CHAPTER 3

RESULTS

1. <u>Comparative study of modified broth disk method and disk</u> diffusion method using BBL disks

The modified broth disk method was determined in comparison with disk diffusion method using 12 BBL disks : SM, TC, CM, CL, NM, CO, KM, FN, FM, GM, AM and SDZ tested against <u>E. coli</u> 23 strains, <u>Salmonella</u> <u>sp</u> 49 strains, <u>Shigella sp</u> 28 strains, <u>V. Cholerae</u> 10 strains and <u>V. parahaemolyticus</u> 15 strains.

The data shown in Table 14, clearly indicate that the results obtained from the modified broth disk method reading at 3, 6, 8, 18 h intervals when compared with those from disk diffusion method are related with small discrepancies, in all tested organisms against TC, CM, CO, PN and AM. For example, there were 26.09% of E. coli susceptible to CM testing with disk diffusion method and 26.09%, 26.09%, 20.09%, 21.74% testing with modified broth disk method detected at the 3, 6, 8, 18 hr. intervals.

The agreement in the results obtained from the two methods were also demonstrated with KM tested against <u>Shigella sp</u>. The percentage susceptibility of which were 78.57% using disk diffusion method and 71.43%,

Modified Broth Lisk Method as compared with isk

Diffusion Lethod using BBL disks

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-		F . 9	eoli (23))		-	Salmo	nelle (4	9)			Shi	ella (28	в)	_
Antibio-	Diek		Hed 1	DD		Diek	12.5	' Med I	щ		Disk		Ned 1	B-D	
1168	Dif.	3	6	8	18	Dif.	3	6	8	18	Dif.	3	6	8	1
5H	39.13	4.35	0	0	0	61,22	4.08	2.0	0	0	21.43	0	0	0	1
TC	26.09	26.09	21.74	21.74	17.39	65.31	63.27	63.27	61.22	59.18	14.86	14.29	17.86	17.86	17
СН	26.09	26.09	26.09	26.09	21.74	69.39	71.43	71.43	69.39	69.39	28.57	28,57	28.57	28.57	28
CL	56.52	86.96	82.61	82.61	73.91	83.67	97.96	95.92	85.71	81.63	89.29	96.55	96.55	96.55	82
NM	47.83	4.35	0	0	0	53.06	34.69	34.69	34.69	20.41	50	35.71	35.71	32.14	28
00	78.26	82.60	82.60	69.57	69.57	89.80	89.80	89.80	83.67	81.63	89.29	92.86	92.86	92.86	92
KM.	52.17	4.35	0	0	0	73.47	4.08	4.08	4.08	2.04	78.57	71.43	71.43	75.00	71
IN	4.35	4.35	4.35	4.35	0	6,12	4.08	4.08	4.08	0	0	0	0	0	0
рн	43.48	95.65	95.65	95.65	95.65	55.10	97.96	95.92	95.92	81,63	57.14	100	100	100	78
GM	78.26	82,60	82,60	73.91	65.22	69.39	6.12	6.12	6.12	4.08	92.86	3.57	0	0	0
AN	13.04	8.70	8.70	8.70	4.35	81.63	77.55	75.51	67.35	60.71	71.43	75	75	71.43	60
SDZ.	13.04	4.35	0	0	0	6.12	0	0	0	0	0	0	0	0	0

Table 14 Continue

Antibio		V. Ch	olera ()	10)		-	V. parah	aemolyti	cus 15	
tics	Disk		Mod 1	B-D		Disk		Mod I	3-D	
	Dif.	3	6	8	18	Dif.	3	6	8	18
SM	30.0	10.0	0	0	0	93.83	73.33	73.33	73.33	13.33
TC	100	100	100	100	100	93.33	100	100	100	100
СМ	90:0	100	100	100	100	86.67	86.67	80.0	80.0	73.33
CL	10.0	10.0	10.0	0	0	6.67	20.0	20.0	20.0	13.33
NM	80.0	60.0	80.0	80.0	50.0	80.0	86.67	93.33	80.0	40.0
co	80.0	80.0	80.0	80.0	80.0	100	93.33	100	93.33	80.0
KM	40.0	20.0	10.0	0	0	86.67	60.0	53.33	53.33	40.0
PN	0	0	10.0	10.0	0	6.67	0	0	0	0
PM	20.0	10.0	10.0	10.0	10.0	. 6.67	13.33	13.33	13.33	13.33
GM	70.0	30.0	40.0	40.0	10.0	80.0	46.67	26.67	20.0	13.33
MA	80.0	90.0	90.0	80.0	60.0	40.0	40.0	40.0	40.0	26.67
SDZ	30.0	40.0	30.0	20.0	20.0	73.33	40.0	6.67	0	0

71.43%, 75%, 71.43% using modified broth disk method reading at 3, 6, 8, 18 hrs. respectively. Again, the agreement also demonstrated with GM tested against E. coli and SD% against Shigells sp, as shown in the Table 14.

There were considerable variation in the results obtained from the two methods with SM against all tester organisms. The nercentage discremancies showed as 39.13% suspectibility of F. coli against SM using the disk diffusion method, and 4.35%, 0%,00%, 0%, from using the modified broth disk method reading at 3, 6, 8, 18 hrs. and vice versa against other organisms. Also, the marked discrepancies between the two method were demonstrated with NM against E. coli, Salmonella sp and Shigella sp. These percentage differences of which, as determined by disk diffusion method and modified broth disk method reading at 3, 6, 8, 18 hrs. intervals, were 47.83% and 4.35%, 0%, 0%, 0% with NM against E. coli: 53.06% and 34.69%, 34.69% 34.68%, 20.41% with NM against Salmonella sp: 50% and 35.71% 35.71%, 32.14%, 28.57% with NM against Shigella sp. The disagreement between the two methods were also shown with KM against E. coli, Salmonella sp, V. cholerae, V. parahaemolyticus: GM against Salmonella sp, Shigella sn, V. cholerae, V. parahaemolyticus and also with SNZ against V. parahaemolyticus. The percentage differences of which were 52.17% and 4.35% 0%, 0%, 0% demonstrated with KM against E. coli as determined by disk diffusion method and to modified broth disk method reading at 3, 6, 8, 18hrs. intervals respectively for instance.

Some disagreement of the two methods were also found with PM and CL against all tested organisms as follow: <u>E. coli</u> showed 56.52% susceptibility to CL as determined by disk diffusion method and 86.96%, 82.61%, 82.61%, 73.91% as determined by modified broth disk method reading at 3, 6, 8, 18 hrs. intervals. <u>E. coli</u> tested against PM, the percentage susceptibility were 43.48% using disk diffusion method and 95.65%, 95.65%, 95.65%, 95.65% using modified broth disk method detected at 3, 6, 8, 18 hrs. intervals. And vice versa for CL and PM against <u>Salmonella</u> sp, <u>Shigella</u> sp, <u>V., cholera</u> and <u>V.</u> parahaemolyticus as given in Table 14.

View from the point of percentage susceptibility reading at different interval in the modified broth disk method, there was good agreement among 3, 6, 8, 18 hrs. readings for the majority of the antimicrobials tested against most of the organisms (Table 14). For example, the readings at 3, 6, 8, 18 hrs. of TC susceptibility were 100%, 100%, 100%, 100% against both <u>V. cholerae</u> and <u>V. parahaemolyticus</u> and 14.29%, 17.86%, 17.86%, 17.86% against Shigella sp.

However, NM was found to show some differences in the percentage susceptibility reading at 3, 6, 8, 18 hrs. as 34.69%, 34.69%, 34.69%, 20.41% tested against <u>Salmonella</u> sp; 35.71%, 35.71%, 32.14%, 28.57% tested against <u>Shigella</u> sp; 60%, 80%, 80%, 50% tested against <u>V. cholerae</u> and 86.67%, 93.33%, 80%, 40% tested against <u>V. parahaemolyticus</u>.

It was important to note that most of the percentage susceptibility obtained from the 18 hrs. reading were about $\leq 5\%$ lower than those from

the earlier readings i.e. Reading at 3, 6, 8, 18 hrs: The susceptibility of E. <u>coli</u> to SM were 4.35%, 0%, 0%, 0%; and the susceptibility of TC tested against <u>Salmonella</u> sp and Shigella sp were 65.27%, 63.27%, 61.22%, 59.18% and 14.29%, 17.86%, 17.86%, 17.86%. However, there were some marked discrepancies of which showed > 10% percentage susceptibility of the 18 hrs. reading lower than the earlier readings in <u>V. parahaemolyticus</u> as 73.53%, 73.33%, 75.33%, 13.33% suscentible to SM reading at 3, 6, 8, 18 hrs. interval: 93.33%, 100%, 93.53%, 80% susceptible to CO: 60%, 53.33%, 53.33%, 40% and 40%, 40%, 26.67% susceptible to CO and AM respectively. The discrepancies were also demonstrated with CL tested against <u>Shigella</u> sp, <u>Salmonella</u> sp.; C0 tested against <u>E. coli</u>: PM tested against <u>Salmonella</u> sp. and <u>Shigella</u> sp.; GM tested against <u>E. coli</u> and <u>V. cholerae</u>; AM tested against <u>Shigella</u> sp. and <u>V. gholerae</u>.

2. <u>Comparative study of BBL disks and local disks as determined by the</u> modified broth disk method and disk diffusion method

Eight local disks : SM, TC, CM, CL, NM, CO, KM and PN were introduced into the study of modified broth disk method and disk diffusion method as in comparison with the BEL disks as data given in Table 15 of which individual percentage susceptibility result using the two disks were compared into 3 categories of agreement as whether the percentage susceptibility result obtained using BEL less than, equal or more than those using local disks. The numbers of agreement in each category were analysed into percentage agreement as data given in Table 16 as determined by disk diffusion method. The majority of agreement in all disks were given to the BEL > local

modified broth disk and disk diffusion methods using BBL

disks compared with the local disks

				2 - S	E. col	<u>i</u> (23) [@]				
Anti-		В	BL disk		-		I	OCAL di	sk	
biotic	Disk		Mod B-	D		Disk		Mod	B-D	
biotic Di Di Di SM 39 TC 26 CM 26 CM 26 CM 26 CM 26 CM 26 CM 56 NM 47 CO 78 KM 52 PN 4 PM 43 GN 78	Dif.	300	6	8	18	Dif.	3	6	8	18
SM	39.13	4.35	0	0	0	52.17	4.35	0	0	0
TC	26.09	26.10	21.74	21.74	17.39	21.74	26.10	26.10	26.10	26.10
СМ	26.10	26.10	26.10	26.10	21.74	26.10	26.10	26.10	26.10	21.74
CL	56.52	86.96	82.61	82.61	73.91	60.87	82.61	82.61	78.26	69.57
NM	47.83	4.35	0	0	0	47.83	4.35	0	0	0
со	78.26	82.60	82,60	69.57	69.57	78.26	52.17	82.61	73.91	73.91
KM	52.17	4.35	0	0	0	47.83	4.35	0	0	0
PN	4.35	4.35	4.35	4.35	0	0	0	0	0	0
РМ	43.48	95.65	95.65	95.65	95.65	-	-	-	-	-
GN	78.26	82.60	82.60	73.91	65.22	-	-	-	-	-
AM	13.04	8.70	8.70	8.70	4.35	-	-	-	-	-
SDZ	13.04	4.35	0	0	0	-	-	-	-	-

@ Number of tested strains

@@ Modified broth disk method

@@@ Besult reading at 3, 6, 8, 18 hrs. after incubation.

Table 15

e 15 Percentage susceptibility of organisms as determined by

modified broth disk and disk diffusion methods using BBL

disks compared with the local disks

				Salı	monella	sp. (49	9)			
Anti-		I	BBL disl	2			1	LOCAL di	sk	
biotic	Disk		Mod B-	-D ^{@@}		Disk		Mod	B-D	
SM TC CM CL NM	Dif.	300	6	8	18	Dif.	3	6	8	18
SM	61.22	4.08	2.04	0	0	57.14	2.04	2.04	2.04	2.04
TC	65.31	63.27	63.27	61.22	59.18	61.22	61.22	61.22	61.22	59.18
СМ	69.39	71.43	71.43	69.39	69.39	61.22	71.43	69.39	67.35	67.35
CL	83.67	97.96	95.92	85.71	83.67	79.59	95.92	91.84	91.84	83.67
NM	53.06	34.69	34.69	34.69	20.41	46.94	30.61	32.65	26.53	20.41
CO	89.80	89.80	89.80	83.67	81.63	89.80	85.71	89.80	83.67	81.63
KM	73.47	4.08	4.08	4.08	2.04	69.39	0	0	0	0
PN	6.12	4.08	4.08	4.08	0	8.16	6.12	4.08	2.04	0
PM	55.10	97.96	95.92	95.92	81.63		-	-	-	-
GN	69.39	6.12	6.12	6.12	4.08	-	-	-	-	-
АМ	81.63	77.55	75.51	67.35	60.71	-51	-	-	-	-
SDZ	6,12	0	0	0	0	-	-	_	-	_

C Number of tested strains

@ Modified broth disk method

CCC Result reading at 3, 6, 8, 18 hrs. after incubation

modified broth disk and disk diffusion methods using BBL

disks compared with the local disks

				Shi	gella s	p. (28)	0			
Anti-		В	BL disk				L	OCAL di	sk	
biotic	Disk		Mod B-	D ^{@@}	1	Disk		Mod	B-D	
	Dif.	<u>9</u> 00	6	8	18	Dif.	3	6	8	18
SM	21.43	0	0	0	0	10.71	3.57	0	0	0
TC	17.86	14.29	17.86	17.86	17.86	32.14	39.29	25.00	25.00	25.00
СМ	28.57	28.57	28.57	28.57	28.57	10.71	7.14	7.14	7.14	7.14
CL	89.29	96.55	96.55	96.55	82.76	78.57	100	96.43	96.43	82.14
NM	50.00	35.71	35.71	32.14	28.57	35.71	35.71	35.71	28.57	14.29
CO	89.29	92.86	92.86	92.86	92.86	82.14	78.57	78.57	78.57	71.43
KM	78.57	71.43	71.43	75.00	71.43	75.00	67.86	71.43	71.43	71.43
PN	0	0	0	0	0	0	0	0	0	0
РМ	57.14	100	100	100	78.57	-	-	-	-	-
GN	92.86	3.57	0	0	0	-	-	-	-	-
AM	71.43	75.00	75.00	71.43	60.71	-	-	-	-	-
SDZ	0	0	0	0	0	-	-	-	-	-

Number of tested strains 0

00 Modified broth disk method

Result reading at 3, 6, 8, 18 hrs. after incubation. 000

modified broth disk and disk diffusion methods using BBL

disks compared with the local disks

				v	. chole	erae (10)	e			
Anti-		BE	L disk				I	OCAL di	sk	
biotic SM TC : CM CL NM CO KM	Disk		Mod B-	.D ^{@@}		Disk		Mod	B-D	
	Dif.	. 300	. 6	. 8	. 18	Dif.	. 3	6	8	18
SM	30	10	0	0	0	30	10	0	0	0
TC	100	100	100	100	100	90	100	100	100	100
См	90	100	100	100	100	100	100	100	90	90
CL	10	10	10	10	10	10	0	0	0	0
NM	80	60	80	80	50	80	40	80	80	40
CO	80	80	80	80	80	80	80	80	80	70
KM	40	20	10	0	0	50	20	10	0	0
PN	0	0	10	10	0	20	20	10	10	0
PM	20	10	10	10	10	-	-	_	-	-
GN	70	30	40	40	10	-	-	-	-	-
AM	80	90	90	80	60	-	-	-	-	-
SDZ	30	40	30	20	20	1	-	_	-	-

@ Number of tested strains

@@ Modified broth disk method

CCC Result reading at 3, 6, 8, 18 hrs. after incubation.

modified broth disk and disk diffusion methods using BBL

disks compared with the local disks

				<u>V</u> . <u>p</u> a	arahaemo	lyticu	<u>s</u> (15)			
CO KM PN PM		1	BBL disl	2			1	LOCAL di	lsk	
biotic	Disk		Mod B-	-D ^{C©}		Disk		Mod	B-D	
biotic SM TC CM CL NM CO KM PN	Dif.	300	6	8	18	Dif.	3	6	8	18
SM	93.33	73.33	73.33	73.33	73.33	86.67	73.33	26.67	26.67	26.67
TC	93.33	100	100	100	100	93.33	100	100	100	100
СМ	86.67	86.67	80.00	80.00	73.33	80.00	86.67	86.67	73.33	66.67
CL	6.67	20.00	20.00	20.00	13.33	6.67	20.00	20.00	20.00	13.33
NM	80.00	86.67	93.33	80.00	40.00	93.33	93.33	93.33	100	93.33
со	100	93.33	100	93.33	80.00	93.33	66.67	86.67	73.33	66.67
KM	86.67	60.00	53.33	53.33	40.00	86.67	60.00	53.33	46.67	26.67
PN	6.67	0	0	0	0	0	0	0	0	0
PM	6.67	13.33	13.33	13.33	13.33	-	-	-	-	-
GN	80.00	46.67	26.67	20.00	13.33		-	-	-	-
AM	40.00	40.00	40.00	40.00	26.67	-	-	-		-
SDZ	73.33	40.00	6.67	0	0	-	_	-	-	

@ Number of tested strains

OC Modified broth disk method

CCC Result reading at 3, 6, 8, 18 hrs. after incubation.

Table 16 <u>Percentage agreement of susceptibility results using BBL</u> <u>disks and local disks as determined by disk diffusion method</u> <u>tested against 125 strains of enteropathogens</u>

Anti-	Pe	ercentage agreement	
biotic	BBL < local disk	BBL = local disk	BBL > local disk
SM	20	20	60
TC	20	20	60
СМ	20	40	40
CL	20	40	40
NM	20	40	40
CO	-	60	40
KM	20	20	60
PN	20	20	20
TOTAL	20	32.5	47.5

Table 17Percentage agreement of susceptibility results using BBLdisks and local disks as determined by modified broth disk

method tested against 125 strains of enteropathogens

					Pe	rcentag	e sgree	ment							
Anti-		3 brs.	12.24	1	6 hrs.			8 hrs.			18 hrs.	5.24	100	Total	-
biotic	BBLく local	BBL= local	BBL> local	BBL < local	BBL= local	BBL > local	HEL < lecal	BBL= local	BBL >	HBL < local	BBL= local	BBL > local	BBL < local	BBL= local	BBL > local
SM	20	60	20	-	100	-	20	60	20	20	60	20	15	70	15
TC	40	40	20	40	40	20	40	60	-	40	-	-	40	50	10
СМ	-	80	20	20	40	40	-	40	60	-	20	80	5	45	50
CL	20	20	60	-	40	60	20	20	60	-	40	60	10	30	60
NM	40	40	20	-	80	20	20	40	40	20	40	40	20	50	30
co	-	20	80	-	60	40	20	40	40	20	20	60	10	35	55
EM,	20	40	40	20	60	20	-	40	60	-	60	40	10	50	40
PN	20	60	20	20	60	20	-	60	40	-	100	-	10	70	20
TOTAL	20	45	35	12.5	60	27.5	15	45	40	12.5	50	37.5	15	50	35

disk category. OF the total numbers of agreement (5 groups of tested organism x 8 antibiotic disks = 40), there were 20% (8/40) of results which showed the lower percentage susceptibility yielded by BBL disk than those from local disk. About thirty three percent of results demonstrated the equal percentage susceptibility and 47.5% of results demonstrated the higher percentage achieved in BBL than local disks. Almost every disk demonstrated the percentage susceptibility obtained using BBL disks were equal or more than those using the local disks i.e. Sixty percent of the agreements showed the higher percentage susceptibility achieved using SM-BBL disks than using SM-local disks, only 20% of agreement showed the lower percentage susceptibility achieved using SM-BBL disks than using SM-local disk or vice versa for other disks as given in Table 16.

However, it was important to note that there were some prominent differences in the percentage susceptibility obtained using the two disks in SM disks tested against <u>E. coli</u> and <u>Shigella</u> and also CM, TC disks tested against <u>Shigella</u> sp. The percentage susceptibility of which shown in Table 14 as <u>E. coli</u> demonstrated 39.13% susceptibility using BBL disk where as 52.17% susceptibility using local disk and so on.

The percentages agreement of susceptibility results using BBL and local disks as determined by modified broth disk method shown in Table 17 were analysed under the same criteria used in Table 16. As demonstrated in Table 17 that, there were 15% (24/160), 50% (80/160, 35% (56/160) of overall agreements of which the susceptibility percentage obtained using BBL were less than, equal or more than those from using

local disk respectively.

Reading at 3 hrs., there were 20% (8/40), 45% (18/40), 35% (14/40) of agreements demonstrated the percentage susceptibility obtained using BBL disks were less than, equal or more than those using local disks respectively. Reading at 6 hrs., the agreements of which demonstrated the equal percentage susceptibility obtained using both disk increased to 60% (24/40). About 13% or 28% agreements demonstrated that the percentage susceptibility obtained using BBL were less than or more than those using local disk respectively and vice versa.

For the individual disk, it was shown that the majority of agreements demonstrated the results obtained using BBL were equal or more than those using local disk i.e. SM demonstrated 15% (3/20), 70% (14/20) or 15% (3/20) of agreement of which the results using BBL were less than, equal or more than those using local disk respectively. For CL disks, there were 10% (2/20), 30% (5/20) or 60% (12/20) of agreements which demonstrated that results obtained using BBL less than, equal or more than those local disk respectively.

However, the TC disk demonstrated the different agreements that there were 40%, 50% or 10% of agreement which the results using BBL were less than, equal or more than those using local disk respectively. Reading at different intervals, the majority of agreements demonstrated that results obtained using BBL were less than or equal those local disks i.e. At the 8 hrs. reading, there were 40% (2/5) and 60% (3/5) of the data demonstrated that results obtained using BBL were less than or equal those local disks and vice verse.

The percentage susceptibility differences obtained using the two disks as demonstrated in Table 15 were within $\pm 10\%$ i.e. Tested against Shigella sp., the percentage susceptibility obtained using CL-BEL disks were 96.55%, 96.55%, 82.76% and the percentage susceptibility obtained using CL-local disk were 100%, 96.43%, 96.43%, 82.14% using modified broth disk reading at 3, 6, 8, 18 hrs. respectively.

However, the marked differences between the percentage susceptibility using the two disks were demonstrated with TC, CM tested against <u>Shigella</u> sp. i.e. The percentage susceptibility obtained using TC-BBL disks were 14.29% reading at 3 hrs. whereas the percentage susceptibility using TC-local disk were 39.29%. Using CM-BBL disk, the percentage susceptibility tested against Shigella sp. were 28.57% whereas those obtained using CM local disk were 7.14% reading at 3 hrs. in the modified broth disk method.

3. Determination of MIC by broth dilution and agar dilution methods

The MIC of <u>Salmonella</u> sp. 30 strains, <u>Shigella</u> sp. 15 strains, <u>E. coli</u> 10 strains and <u>V. parahaemolyticus</u> 6 strains to SM, TC, CM, PN, GM, KM, PN, AM and NM was determined using the agar dilution and broth dilution method.

The MIC obtained by the two dilution methods were compared in Table 18-21 with the percentage of sensitive strains to individual MIC. Except for one strain of <u>E. coli</u> tested with FN, all organisms had identical MIC's or, as demonstrated in Fig. 5-6. varied by one or two dilutions when tested by the two methods.

		Minimum	Inhibiter	y Cencent	ration .	- MIC (pg	/ ml)						
Anti- biotic	Tested	0.02	0.039	0.078	0.156	0.313	0.625	1.25	2.5	5.0	10	20 25	20
54	A-D B-D	-	-	-	-	-	- 3.33*	13.33 13.33	43.33 50.00	6.67 3.33	6.67 3.33	30.0 0 26.67	-
TC	A-D B-D	-	-	-	-	6.67 6.67	10.00 13.33	13.33 13.33	33.33 43.33	3.33 6.67	13.33 3.33	20.00 13.33	-
CM	A-D	-	-	-	-	-	-	-	46.67	20.00	6.67	26.67	-
PM	A-D B-D	-	• -	- 3.33	6.67 3.33	1000	40.00 46.67	13.33 10.00	6.67	16.67 3.33	3.33 3.33	-	-
GM	A-D	-	-	-	10.00	10.00	10.00	36.67	-	-	10.00	23.33	-
EM	A-D B-D	-	-	-	-	-	-	13.33 23.33	56.67 50.00	- 6.67	13.33 3.33	16.67 16.67	-
PN	A-D B-D	-	-	-	-	-	-	5.53	- 6.67	-	26.67 16.67	50.00	20.00
AM	A-D B-D	-	-	-	-	-	20.00 26.67	50.00	3.33	-	6.67 10.00		-
NM	- B-D	-	-	-	-	-	6.67	- 13.33	- 26.67	- 3.33	- 6.67	43.33	-

Table 18 The Minimum Inhibitory Concentration of 30 strains of Salmonella sp.

as determined by broth dilution and agar dilution method

A-D = Agar dilution

B-D . Broth dilution

. Percentage of sensitive strains to marticular concentration

- - Visible growth

65

.

Anti-	Tested	Minimum	Inhibitor	y Concen	tration	- MIC (pg	/m1)						
biotic	method	0.02	0.039	0.078	0.156	0.313	0.625	1.25	2.5	5.0	10	20 25	20
SM	A-D B-D	-	-	-	-	-	-	- 6.67 [*]	20.00 [*] 13.33		13.33 20.00	66.67 60.00	-
TC	A-D B-D	-	-	1.1	-		-	-	13.33 13.33	-	13.33 26.67	73.33 60.00	-
CM	A-D -	-	-	-	-	-				6.67	26.67	66.67	
PM	A-D B-D		-	13.33 20.00	13.33		13.33 13.33	6.67 -	13.33	26.67 40.00	13.33 13.33	-	-
GM	A-D -	-	-	-	-	-	20.00	60.00	-	6.67	13.33	-	-
KM	A-D B-D		-	-	-	-	-	13.33 33.33	40.00 40.00	-		26.67 26.67	-
PN	A-D B-D	-	-	-	-	-	-	-	-	-	53.33	6.67 20.00	40.00
AM	A-D B-D	-	-	-	-	-	-	66.67 53.33	-	-	20.00 26.67	20.00 6.67	-
NM	- B-D	-	-	-		-	-	-	- 33.33	- 26.67	-	- 26.67	-

Table 19 The Minimum Inhibitory Concentration of 15 strains of Shigella sp

as determined by broth dilution and agar dilution method

A-D = Agar dilution

B-D = Broth dilution

* = Percentage of sensitive strains to marticular concentration

- = Visible mouth

66

TTAN

Table 20 The Minimum Inhibitory Concentration of 10 strains of E. coli

as determined by broth dilution and agar dilution method

Anti-	Tested	Minimum	Inhibitor	y Concen	tration	- MIC (pg	;/ml)						
biotic	method	0.02	0.039	0.078	0.156	0.313	0.625	1.25	2.5	5.0	10	20	20
SM	A-D B-D	-	-	-	-	-	-	-	* 20.00 30.00 [*]	10.00	10.00	60.00 50.00	-
TC	A-D B-D	-	-	-	-		-	- 10.00	10.00 10.00	10.00	30.00 20.00	50.00	-
СМ	A-D -	-	-	-	1.1	1.1	-	-	-	20.00	10.00	70.00	-
РМ	A-D B-D	-	-	-	-	20.00	20.00	10.00 10.00	10.00	50.00 40.00	-	10.00	-
GM	AD -	-	-	-	-	-	20.00	50.00	-	-	10.00	20.00	-
КМ	A-D B-D	-	-	-	-	-	-	-	40.00	10.00	-	50.00 50.00	-
PN	A-D B-D	-	-	-	-	1	-	- 10.00	-	-	30.00	40.00	70.00
AM	A-D B-D	-	-	-	-	-	1 1	10.00 10.00	-	10.00 10.00		70.00	-
NM	B-D	-	-		-	-	-	-	40.00	- 10.00	-	30.00	-

A-D = Agar dilution

B-D = Broth dilution

* = Percentage of sensitive strains to particular concentration

Table 21 The minimum Inhibitory Concentration of 6 strains of Vibrie para-

Anti-	Tested	Minimum	Minimum Inhibitery Concentration - MIC (ng/ml)										
bietic	method	0.02		0.078	0.156	0.313	0.625	1.25	2.5	5.0	10	20	>20
SM	A-D B-D	-	-	-		-	-	66.67 [*] 66.67 [*]	53.33 33.33	-	-	-	-
TC	A-D B-D	-	-	-	-	-	-	- 16.67	83.33 66.67	-	16.67 16.67	-	-
СМ	A-D -	-	-		-	-	-	-	-	66.67	16.67	16.67	-
PM	A-D B-D		-	-	-	-	-	-	-	16.67 16.67	-	-	83.33 83.33
GH	A-D -	-	-	-	-	16.67 -	16.67	33.33 -	-	-	16.67	16.67	-
KM	A-D B-D	-	-		-	-	-	-	50.00 83.33	33.33 -	-	16.67 16.67	-
PN	A-D B-D		-		-	-	-	- 16.67	16.67	-	-	66.67 33.33	16.67
AM	A-D B-D	-	-	-	-	-	- 16.67	53.53 33.33	16.67	-	16.67 33.33	33.33 16.67	-

haemolyticus as determined by broth dilution & agar dilution method

A-D = Agar dilution

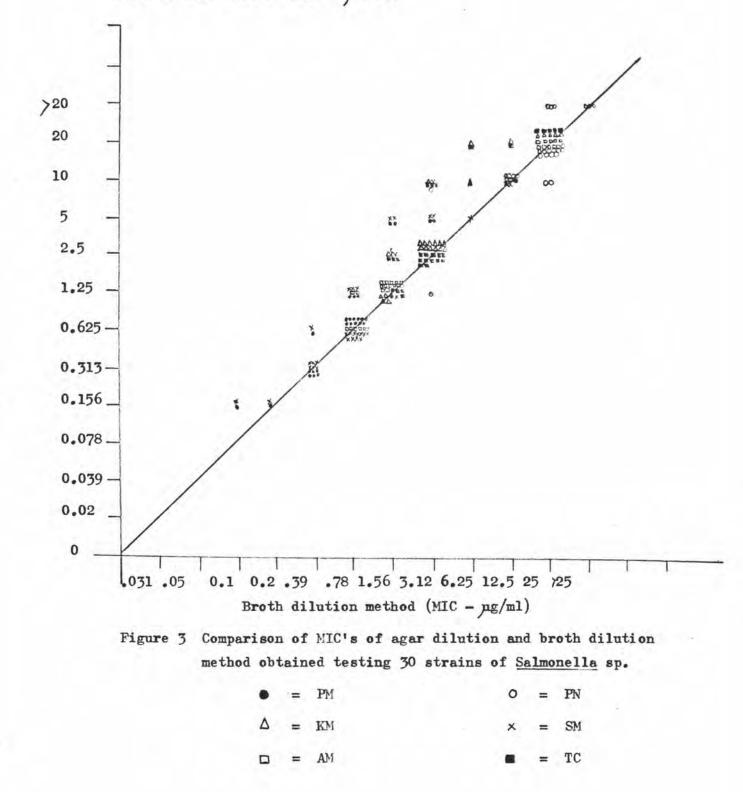
4

B-D = Broth dilution

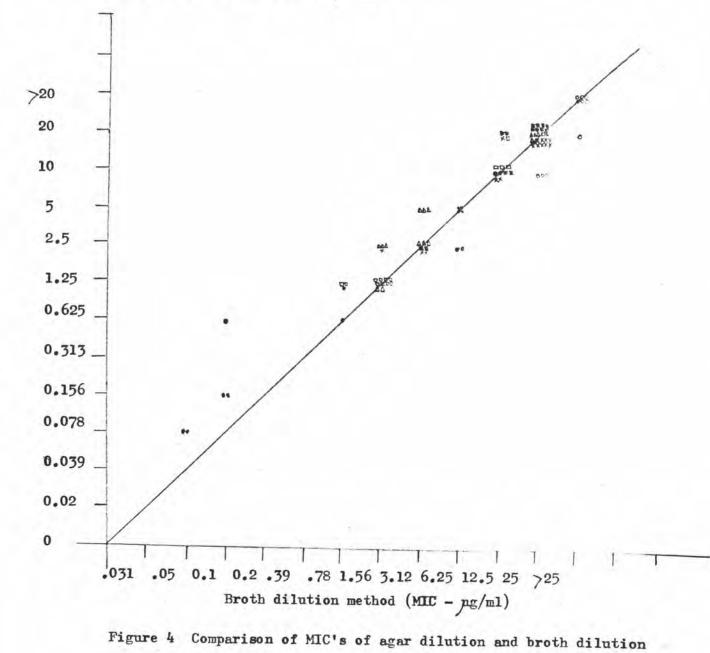
* = Percentage of sensitive strains to marticular concentration

- = Visible growth

Agar dilution method (MIC - pg/ml)

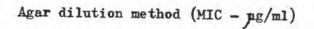


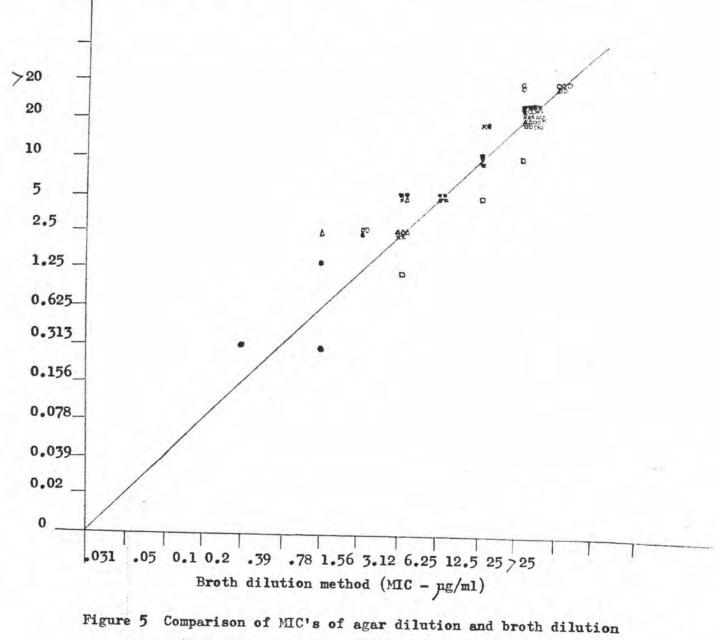
Agar dilution method (MIC - µg/ml)



method obtained testing 15 strains of Shigella sp.

•	=	PM	0	=	PN
۵	=	KM	×	=	SM
9	=	AM			TC





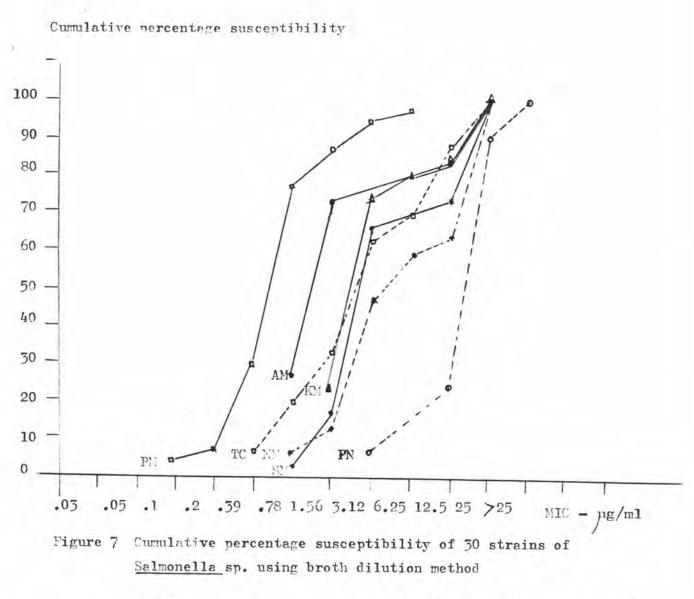
method obtained testing 10 strains of E. coli

•	=	PM	0	=	PN	
۵	=	KM	×	=	SM	
D	=	AM		=	TC	

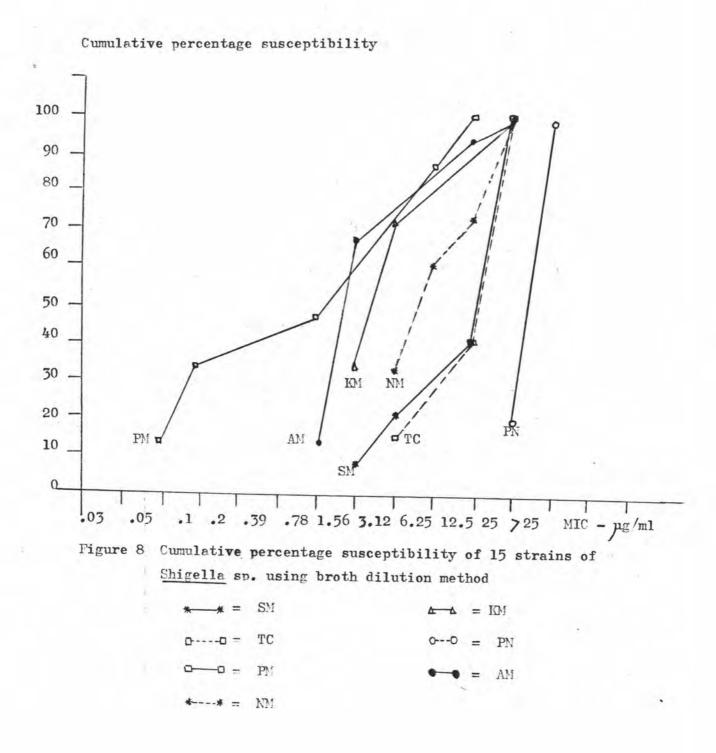
Agar dilution method (MIC - µg/ml) 720 20 10 5 44 2.5 1.25 ۵ 0.625 0.313 0.156 0.078 0.039 0.02 0 0.1 0.2 .39 .78 1.56 3.12 6.25 12.5 25 725 .031 .05 Broth dilution method (MIC - ng/ml) Comparison of MIC's of agar dilution and broth dilution Figure 6

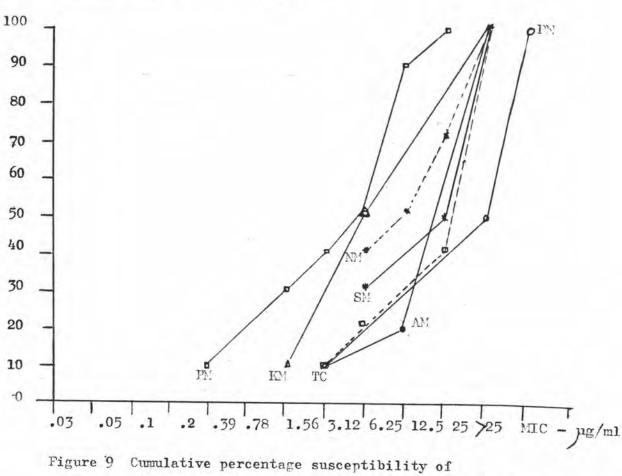
method obtained testing 6 strains of V. parahaemolyticus.

•	=	PM	0	=	PN	4
Δ	=	KM	×	=	SM	
0	=	AM		=	TC	



**	-	SM	ΔΔ =	\mathbf{K}
00			o0 =	\mathbf{PN}
0-0	=	PN		A21
4*	-	NM		

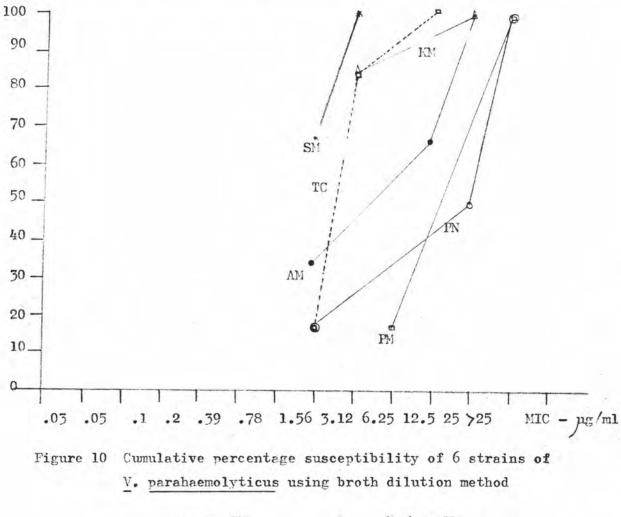




Cumulative percentage susceptibility

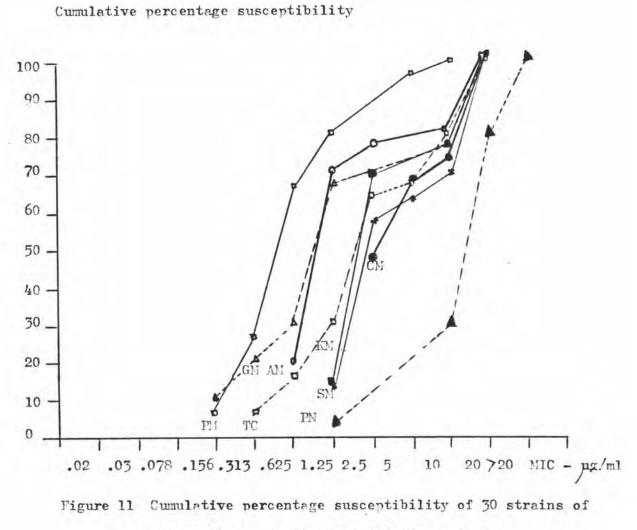
10 strains of <u>E. coli</u> using broth dilution method

* *	=	SM	$\Delta - \Delta = K$	M
DD	=	TC	00 = P	NT.
0-0	=	PM	• = Al	М
**	=	NM		



Cumulative percentage susceptibility

* *	=	Sh	<u>Δ</u> _Δ	=	KM
00	-	TC	00		PN
0-0	=	PM		=	AM
**	-	X2.			



Salmonella sp. using agar dilution method.

* *		SM	ΔΔ	Ξ	GM
DD		TC		H	K.
0-e	н	CM	A4	-	$_{\rm PN}$
D O		PM		-	AM

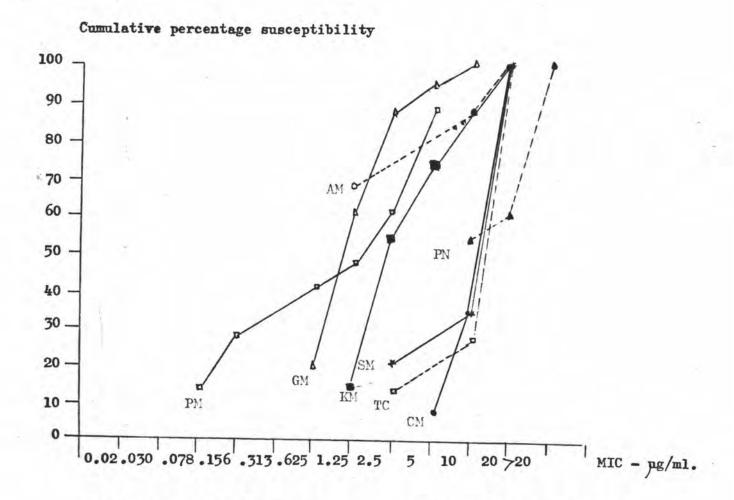


Figure 12 Cumulative percentage susceptibility of 15 strains of Shigella sp. using agar dilution method.

= SM = TC = CM = PM = PM = GM = KM = PN = PN

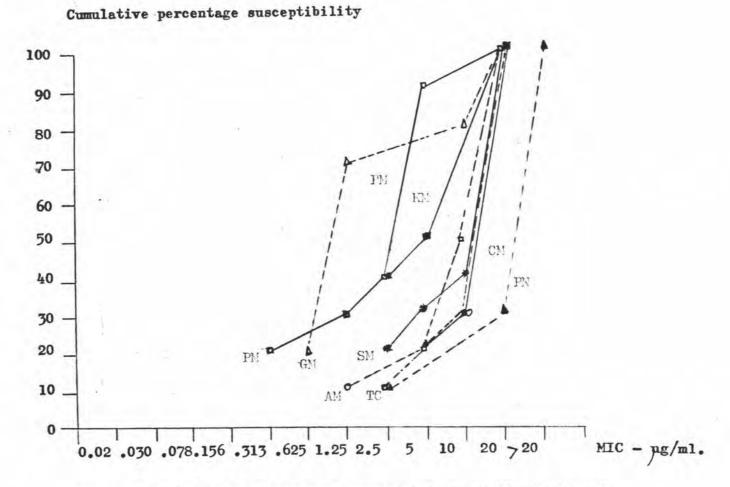


Figure 13 Cumulative percentage susceptibility of 10 strains of

E. coli using agar dilution method.

SM TC -0 = CM = PM -0 = GM ----= 4 KM PN AM 0----0 =

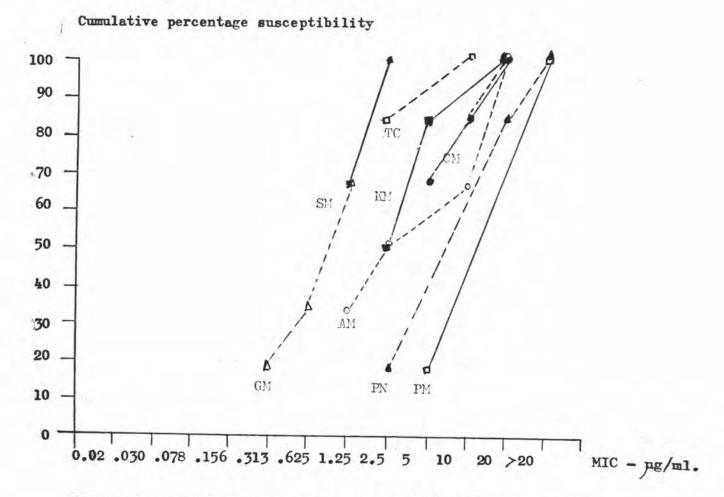


Figure 14 Cumulative percentage susceptibility of 6 strains of

V. parahaemolyticus using agar dilution method.

SM TC -0 CM PM D -0 A ---- A GM = KM = PN AM 0---0 =

The MIC's of <u>Salmonella</u> sp. to SM, given in Table 18, were 1.25-20 µg/ml as determined by agar dilution method and 0.78-25 µg/ml as determined by agar dilution method. There were 13.33% and 30% of <u>Salmonella</u> strains with the MIC's of 1.25 and 20 µg/ml respectively by agar dilution method, whereas 3.33%, 13.33% and 26.67% of strains had the MIC's of 0.78, 1.56 and 25 µg/ml respectively by broth dilution method. The MIC's of <u>E. coli</u> to FN (Table 20) were 10 - > 20 µg/ml determined by agar dilution method compared with 1.56 -> 25 µg/ml determined by broth dilution method.

The MIC's obtained by the two methods were compared in Fig.3 (Salmonella sp.), Fig.4 (Shigella sp.), Fig.5 (E. coli) and Fig.6 (V. parahaemolyticus). The line of best fit for the relationship of the two methods in all tested organisms, when regressed, was a linear graph, of which demonstrated the identical MIC in most strains tested by the two method. Relationship of the MIC's tested by the two method in Fig.3 demonstrated that 76% (23 strains out of total 30 strains) of <u>Salmonella</u> sp. tested against SM, 70% (21/30) tested against TC, 70% (21/30) tested against EN, 76% (23/30) tested against PN and 90% (27/30) tested against AM had the identical MIC's by the two methods. The number of sensitive strains at different MIC in both methods were then interpreted as either sensitive or resistant according to the correlated MIC using the interpretive standards given in Table 12 P.43 for example. Ampicillin when testing gram negative enteric organisms, only the strains with MIC equals or lower than 8 µg/ml will be interpreted as susceptible to AM. With the MIC

obtained from the two dilution methods in Table 18-21 there were 74% & 74% of Salmonella sp., 67% & 67% of Shigella sp., 20% & 20% strains of E.coli and 50% & 50% of V. parahaemolyticus as determined by agar dilution and broth dilution methods respectively which had the MIC lower than 8 ug/ml and could be interpreted as suscentible to the related antibiotic. With other antibiotics, the interpretion was also based on the interpretive standard given in Table 12 and interpreted under the same criteria as mentioned above for AM. As demonstrate from the cumulative percentage susceptibility vs. correlated MIC graph in Fig. 7-10 (broth dilution method) and Fig. 11-14 (agar dilution method), the percentage of sinsitive organism inhibited at different MIC's. For example, there were 13%, 57%, 63%, 70% and 100% of Salmonella sp. were inhibited by SM at the MIC level of 1.25, 2.5, 5.0, 10.0, 20.0, >20 mg/ml as determined by agar dilution method.

The commarison of modified broth disk, disk diffusion and the two dilution methods were demonstrated in Table 22 (Salmanella sp.), 23 (Shigella sp.), 24 (E. coli) and Table 25 (V. parahaemolyticus). Comparing the three conventional methods (disk diffusion, broth dilution and agar dilution method). There were significant agreement in the obtained percentage susceptibility for all tested organisms against all, except PM, antibiotics as data shown in Table 22-23 such as Salmonella sp. tested against SM (57.14% to 66.67%, p(0.20), TC (61.22% to 66.67%, p(0.20), CM (61.22% to 71.43%, p(0.10), GM (69.39% to 66.67%, p(0.20), KM (69.39% to 73.47%, p(0.20), PN(3.33% to 8.16%, p(0.2)) and AM (81.63% to 73.33%,

p(0.10) and so on.

However, the agreement of modified broth disk method with other three conventional method were only demonstrated in Salmonella sp., <u>Shigella</u> sp., E. coli., <u>V. parahaemolyticus.tested</u> against TC, CM, PN, AM ($p\langle 0.2 \rangle$) ie the percentage susceptibility of <u>Salmonella</u> sp. which determined by disk diffusion, modified broth disk, using BBL and local disk, broth dilution and agar dilution method were 65.31%, 63.27%, 61.22%, 61.22%, 63.33%, and 66.67% tested against TC respectively.

The agreement in all methods were also demonstrated with <u>Shigella</u> sp. tested against KM (p(0.20)) The percentage susceptibility of which shown as 78.57%, 71.43%, 75.00%, 71.43%, 73.33% and 73.33% respectively determined by disk diffusion, modified broth disk; using BBL and local disk, broth dilution and agar dilution method.

Table 22 Percentage susceptibility of <u>Salmonella</u> sp. as determined by <u>disk diffusion, modified broth disk, broth dilution and agar</u> <u>dilution methods using BBL and local disks</u>.

Anti-	Salmonella sp.								
biotics	BBL d	isk	local	disk		Agar-D (30)			
	Disk Dif. (49)	Mođ B-D (49)	Disk Dif. (49)	Mod B-D (49)	Broth-D				
SM	61.22	2.00	57.14	2.00	66.67	63.33			
TC	65.31	63.27	61.22	61.22	63.33	66.67			
СМ	69.39	71.43	61.22	69.39	-	73.33			
NM	53.06	34.69	46.94	32.65	50.00	-			
KM	73.47	4.08	69.39	0	73.33	70.00			
PN	6.12	4.08	8.16	4.08	0	3.33			
PM	55.10	95.92	-	-	107.00	100.00			
GM	69.39	6\$12	-	-	-	66.67			
AM	81.63	75.51	-	-	73.33	76.67			

- () Number of tested strains
 - * Percentage reading at 6 hrs.
 - No test

*

Table 23 Percentage susceptibility of <u>Shigella</u> sp. as determined by <u>disk diffusion</u>, modified broth disk, broth dilution and agar dilution methods using BBL and local disks.

Anti-	Shigella sp.								
biotices	BBL d	lisk	local	disk		Agar-D			
	Disk Dif.	Mod [*] B.D	Disk Dif.	Mod*B.D	Broth-D				
	(28)	(28)	(28)	(28)	(15)	(15)			
SM	21.43	0	10.71	0	20.00	20.00			
TC	17.86	17.86	32.14	25.00	13.33	13.33			
СМ	28.57	28.57	10.71	7.14	-	33.33			
NM	51.00	35.71	35.71	35.71	60.00	-			
KM	78.57	71.43	75.00	71.43	73.33	73.33			
PN	0	0	0	0	n	0			
PM	57.14	100.00	-	-	100.00	100.00			
GM	92.86	0	-	-	-	93.33			
AM	71.43	75.00	1-1-1	-	66.67	66.67			

() Number of tested strains

Percentage reading at 6 hrs.

No test

Table 24 Percentage susceptibility of E. Coli as determined by disk diffusion, modified broth disk, broth dilution and agar

Anti-	E.coli								
biotics	BBL d	isk	local	disk	1.0.101.0				
	Disk Dif.	Mod B-D	Disk Dif.	Mod [*] B-D	Broth-D	Agar-D			
	(23)	(23)	(23)	(23)	(10)	(10)			
SM	39.13	0	52.17	0	30.00	30.00			
TC	26.10	21.74	21.74	26.10	20.00	10.00			
СМ	26.10	26.09	26.10	26.10	-	30.00			
NM	47.83	0	47.83	0	50.00	-			
KM	52.17	0	47.83	0	50.00	50.00			
PN	4.35	4.35	0	0	0	0			
PM	43.48	95.65	-	-	100.00	100.00			
GM	78.26	82.60	-	-	-	70.00			
AM	13.04	8.70	-	_	20.00	20.00			

dilution methods using BBL and local disks.

- () Number of tested strains
 - * Percentage reading at 6 hrs.
 - No test

Table 25 Percentage susceptibility of <u>V.parahaemolyticus</u> as determined by disk diffusion, modified broth disk, broth dilution and agar dilution methods using BBL and local disks.

Anti-	V. parahaemolyticus								
biotics	BBL d	isk	local	disk	1	1.			
	Disk Dif. (15)	Mod B-D (15)			Broth-D				
SM	93.33	73.33	86.67	26.67	100.00	100.00			
TC	93.33	100.00	93.33	100.00	83.33	83.33			
CM	86.67	80.00	80.00	86.67	-	83.33			
NM	80.00	93.33	93.33	93.33	83.33	-			
KM	86.67	53.33	86.67	53.33	83.33	83.33			
PN	6.67	0	0	0	0	0			
PM	6.67	13.33	-	-	16.67	16.67			
GM	80.00	26.67	-	-	-	66.67			
AM	40.00	40.00	-	-	50.00	50.00			

- () Number of tested strains
 - * Percentage reading at 6 hrs.
 - No test