ISSN 1021-366X

Analysis of the Trend of Drug Resistance of *Salmonella typhimurium* in Taiwan, 1991-2001

Abstract

The present study collected for analysis 114 strains of clinically isolated sporadic *Salmonella typhimurium* in the ten years between 1991 and 2001. Disk susceptibility test, Vitek's drug susceptibility assessment card (GNS-206), and E-test were used for the analysis of drug resistance and its trend of different drugs. Findings of the disk susceptibility test showed that drug resistance ratio for single drug was the highest for streptomycin at 84.2%, followed by tetracycline at 82.5%, chloramphenical at 71.9%, ampicillin at 70.2%, and nalidixic acid at 18.4%; and that, resistance ratio to three (inclusive) drugs was 64%, to five drugs was 25%, and to seven and more drugs was 11%. Analysis of the drug resistance strains by year to the commonly used drugs, ampicillin, nalidixic acid, ciprofloxacin and ceftiriaxone, for their changes in drug resistance concentrations at two different time points, 1994-1995 and 1999-2000 showed that, the ratio of strains isolated in 1999-2000 was much higher than that in 1994-1995, showing a significant increase in the number of drug resistant strains. A further analysis of the changes in drug resistance concentrations of more

recent drugs, ciprofloxacin and ceftiriaxone, showed a gradual increase in their drug resistance concentrations. By comparing the associations of the drug resistance concentrations of the three different drug susceptibility tests showed that, when ampicillin and nalidixic acid were cross-compared by disk susceptibility test, MIC of the Vitek test and E-test, different tests had certain differences in drug resistance concentrations; and that, the E-test showing gradual distribution of concentrations was more accurate in assessing dosage of drug therapy and drug resistance concentrations. Findings of the study showed that the front-line drugs used for the treatment of *Salmonella typhimurium* infections in Taiwan had already a high drug resistance of 70-80%, their therapeutic effect was almost nil. With increase in the ratio of multi-drug resistance, 17.5% (20/114) of ASSuCT type drug resistant strains for instance, the ratios of drug resistance and drug resistance concentrations of some commonly used and new-generation drugs had gradually increased, the issue of drug resistance had become more serious. Different susceptibility tests had different concentrations; they should be more precisely assessed in the monitoring of the drug resistance concentrations of strains and dosages of clinical treatment.

Introduction

Salmonellosis is a food and water-borne bacterial infection of men and animals. Of all serotypes of *Salmonella*, with the exception of *Salmonella typhi* and *Salmonella paratypi* which are agents of notifiable diseases, the rest are common serotypes of *Salmonella*. Of all food-borne poisoning and sporadic infections in Taiwan, *Salmonella typhimurium* is the most common agent. Data of serotypes of *Salmonella* in Taiwan reported by Wang et al. of the Center for Disease Control of Taiwan shows that in the ten years between 1983 and 1993, of

the 1,647 strains involved in food poisoning incidents, *Salmonella* O_4 and O_9 serotypes were more common, accounting for 44% and 32.5% respectively. Of the serotype O_4, *Salmonella typhimurium* is more common; and of the serotype O_9, *Salmonella typhi* is more common[1]. A further analysis of strains in 1994-1999 shows that *Salmonella enteritidis*, of O_9, has been increasing in number since 1991. The pathogenic agents of group food poisoning incidents in the years between 1993 and 1997 were primarily *Salmonella enteritidis* and *Salmonella typhimurium*.

Since the reporting of *Salmonella typhimurium* Definitive Type 104 (DT104) in the United Kingdom in the 1990s[4], 90% of this serotype is found to be drug resistant to five drugs of ampicillin, streptomycin, sulfonamides, chloramphenical and tetracycline (ASSuCT, R-type). Findings from international disease surveillance networks show that cases of multi-drug resistant *Salmonella* infections have higher hospitalization rates and mortality[6,7]. In the last ten years, drug resistance of *Salmonella typhimurium* has significantly increased internationally; and DT104 resistant to new drugs such as trimethoprin-sulfamethoxazole and ciprofloxacin has also appeared and caught the attention of the health authorities around the world[2,6,8]. In the 1996 drug resistance surveillance, the US CDC reported that, of the 3,903 *Salmonella* strains isolated, 976 (25%) were *Salmonella typhimurium*; and of them, about 28% were confirmed *Salmonella typhimurium* DT104 serotype, higher than the 7% of 1990[3,5]. The issue of drug resistance of *Salmonella typhimurium* has become more serious around the world.

Pathogenic agents isolated in Taiwan generally have a serious problem of drug resistance, *Streptococcus pneumoniae* resistant to penicillin for instance. The issue of bacteria resistance to drugs is complex. Factors such as drug use

(abuse and excess use), inadequate control or monitoring of antibiotics, or ineffective treatment may result in drug resistance. Changes in farming practice (adding of antibiotics to animal feeds for instance) may result in drug residues on meat. Long-term consumption of such meat could also produce drug resistance. Data on the surveillance of drug resistance of *Salmonella typhimurium* in Taiwan is not quite available, medical circles are less concerned about drug resistance, and epidemiological data of incidents is also less available. It is, therefore, impossible to analyze and compare differences in drug resistance between *Salmonella* isolated in animals and poultry and their meat products and the strains isolated clinically from humans. It is neither possible to clear the molecular-associated evidence in the route of transmission of poisoning incidents caused by food or meat contaminated by *Salmonella*. These are issues of *Salmonella typhimurium* that require further investigation.

The present study analyzes the status and trend of drug resistance of clinically isolated *Salmonella typhimurium* in the recent years. The results show that the front-line drugs commonly used in Taiwan such as ampicillin, tetracycline and chloramphenicol are 70-80% drug resistant, and the ratio of multi-drug resistance has increased to as high as 17.5% of strains to ASSuCT. Drug resistance ratios and concentrations for some commonly used antibiotics and new antibiotics have also gradually increased, showing seriousness of drug resistance. The issues require closer attention of the medical circles and disease control authorities. Different susceptibility tests show certain differences in concentration, dosage for clinical treatment and monitoring of drug resistance should be more carefully assessed.

Materials and Methods

1. Isolation of Strains and Biochemical and Serological Assessment

The present study collected for analysis 114 strains of *Salmonella typhimurium* clinically isolated in the ten years between 1991 and 2001. The strains were cultured in SS-medium (*Salmonella-Shigella* agar) at 36℃ for 18-20 hours for basic biochemical test (Triple Sugar Iron, TSI agar) and serum O, H antigen assay, and then kept under -20℃.

2. Vitek System

A fresh colony was picked up and mixed with 1.8 cc of 0.45% normal saline, stirred with colormeter to McFarland No. 1 barium sulfate standard fluid. GNI was used for the biochemical assay of strains.

3. Disk Susceptibility Test

Antibiotics meeting the NCCLS standard and recommended by the Taiwan Infectious Disease Association were selected. They included antibiotics of extended spectrum such as ampicillin (AM), the quinolones group such as nalidixic acid (NA) and ciprofloxacin (CIP), the cephalosporins group such as ceftiriaxone (CRO), the third-generation cephalosporin, the tetracycline group such as tetracycline (T), and the sulfonamides group such as sulfamethoxazole (Su) and chloramphenical (C). They were used for the monitoring and assessment of drug resistance concentrations. Fresh colony fluid was mixed to McFarland 0.5 (about $1.5x10^8$ CFU/ml) barium sulfate suspension, placed it evenly on Mueller-Hinton agar (M-H medium), added disk with dispenser, placed at 37℃ for 18-20 hours for reading by the standard supplied by the manufacturer.

4. GNS-206

The GNS-206 of Vitek has 18 antibiotics such as ampicillin, tetracycline, ciprofloxacin and cephalosporins. Fresh strains were mixed with 0.45% normal saline to McFarland 0.2 barium sulfate suspension, put carefully on assessment card, and placed in the Vitek reader to test MIC.

5. E-test

Fresh strain fluid was mixed to McFarland 0.5 barium sulfate suspension, put evenly on M-H medium, added carefully E-test papers of different antibiotics concentrations, placed at 37℃ for 19-20 hours and read by the standard supplied by the manufacturer.

Results

Disk susceptibility test of 114 strains of sporadic infections showed that, for drug resistance by single strain, streptomycin had the highest drug resistance ratio of 84.2% (96/114), followed by tetracycline at 82.5% (94/114), chloramphenicol at 71.9% (82/114), amppicillin at 70.2% (80/114), nalidixic acid at 18.4% (21/114) and ciprofloxacin at 3.8% (3/114) (Figure 1). For multi-drug resistance, the ratio of resistance to more than three drugs (inclusive) was 64% (73/114), that to five drugs was 25% (29/114), that specific to ASSuCT was 17.5% (20/114), and that to seven and more drugs was 11% (13/114) (Figure 2).

Analysis of ampicillin, nalidixic acid, ciprofloxacin and ceftiriaxone in two periods of 1994-1995 and 1999-2000 by disk susceptibility test to decide whether they were drug resistant or intermediate type strains showed that drug resistance was increasing (Figure 3). Analysis of changes in drug resistance concentrations by E-test showed that concentration of ciprofloxacin had gradually increased from a low 0.016 μg/ml to 0.38 μg/ml; the concentration of ceftiriaxone had also increased from 0.19 μg/ml to 2 μg/ml (Figure 4, Table 2).

Strains of ampicillin and nalidixic acid susceptible to disk susceptibility test (S-type) were selected for MIC test with Vitek and E-test. The MIC values were S≦0.25 μg/ml for ampicillin, and S≦16 μg/ml for nalidixic acid. The MIC values of E-test were 1-4 μg/ml and 1-1.5 μg/ml respectively. When R-type

strains of the two drugs were used, their MIC and E-test MIC values were ≧32 μg/ml for MIC, and >256 μg/ml for W-test (Table 1).

Analysis of 21 susceptible strains of ciprofloxacin (S=21-22 mm) showed that, their E-test MIC ranged from 0.016 to 0.19 μg/ml; that for the six intermediate type strains (I=17-20 mm) ranged from 0.25 to 0.38 μg/ml; and that for three R-type strains (R=14 mm) was 0.38 μg/ml. Analysis of 11 intermediate type strains of ceftiriaxone (I=17-20 mm) showed that, their E-test MIC ranged from 0.38 to 2 μg/ml; and that for 23 susceptible strains (S=21-24 mm) ranged from 0.19 to 0.25 μg/ml (Table 2).

Discussion

Multi-drug resistance of *Salmonella typhimurium* involves many aspects. The abuse and excess use of drugs on the part of the public, inadequate control or monitoring of antibiotics, and ineffective therapy of medical practice are some of the factors. Agricultural and fishery farming practices of adding drugs in animal feeds leaving behind some drug residues on meat may also result in more drug resistant bacterial strains. Of all food-borne and sporadic infections in Taiwan, more are induced by *Salmonella typhimurium*. Indigenous data on the current status of drug resistance of *Salmonella typhimurium*, and its changes in drug resistance concentrations and trend of development are therefore most essential. Further analysis of some specific multi-drug resistant strains (the ASSuCT types) and status of drug resistance to new antibiotics should serve as a reference and warning to the medical circles and disease control authorities.

The NCLLS disk susceptibility test, for its simplicity and low cost, has been widely accepted by hospitals for qualitative analysis of drugs. Standardization of the technique should affect the accuracy and reliability of testing for drug resistance. Common reasons of error are inadequate storage or expiration of the

Mueller-Hinton agar; inadequate storage or expiration of disks; inadequate preparation of the McFarland 0.5 barium sulfate suspensions; and inadequate calculation of the inhibition ring. When the dosage of antibiotics requires adjustment or the disk finding shows intermediate value, quantitative analysis of the MIC is necessary to provide physicians with information for the prescription of drugs. NCLLS and the Taiwan Infectious Disease Association both recommend that, for *Salmonella*, drug susceptibility test for drug resistance is only required for ampicillin, the quinolones group, the third-generation cephalosporins, sulfamethoxazole, and chloramphenical. It should be noted, however, that laboratory testing of the aminoglycosides group such as gentamycin, streptomycin and amikacin though is susceptible, they are clinically ineffective. The first and the second generation drugs of the cephalosporins group such as cephalothin and cefamondole are the same.

Disk susceptibility test of 114 isolated strains of sporadic infections showed that the conventional front-line drugs such as ampicillin, tetracycline, chloramphenicol and others were 70-80% drug resistant. They are no longer therapeutically effective and are not used any more clinically. Nalidixic acid of the quinolones group commonly used for intestinal tract infections was 18.4% drug resistant; and ciprofloxacin of the floronquinolones group was 3.8% drug resistant (Figure 1). Ceftiriaxone, the third generation drug of the cephalosporins group, though had no R-types, there were some intermediate types (I-types, N=17), suggesting that new generation drugs were producing drug resistance. Multi-drug resistance showed that resistance to three (inclusive) and more drugs was 64%, that to five drugs was 25%, and that to ASSuCT was 17.5%, similar to the report of the US in 1996, and that to seven and more drugs was 11% (Figure 2), suggesting that multi-drug resistance of *Salmonella typhimurium* in Taiwan is rather serious.

In the analysis of the trend of drug resistance, drugs such as ampicillin, nalidixic acid, ciprofloxacin and ceftiriaxone were selected for study in two periods of 1994-1995 and 1999-2000 for their distribution of drug resistant strains. Disk susceptibility test was used to decide their R- and I-type strains. The results were that the number of drug resistant strains was significantly higher in 1999-2000 than in 1994-1995 (Figure 3). Changes in drug resistance concentrations of *Salmonella typhimurium* in the recent years were studied by using new generation antibiotics such as ciprofloxacin and ceftiriaxone. From reports, ciprofloxacin is known as a synthesized antibiotic of extended spectrum. It inhibits the metabolism of bacteria by DNA gyrase in the course of DNA cloning. Its drug resistance is gradual, and will not develop drug resistance by plasmid-mediated β-lactam type antibiotics[(9)]. Ceftirizxone inhibits primarily the formation of cell walls, and join the albumin in blood to become a long-effect antibiotic. Both of them are used for the treatment of respiratory and intestinal tract infections. Analysis by E-test of their drug resistance concentrations with 34 strains of different concentrations showed that, of the R-type (R=14 mm, n=3) strains of ciprodloxacin, the E-test MIC was 0.38 μg/ml; of the intermediate type (I=17-20 mm, n=6), it was 0.25-0.38 μg/ml; and of the rest susceptibility strains (S=21-25 mm, n=25), it was 0.016-0.19 μg/ml. For ceftiriaxone, the E-test MIC of the intermediate strains (I=17-20 mm, n=11) was 0.82-2 μg/ml; that of the susceptibility type strains (S=21-25 mm, n=23) was 0.19-0.38 μg/ml (Table 2). Trend of the drug concentration of the two drugs (Figure 4) showed an increase. They were not yet fully drug resistant, their monitoring should be more careful.

Disk susceptibility test reads the quantitative resistance of drugs by the size of the inhibition ring; the method does not meet the practical need of therapy. In deciding on the dosage for therapy, the drug susceptibility and resistance of

antibiotics are converted into MIC. As the dosage and route of drug administration affect the maximum concentration of drug in blood, the maximum dosage should be 2-8 times larger than MIC to be effective, the measurement of MIC, therefore, is most important in deciding the dosage of drug for treatment. The Epsilometer used in the study is a combined improvement of the disk diffusion and dilution tests. By 2^{20} dilutions, different degrees of concentration were spread over the meter. There should be at least three groups of concentration for different drugs, 0.002-32 μg/ml, 0.016-256 μg/ml, and 0.064-1,024 μg/ml. MIC was read by the standard oval ring. The method though is simple and precise, it is costly. The Vitek susceptibility card (GNS 206) contains 18 antibiotics such as ampicillin, ciprofloxacin and the cephalosporins group. It has many panels for different purposes and different laboratories. It is a more rapid and convenient tool for the monitoring of drug concentration.

The study chose two commonly used antibiotics, ampicillin and nalidixic acid for the investigation of drug concentration issues by three different drug susceptibility methods, the disk susceptibility test, Vitek susceptibility card and E-test. Strains decided by the disk susceptibility test to be susceptible were tested by Vitek and E-test. MIC showed that the Vitek MIC value of ampicillin was S$\leqq$0.25 μg/ml; the E-test MIC value was 1-4 μg/ml; and for nalidixic acid, they were S$\leqq$16 μg/ml and 1-1.5 μg/ml. Drug concentrations showed that, when susceptible strains were of one single concentration, the Vitek MIC had a certain association of MIC; and the E-test showed a wider range of MIC. They should be helpful clinically in assessing more precisely drug concentration and dosage. In contrast, strains decided to be drug resistant (R-types, n=10) by the disk susceptibility test were tested with the three drugs for MIC and E-test. The result showed that the Vitek MIC of ampicillin and nalidixic acid showed a

maximum value of ≧32 μg/ml; and the E-test also gave a maximum value of >256 μg/ml, suggesting that these strains had no responses to these drugs at all.

Vitek though can provide clinicians with information on the lowest inhibition concentration, further information on changes in the fine drug resistance concentration between strains, trend of drug resistance in drugs, and adjustment or control of drug dosage for treatment can only be provided by E-test. 18 susceptible type strains of ciprofloxacin (Vitek MIC value ≦0.5-1 μg/ml) were tested 0.012-0.032 μg/ml by the E-test; and 23 susceptible strains of ceftiriaxone (Vitek MIC value ≦8 μg/ml) were tested 0.125-1 μg/ml by the E-test (Table 2), suggesting that when Vitek was a single concentration, there could be differences in the E-test MIC. This difference should be noted with care in the clinical monitoring of drug resistance.

Conclusion

1.70-80% of the conventionally used front-line drugs in Taiwan are drug resistant. 25% of strains are resistant to five drugs, 17.5% to ASSuCT, and 11% to more than seven drugs, suggesting that *Salmonella typhimurium* responsible for food-borne infections in Taiwan has a serious problem of multi-drug resistance.

2. Analysis of the ratios of strains resistant to ampicillin, nalidixic acid, ciprofloxacin and ceftiriaxone in different time periods showed that the ratio of strains isolated in 1999-2000 was significantly higher than those isolated in 1994-1995. Increase in the drug resistance concentrations of ciprofloxacin and ceftiriaxone also suggested that the new generation drugs were producing drug resistant strains. More attention should be given to.

3. The association in drug resistance concentrations of three susceptibility testes was studied. Cross comparison of MIC changes in disk susceptibility test,

Vitek and E-test by using ampicillin and nalidixic acid showed differences in concentrations by different tests. Drug concentrations of ciprofloxacin and ceftiriaxone also suggested that the differences in drug concentration should be more precisely assessed in the monitoring of drug resistance among strains and in drug dosage for clinical treatment.

4. Each method has its merits. NCLLS disk susceptibility test though is simple and economic, provides only information on the qualitative concentration of drugs. Vitek though provides MIC for clinical use, it does not provide information on the fine changes in drug concentration between strains and the trend of drug resistance. E-test gives distribution of different concentrations, and is helpful in the assessment of drug concentration and dosage in clinical use, it is costly. Its clinical use is thus restricted.

Recommendations

1. Surveillance of the drug resistance of food-borne *Salmonella* should be continued to provide information on the status of drug resistance and its trend.
2. Molecular epidemiological methods should be used to understand the routes of transmission of clinical food-borne poisoning by food or meat contaminated by *Salmonella*, and to investigate the difference in drug resistance patterns of *Salmonella* isolated from animals and poultry and clinically from humans, to decide if increase in drug resistance is associated with agricultural and fishery farming practices (addition of antibiotics in animal feeds, for instance).
3. Investigation should be made on the characteristics of multi-drug resistant strains and their drug resistance mechanism to face the increasingly serious problem of drug resistance.

Prepared by：KL Chen, CN Yeh, HC Lee, CL Tsai, HY Chiu, TK Wang, CL Lee, HP Su, HS Wu, TH Lin

Intestinal Tract Bacteria Laboratory, Division of Research and Laboratory Testing, CDC, DOH

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Figure 1. Rates of Resistance of *Salmonella typhimurium* to Various Antibiotics Resistance rate (%)

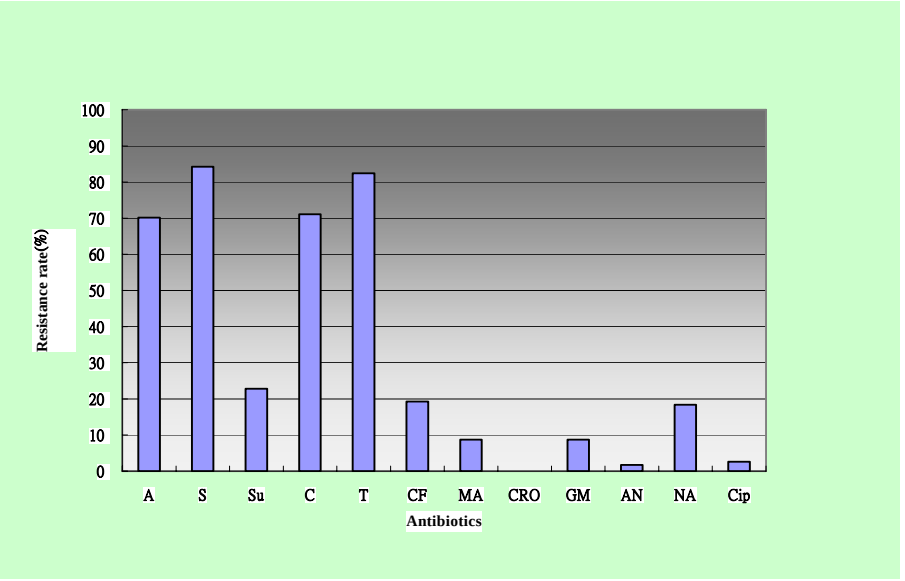


Figure 2. Analysis of Multi-Drug Resistance of *Salmonella typhimurium*

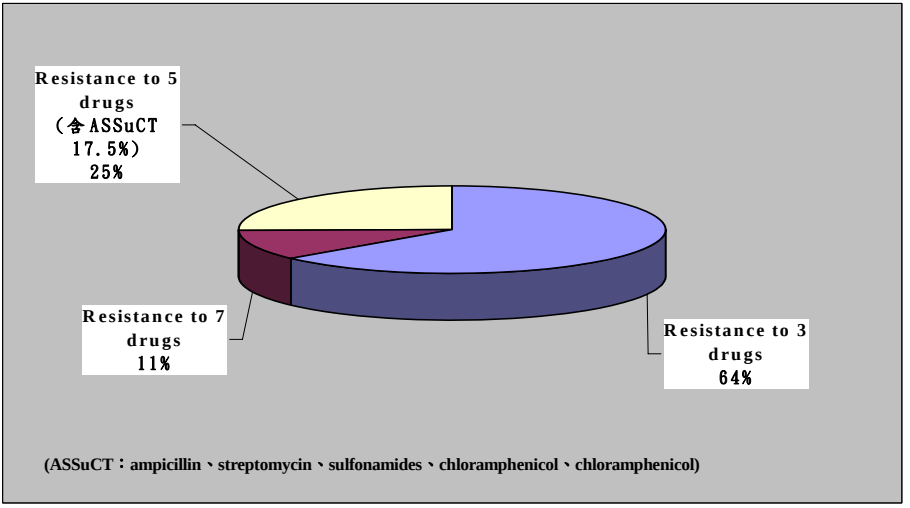
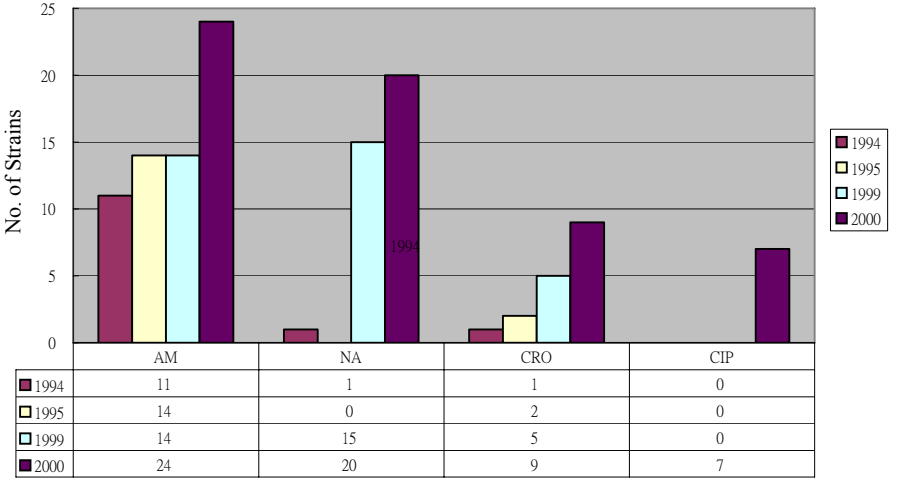


Figure 3. Rates of Resistance of *Salmonella typhimurium* to Some Commonly Used Antibiotics in two Different Time Periods (1994-1995 and 1999-2000)



	AM	NA	CRO	CIP
1994	11	1	1	0
1995	14	0	2	0
1999	14	15	5	0
2000	24	20	9	7

Figure 4. Trend of Antibiotic Resistance Concentrations of Ciprofloxacin and Ceftiriaxone

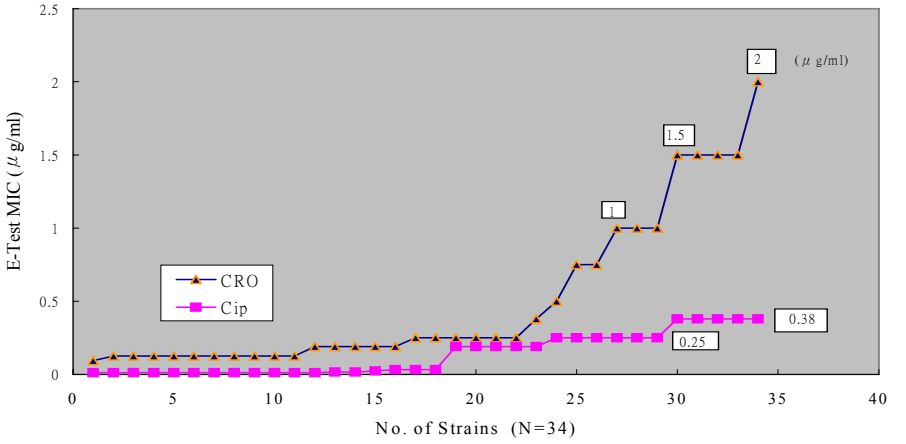


Table 1. Associations of MIC of Ampicillin, Nalidixic Acid and Gentamycin by Disk Susceptibility Test, Vitek and E-test

		AM			NA		
		Disk	Vitek	E-test	Disk	Vitek	E-test
No	Date isolated	13,14-16,17	≧32-≦0.25	0.016-256	13,14-16,17	≧32-≦16	0.016-256
4	5/24/90	S(19)	≦0.25(S)	2	S(21)	≦16(S)	1
17	62	S(17)	≦0.25(S)	4	S(20)	≦16(S)	1
00-17	4/12/89	S(18)	≦0.25(S)	2	S(19)	≦16(S)	1
00-23	5/24/89	S(19)	≦0.25(S)	4	S(20)	≦16(S)	1.5
00-28	5/24/89	S(18)	≦0.25(S)	1.5	S(19)	≦16(S)	1
92- -1	8/4/81	S(19)	≦0.25(S)	1	S(20)	≦16(S)	1.5
94- -11	1/2/83	S(18)	≦0.25(S)	1	S(20)	≦16(S)	1.5
94- -13	1/2/83	S(18)	≦0.25(S)	1.5	S(19)	≦16(S)	1
95- -8	5/25/84	S(19)	≦0.25(S)	3	S(20)	≦16(S)	1.5
95- -9	6/29/84	S(18)	≦0.25(S)	4	S(20)	≦16(S)	1.5
16	5/13/88	R(-)	≧32(S)	>256	R(-)	≧32(S)	>256
19	5/13/88	R(-)	≧32(S)	>256	R(-)	≧32(S)	>256
21	9/4/88	R(-)	≧32(S)	>256	R(-)	≧32(S)	>256
25	9/4/88	R(-)	≧32(S)	>256	R(-)	≧32(S)	>256
26	10/4/88	R(-)	≧32(S)	>256	R(-)	≧32(S)	>256
00-20	6/15/89	R(-)	≧32(S)	>256	R(-)	≧32(S)	>256

Table 2. Associations of MIC of Ceftiriaxone and Cirpofloxacin by Disk Susceptibility Test, Vitek and E-test

drug/methods / strains		Ceftiriaxone(CRO)						drug/methods / strains		Ciprofloxaacin(Cip)					
		Disk			Vitek		E-test			Disk			Vitek		E-test
		R	I	S	R	S				R	I	S	R	S	
No	Date isolated	≤13	14-20	≥21	≥64	≤8	0.016-256	No	Date isolated	≤15	16-20	≥21	≥4	≤0.5	0.016-256
00-26	6/2/89	I(17)			I(16)		2	16	5/13/88	R(15)			S(≤1)		0.38
19	5/13/88	I(18)			I(16)		1.5	19	5/13/88	R(14)			S(≤1)		0.38
25	9/28/88	I(18)			I(16)		1.5	21	9/4/88	R(14)			S(≤1)		0.38
00-20	6/15/89	I(18)			I(16)		1.5	00-32	5/18/89	I(18)			S(≤1)		0.38
00-29	6/15/89	I(18)			I(16)		1.5	25	9/28/88	I(17)			S(≤0.5)		0.38
26	10/4/88	I(20)			I(16)		1.0	00-20	6/15/89	I(18)			S(≤1)		0.25
00-24	6/15/89	I(19)			S(≤8)		1.0	00-27	6/20/89	I(17)			S(≤1)		0.25
00-32	5/18/89	I(19)			S(≤8)		1.0	26	10/4/88	I(20)			S(≤0.5)		0.25
00-51	10/18/89	I(20)			S(≤8)		0.75	00-33	6/22/89	I(20)			S(≤0.5)		0.25
16	5/13/88	I(19)			S(≤8)		0.75	00-29	6/15/89	S(22)			S(≤0.5)		0.19
00-33	6/22/89	I(20)			S(≤8)		0.38	18	5/16/88	S(22)			S(≤0.5)		0.19
00-30	6/19/89	S(24)			S(≤8)		0.25	00-26	6/2/89	S(22)			S(≤0.5)		0.19
00-37	4/5/89	S(22)			S(≤8)		0.25	00-46	9/13/89	S(21)			S(≤0.5)		0.19
94-2	8/15/83	S(21)			S(≤8)		0.25	00-23	5/24/89	S(25)			S(≤0.5)		0.032
94-15	1/2/83	S(21)			S(≤8)		0.25	00-31	5/18/89	S(25)			S(≤0.5)		0.032
00-27	6/20/89	S(24)			S(≤8)		0.19	94-14	1/2/83	S(23)			S(≤0.5)		0.032
00-43	8/4/89	S(23)			S(≤8)		0.19	00-4	6/22/89	S(24)			S(≤0.5)		0.023
00-34	6/22/89	S(22)			S(≤8)		0.19	00-41	7/11/89	S(25)			S(≤0.5)		0.016
00-52	10/13/89	S(22)			S(≤8)		0.19	00-42	8/4/89	S(25)			S(≤0.5)		0.016