years.

Proteus spp. (Figure 4)

The susceptibility rate of *Proteus spp.* to amoxicillin-clavulanate increased between 1992

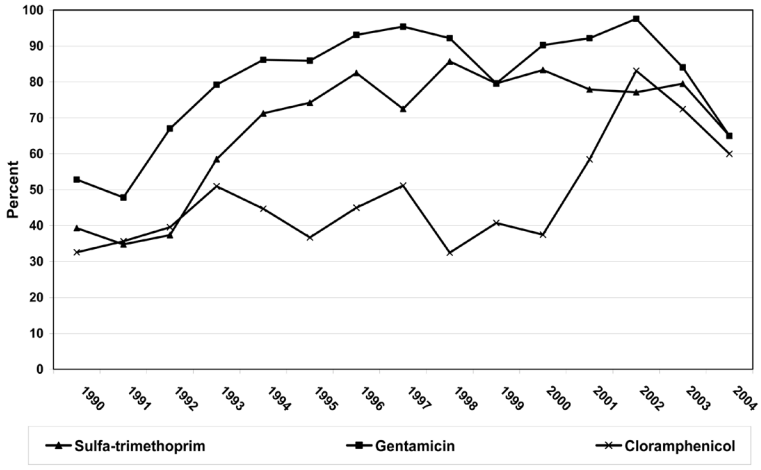

Figure 1: Percent of *S. enterica* serogroup B susceptible to sulfamethoxazole-trimethoprim, gentamicin and chloramphenicol

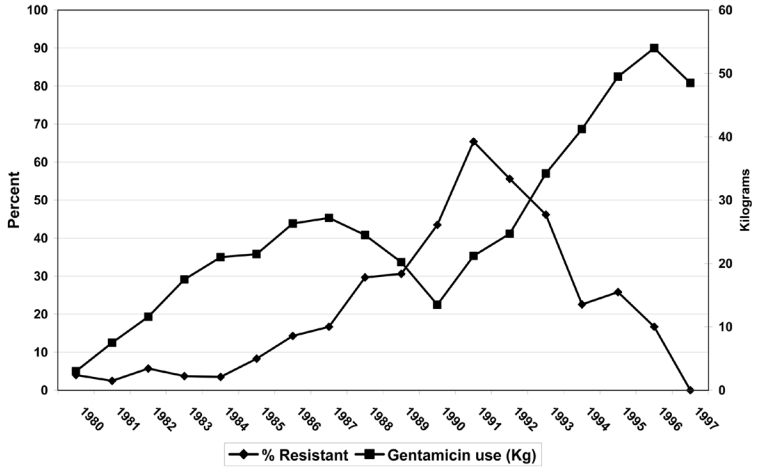

Figure 2a: Gentamicin use (kg) and prevalence of bovine *Salmonella* Typhimurium isolates resistant to the drug

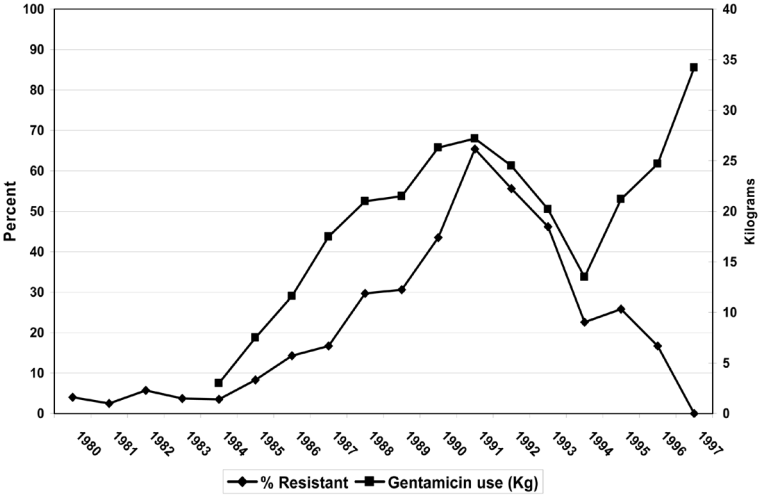

Figure 2b: Gentamicin use (kg) and prevalence of bovine *Salmonella* Typhimurium isolates resistant to the drug – five year time lapse

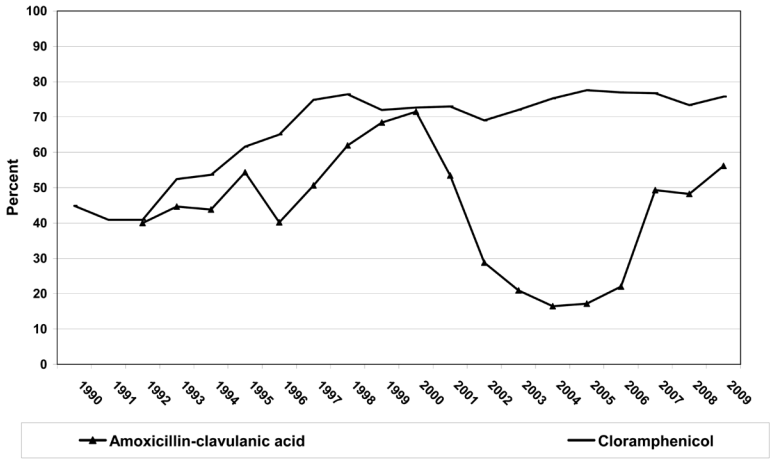

Figure 3: Percent of *Escherichia coli* susceptible to amoxicillin-clavulanate and chloramphenicol

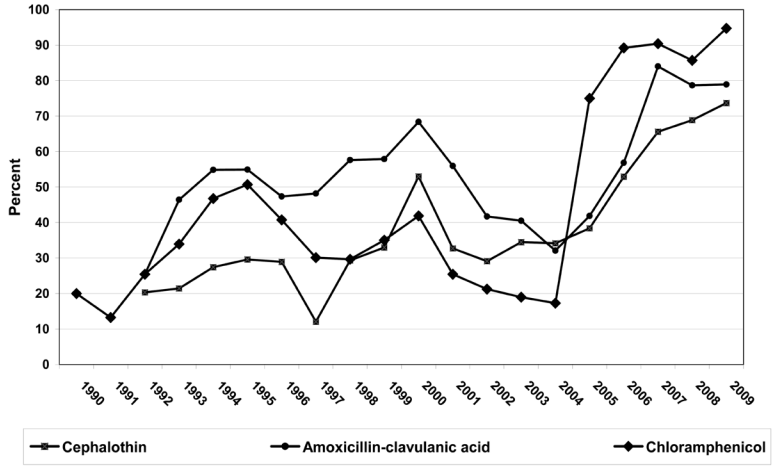

Figure 4: Percent of *Proteus spp*. susceptible to amoxicillin-clavulanate and cephalothin

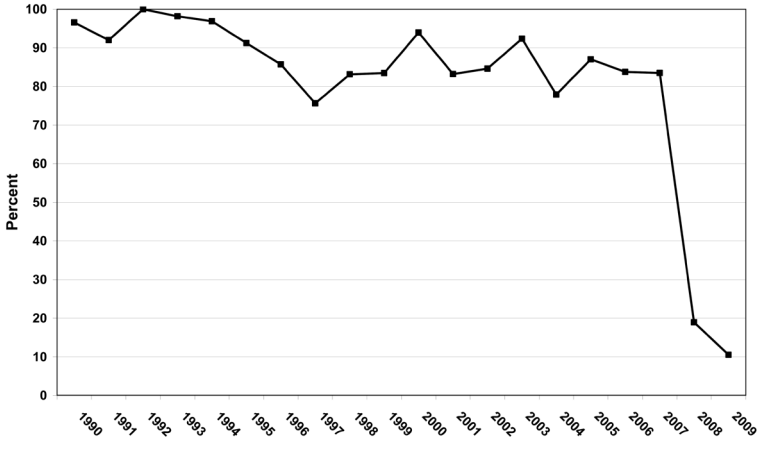

Figure 5: Percent of *Pseudomonas aeruginosa* susceptible to fluoroquinolones

and 2000, decreased steeply until 2004 and increased in 2007, followed by 2 years of moderate decrease with a tendency towards stability. This curve was very similar to that observed for the same drug with *E. coli* (Figure 2). The susceptibility rate of *Proteus spp*. to cephalothin increased between 1992 and 2009 from 20.3% to 73.7% ($p<0.01$). Similarly, the susceptibility of *Proteus spp*. to chloramphenicol remained generally unchanged between 1990 and 2004, but increased steeply in the following two years (2005-2006), a tendency that was sustained, albeit more moderately, between 2007 and 2009.

Pseudomonas aeruginosa (Figure 5)

While being the drug group that was the most affected by emerging resistance (Table 8) the susceptibility rate of *P. aeruginosa* to fluoroquinolones declined slowly but steadily between 1990 (96.5%) and 2007 (83.5%). In 2008 the susceptibility rate dropped abruptly to 18.9% further decreasing in 2009 to 10.5%,

Pasteurella multocida (Figure 6)

The susceptibility of this microorganism to tetracyclines declined between 1990 and 1994 but the trend was reversed between 1994 and 2000, remaining stable thereafter (Figure 6).

Susceptibility curves of domestic carnivores were similar to those observed in ruminants (Figure 7).

Mannheimia haemolytica (Figure 8)

The microorganism's susceptibility to tetracycline decreased between 1990 and 1997, increasing thereafter slightly until 2006 and more abruptly until 2009 to levels slightly lower than those observed 20 years earlier.

DISCUSSION

The expression "long term" has been used for surveys spanning from 2 to 8 years (5, 6, 7) or for the comparison between two or more periods (8, 9). As demonstrated in our survey, the shorter the survey period, the greater the likeli-

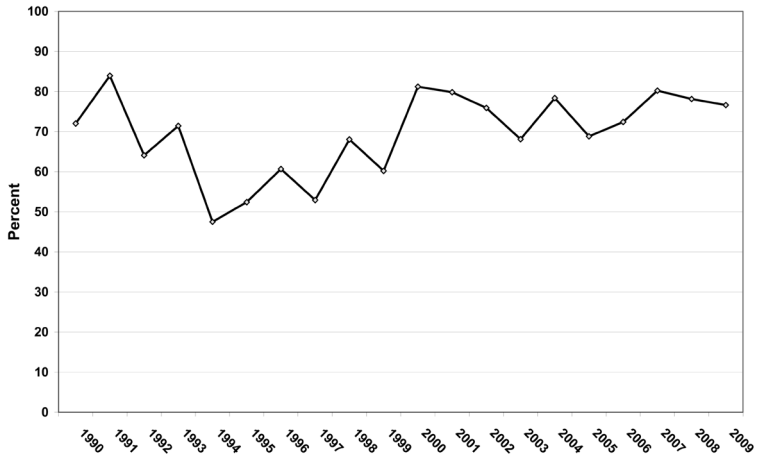

Figure 6: Percent of *Pasteurella multocida* isolates susceptible to tetracyclines

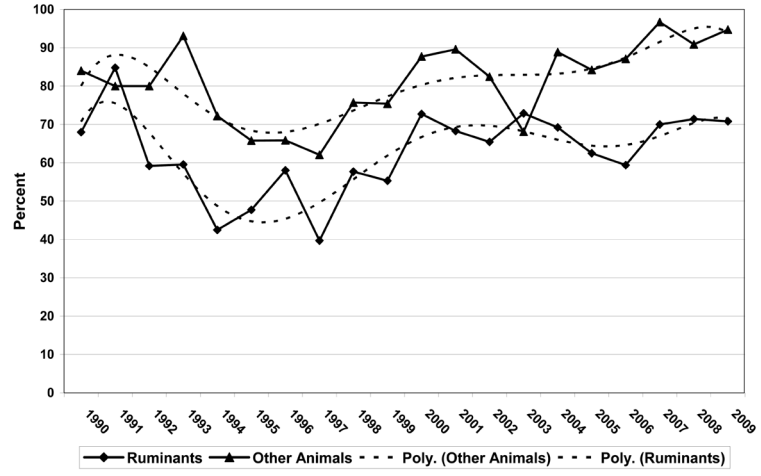

Figure 7: Percent of *Pasteurella multocida* isolates susceptible to tetracyclines grouped by source animal. Dotted lines: polynomial trendlines

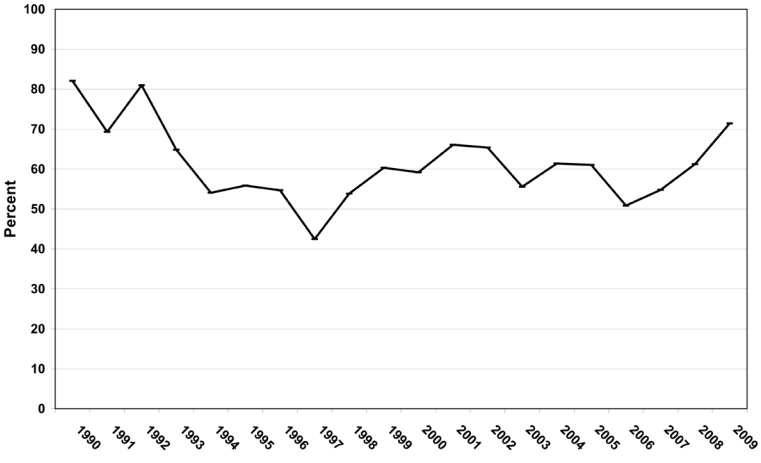

Figure 8: Percent of *Mannheimia haemolytica* isolates susceptible to tetracyclines

hood of misinterpreting fluctuations as trends. Examining split periods, on the other hand, may provide a valid picture if the trends are linear for the whole period, including the interval, but not if the changes are represented by a curve (such as those found in our study for the susceptibility rates of *P. multocida* and *M. haemolytica* to the tetracyclines). In fact, no study can guarantee to be able to have a consistent predictive value. The longer the study, however, the higher the likelihood that it will provide a dependable depiction of a specific drug/bacterium interaction. Nevertheless, studies of antibacterial susceptibility spanning two decades are rare. Recently two other surveys, spanning two or more decades, were published (10, 11).

It is becoming more apparent that the equation linking extensive use of an antibacterial drug and the increase in the prevalence of strains resistance is too simplistic. The dynamics of antibacterial drug resistance are highly complex involving among other factors selective pressures and "penalties" sometimes imposed upon resistant strains controlling their spread, such as hygienic conditions. Attempts to develop reliable models simulating these processes have been made during the last decade (12).

The results of our analysis seem to disprove some accepted paradigms regarding the evolution of antibacterial resistance. We found that susceptibility remained mostly unchanged and even if periodic decreases occurred, they were followed by increases. Moreover, several drug/bacterium combinations showed a significant increase in susceptibility. Highly significant ($p<0.01$) decreases were the exception and seen in only 3 combinations, all involving fluoroquinolones. The results of the susceptibility of the various bacteria to this drug underline the difficulty in defining universal rules for the evolution of resistance over time. A decrease was continuous for two *Enterobacteriaceae* (*E. coli* and *Proteus spp.*) while it decreased rapidly during a single year for *P. aeruginosa*. At the same time

the susceptibilities of *S. enterica* sgr. B (belonging also to the *Enterobacteriacea*) and of *P. multocida* and *M. haemolytica* remained unchanged.

Another paradigm which was not substantiated by our results is the dependence of resistance induction on the intensity of exposure to the respective antibacterial drug. While detailed information of drug use during the study period is not available, general knowledge of therapeutic practices are known. Our results reveal other examples that seem to substantiate that factors other than drugs use may influence the evolution of resistance. Trimethoprim potentiated sulfonamides are widely used to treat farm animals in Israel. Nevertheless, the susceptibility of *S. enterica* sgr. B remained stable since 1997, after 7 years of an increase in susceptibility. A similar observation was made for P. *multocida* and *M. haemolytica*, both of which are frequently exposed to prophylactic tetracyclines, administered to ruminants in food. This did not prevent an increase in the susceptibility of these microorganisms in recent years, even though there was no significant change in the use of the drug.

The replacement of susceptible bacterial populations with resistant strains or vice versa sometimes occurred very abruptly. The susceptibility of *Proteus spp*. to cephalothin, amoxicillin-clavulanic acid and chloramphenicol increased steeply between 2004 and 2007 whereas that of *P. aeruginosa* to fluoroquinolones decreased from 83.3% susceptible isolates in 2007 to 18.9% in 2008, to 10.5% in 2009, transforming it from drug of choice to being practically useless in treating *P. aeruginosa* infections.

The oral treatment of feedlot calves with tetracyclines in order to prevent respiratory infections is widespread in Israel. Consequently, based exclusively on the microorganism's exposure to subtherapeutic doses, a continuing decline in susceptibility would be expected. Domestic carnivores, on the other hand, are subjected to tetracycline treatment mostly for therapeutic purposes, which is less likely to induce resistance. The similarity between trendlines based on the susceptibility curves for *P. multocida* from these two populations indicate that the exposure to tetracyclines either at subtherapeutic or therapeutic doses did not influence significantly the development to resistant strains.

The correlation between the use of gentamicin and the prevalence of resistant bovine *S.* Typhimurium isolates reveals several noteworthy findings. The time lapse required to reach the highest correlation coefficient between the gentamicin use and that of the resistant *S.* Typhimurium isolates was 5 years. This might be an indication of the time required for the drug to influence the microorganism's susceptibility on a national basis. Another interesting observation is that after an initial matching course of the curves, they diverge and an increase in the use of the drug did not correspond to an increase in resistance (Figure 2b). A similar observation was made by Imberechts *et al*. (13), regarding the apparent loss of resistance of *S*. Typhimurium to enrofloxacin. This phenomenon is a possible indication of susceptible strains, considered inferior under antibiotic selective pressure, being able to supersede the resistant ones by a mechanism still to be determined.

A recent publication (14) underlines the complexity of the balance between susceptible and resistant microbial populations, once the selective pressure of the drug is withdrawn. While penalties stemming from maintaining resistance mechanisms that are a waste of energy in the absence of the antibacterial compound and eventual decreases in their capabilities to complete their life cycle (fitness) will tend to reduce their prevalence, compensatory mutations that improve their energetic efficacy and/or fitness may reduce or annul such penalties. This is corroborated by our observations on the reaction of *Salmonella enterica* sgr. B, *E. coli* and *Proteus spp*. to the withdrawal of chloramphenicol in food animals in 1992. The susceptibility of *Salmonella enterica* sgr. B to the drug remained unchanged for eight years (Figure 1), despite the origin of the isolates, farm animals that should have been the most influenced by the withdrawal. The susceptibility of *E. coli* to the drug, on the other hand, increased the same year the drug was removed from use (Figure 3), this despite the fact that the large majority of our isolates came from domestic carnivores, mostly unaffected by the ban. At the same time, the susceptibility of *Proteus spp*., isolated primarily from the same population, showed no such change in susceptibility, that started to increase only in 2005 (Figure 4).

In conclusion, our findings did show that some of the paradigms associated with antibacterial drug use and resistance development, such as the one claiming that continuous prolonged use of a drug will reduce the rate of susceptible microorganisms, may be true in some cases but not all, especially if the survey period is extended enough to reveal long term fluctuations and trends.

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