III- Results of Antibiotic's , Action Singly :

The susceptibility of the six strains of L. monocytogenes isolated in this study were examined for the action of the following antibiotics : **Ampicillin , Gentamicin and Cefotaxime .**

Figure 4.1 and 4.2 show the Minimum Inhibitory Concentrations (MICs) and Minimum Bateriocidal Concentrations (MBCs) of single antibiotics tested namely : **Ampicillin , Gentamicin and Cefotaxime** for all the six strains of L. monocytogenes which had been isolated in this investigation .

Table 4.3 lists the values of MICs and MBCs of these antibiotics for the isolated strains . All drugs when assayed alone had MICs in a clinically achievable range with all strains tested .

The MIC of **Ampicillin** for the L. monocytogenes isolates ranged from 0.5-1 mg/l while the MBC ranged from 1-4 mg/l using Müller Hinton broth . The values were similar for the two types of media namely Müller Hinton broth and Brain Heart infusion except for two strains where for one of which the MIC was 0.5 mg/l and the MBC was 1 mg/l using Müller Hinton broth while 1 mg/l and 2 mg/l using Brain Heart infusion . For the other , MIC was the same using the different media while the MBC was 1 mg/l with Muller Hinton broth and 2 mg/l with the Brain Heart infusion **(Table 4.4)** .

The MIC of **Gentamicin** ranged from 0.5-1 mg/l and the MBC ranged from 0.5-2 mg/l using Muller Hinton broth . The values were higher with 1 to

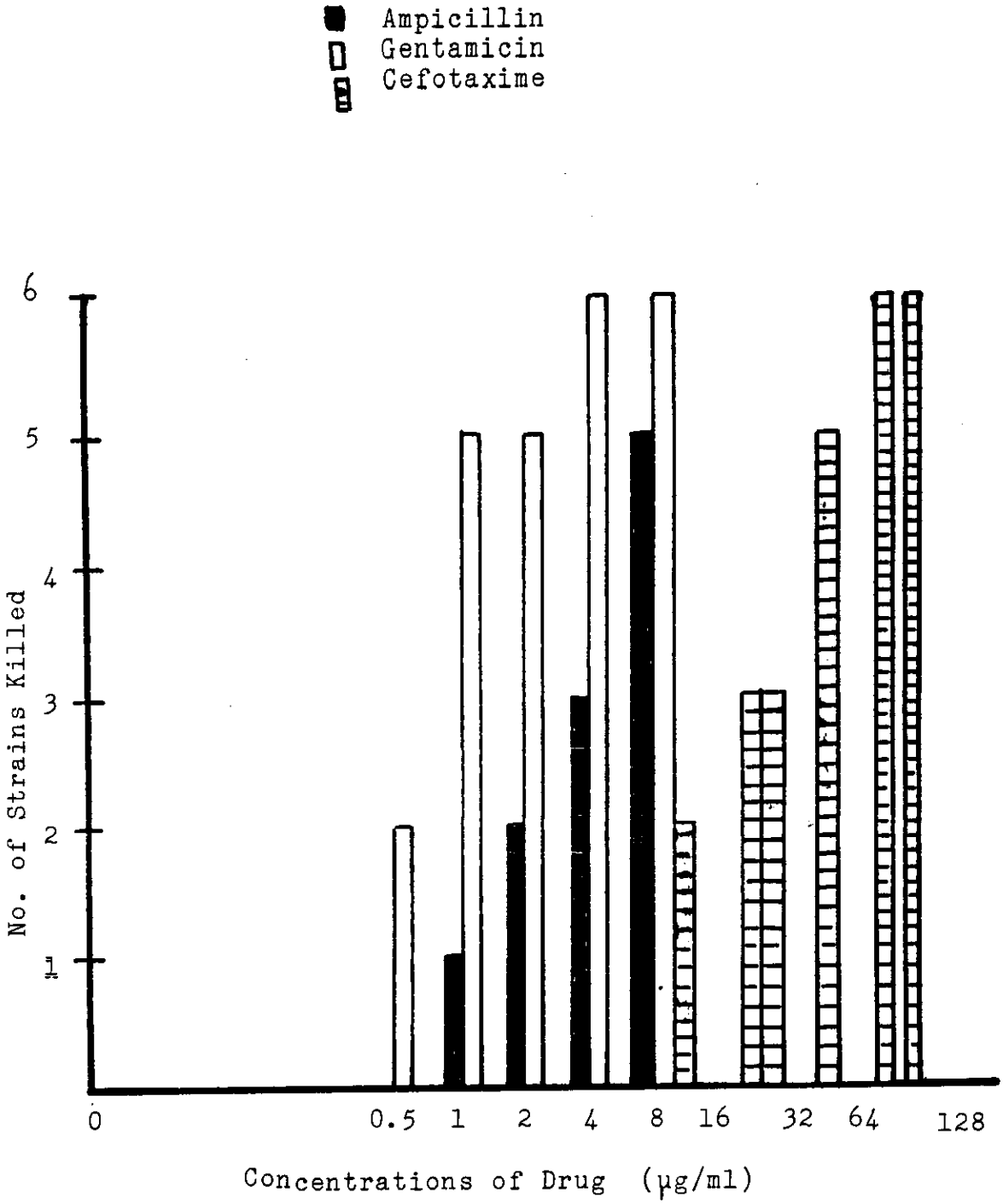


Fig. 4.1 MBCs of the Six L. Monocytogenes Isolates .

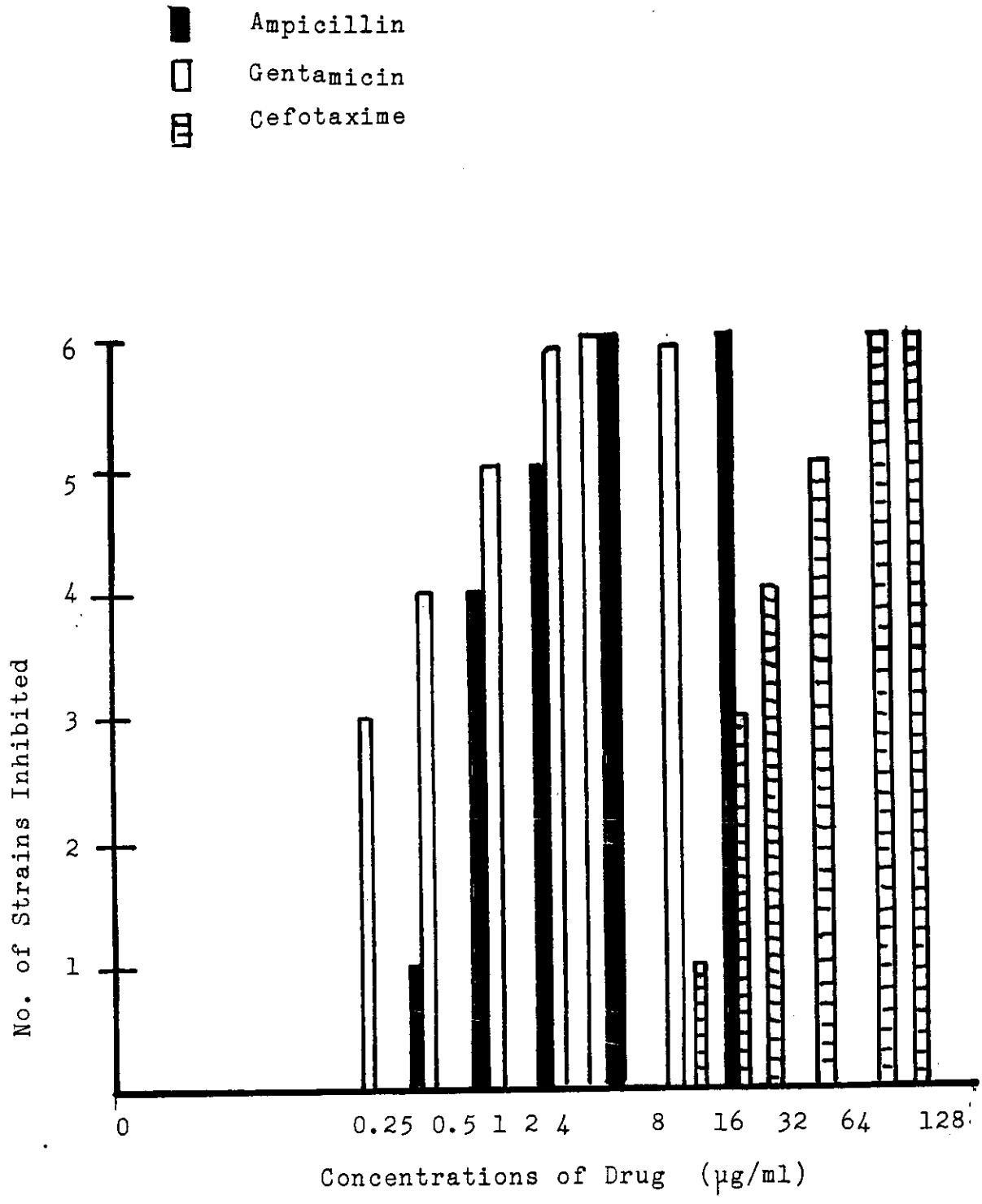


Fig. 4.2 MICs of the Six L. Monocytogenes Isolates .

TABLE 4.3 MIC AND MBC OF AMPICILLIN AND GENTAMYCIN

AND CEFOTAXIME FOR THE 6 LISTERIA MONOCYTOGENES ISOLATES

USING TRYPCASE SOY BROTH.

Listeria Monocytogenes	Ampicillin		Gentamicin		Cefotaxime	
strain	MIC mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)	MIC (mg/L)	MBC (mg/L)
L1	1	1	0.5	0.5	10	20
L2	1	4	1	1	25	100
L3	0.5	1	0.5	1	20	50
L4	1	4	1	1	25	100
L5	0.5	2	0.5	2	20	100
L6	0.5	1	0.5	1	20	100

MIC = minimal inhibitory concentration

MBC = minimal bacteriocidal concentration

2 dilutions using Brain Heart infusion (**Table 4.4**) .

With regards to **Cefotaxime** the MIC ranged from 10-25 mg/l and MBC ranged from 20-100 mg/l using Müller Hinton broth . The values were similar for 3 strains using Brain Heart infusion while the MIC of the other 3 strains were less by 1 dilution using Brain Heart infusion (**Table 4.4**) .

The comparison between the use of the two media : Müller Hinton broth and Brain Heart infusion in obtaining the MIC and MBC of the studied antibiotics for the L. monocytogenes isolates are shown in **Fig. 4.3** .

The change in the 24 and 48 hrs. MBC and MIC of **Ampicillin** , **Gentamicin** and **Cefotaxime** for the L. monocytogenes isolates are shown in **Fig. 4.4** . There was an increase of 2 dilutions in the MIC as well as the MBC of **Gentamicin** , **Ampicillin** and **Cefotaxime** for two strains only while four strains had the same values unchanged . This is presented in **Table 4.5** .

Figure 4.5 shows the kill curve of the six strains of L. monocytogenes (the inoculum was prepared from an 18 hrs. culture), with **Gentamicin** 0.5 mg/l **Cefotaxime** 5 mg/l and 10 mg/l and **Ampicillin** 10 mg/l, singly and in combination .

As regards **Gentamicin** , the use of 0.5 mg/l alone , led to death of only one strain after 4 hrs. While after 6 hrs. , four strains were killed and two strains were resistant . After 24 hrs. there was a regrowth of the organism so that only two strains remained killed .

Referring to **Cefotaxime** no strains were killed till 8 hrs. whether

TABLE 4.4 MIC AND MBC OF AMPICILLIN AND GENTAMYCIN

AND CEFOTAXIME FOR THE 6 LISTERIA MONOCYTOGENES ISOLATES

USING MULLER HINGTON BROTH AND BRAIN HEART INFUSION MEDIA

Listeria Monocytogenes	Ampicillin				Gentamicin				Cefotaxime			
strain	MIC mg/L)		MBC (mg/L)		MIC (mg/L)		MBC (mg/L)		MIC (mg/L)		MBC (mg/L)	
	MHB	BHi	MHB	BHi	MHB	BHi	MHB	BHi	MHB	BHi	MHB	BHi
L1	1	1	1	1	0.5	2	0.5	1	10	10	20	20
L2	1	1	4	4	1	2	1	2	25	20	100	100
L3	0.5	1	1	2	0.5	1	1	2	20	10	50	50
L4	1	1	4	4	1	4	1	2	25	20	100	100
L5	0.5	0.5	2	2	0.5	2	2	4	20	20	100	100
L6	0.5	0.5	1	2	0.5	2	1	2	20	20	100	100

MIC = minimal inhibitory concentration

MBC = minimal bacteriocidal concentration

MHB = Muller Hington Broth

BHi= Brain heart infusion..

TABLE 4.5 MIC AND MBC OF AMPICILLIN AND GENTAMYCIN

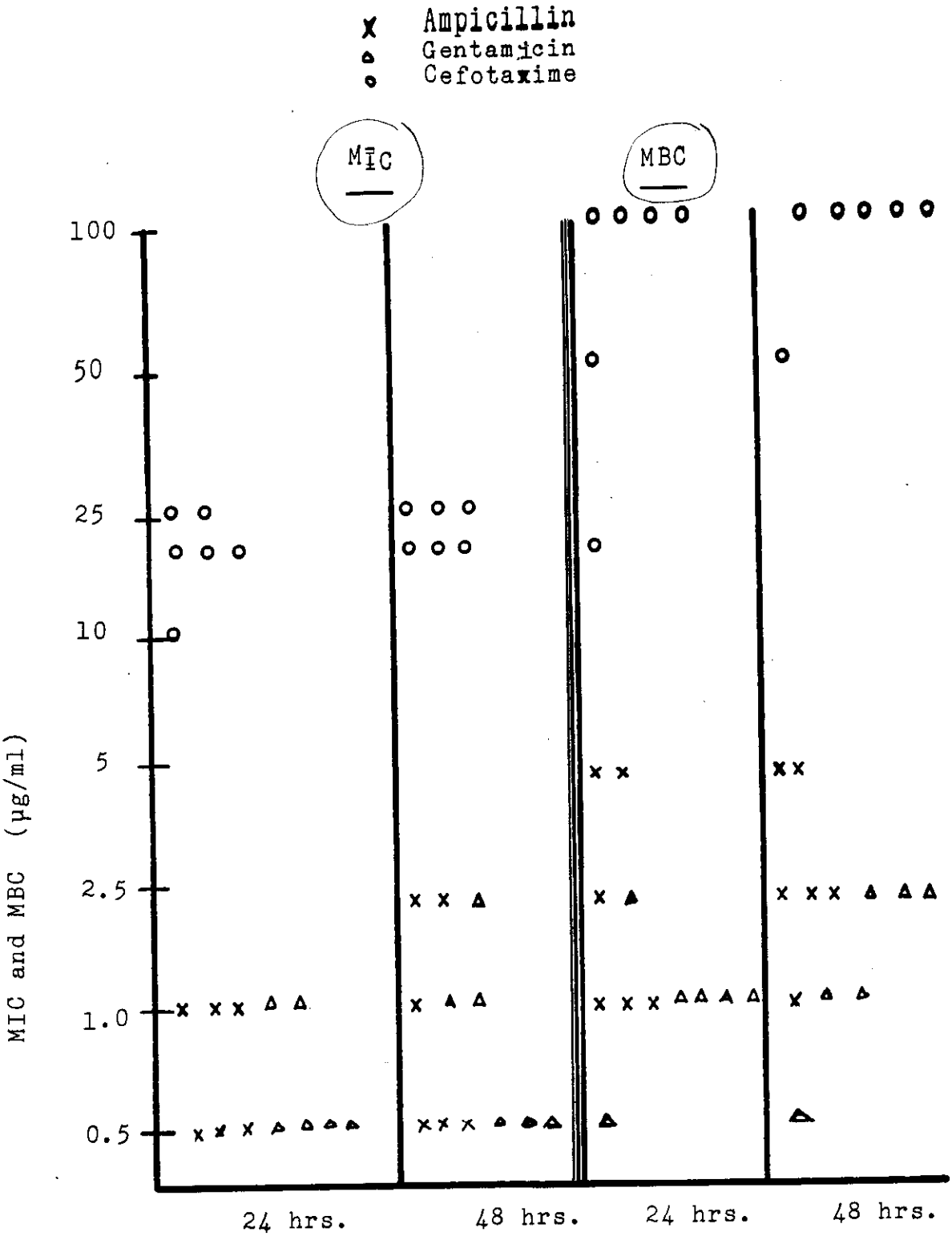
AND CEFOTAXIME FOR THE 6 LISTERIA MONOCYTOGENES ISOLATES

AFTER 24 HOURS AND 48 HOURS

Listeria Monocytogenes	Ampicillin				Gentamicin				Cefotaxime			
strain	MIC mg/L)		MBC (mg/L)		MIC (mg/L)		MBC (mg/L)		MIC (mg/L)		MBC (mg/L)	
	24H	48H	24H	48H	24H	48H	24H	48H	24H	48H	24H	48H
L1	1	2	1	2	0.5	0.5	0.5	0.5	10	20	20	50
L2	1	1	4	4	1	1	1	1	25	25	100	100
L3	0.5	0.5	1	2	0.5	1	1	2	20	20	50	100
L4	1	2	4	4	1	2	1	2	25	25	100	100
L5	0.5	0.5	2	2	0.5	0.5	2	2	20	25	100	100
L6	0.5	0.5	1	1	0.5	0.5	1	1	20	20	100	100

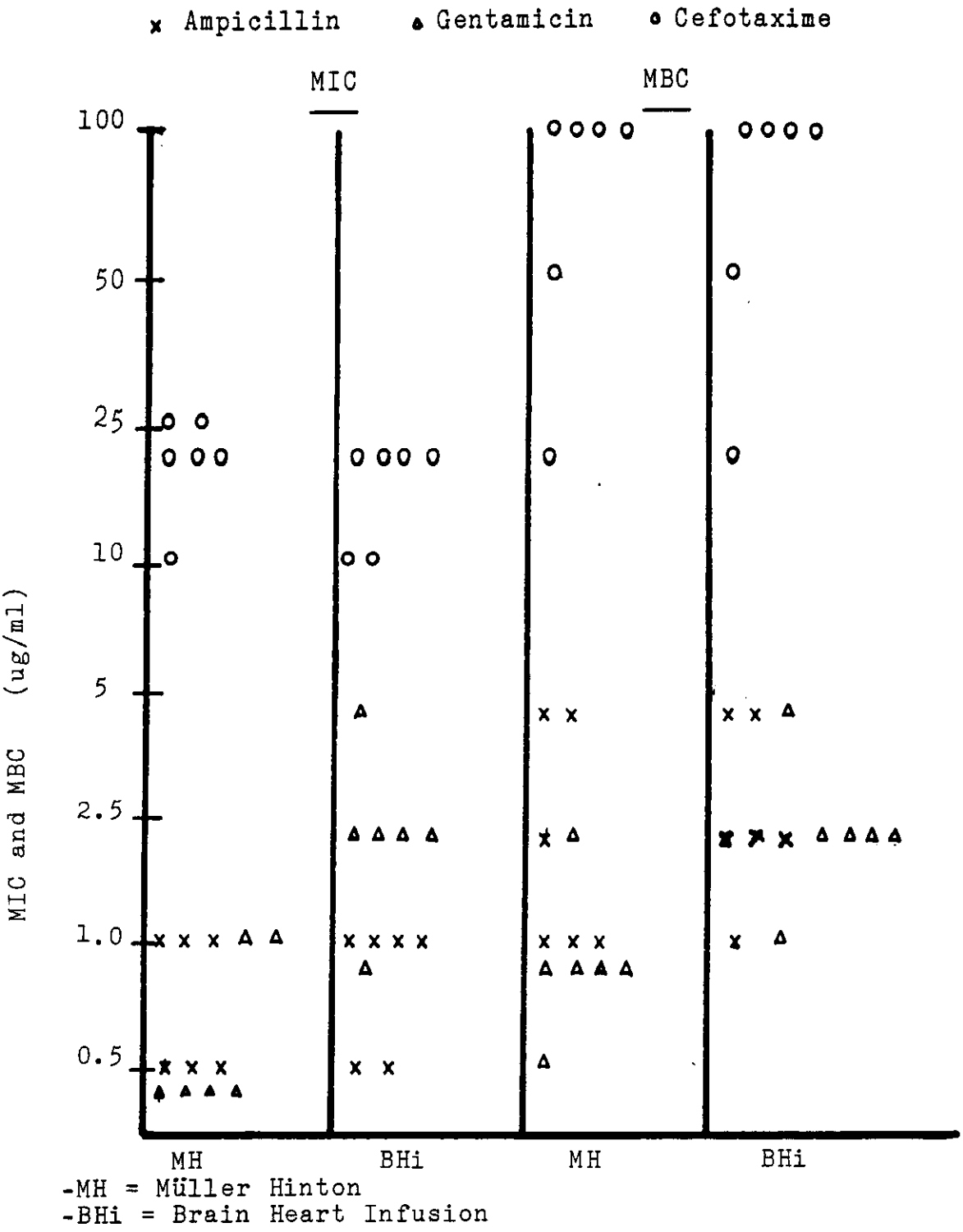
MIC = minimal inhibitory concentration

MBC = minimal bacteriocidal concentration



4.4

Fig. 4.3 Change in MBCs and MICs of Ampicillin , Gentamicin and Cefotaxime for L. Monocytogenes Isolates with Incubation time 24 h. and 48 h.



-MH = Müller Hinton
-BHi = Brain Heart Infusion

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Fig. 4.4 Change in MBCs and MICs of Ampicillin , Gentamicin and Cefotaxime for L. Monocytogenes Isolates with Muller Hinton Media and Brain Heart Infusion Media .

using a concentration of 5 mg/l or 10 mg/l . After 24 hrs. only one strain was killed with **Cefotaxime** of 5 mg/l concentration and two strains with **Cefotaxime** of 10 mg/l concentration .

Studying the bacteriocidal effect of **Ampicillin** in a concentration of 10 mg/l we found that no strain was killed on the first 8 hrs ; while only two strains out of the six were killed after 24 hrs.

IV- Results of Antibiotic Combinations :

The combinations of antibiotics studied were :

1- **Ampicillin** 10 mg/l

\+ **Gentamicin** { 1 mg/l ; 0.5 mg/l ; 0.25 mg/l }

2- **Cefotaxime** 5 mg/l and 10 mg/l

\+ **Gentamicin** { 1 mg/l ; 0.5 mg/l ; 0.25 mg/l }

The combination of **Gentamicin** 0. 5 mg/l with **Cefotaxime** 5 gm/l gave better bacteriocidal effect than each drug alone . After 4 hrs. , two strains were killed , four were killed after 6 hrs. and after 8 hrs. 5 strains were killed **Fig. 4.5** .

The combination of **Gentamicin** 0.5 mg/l with **Cefotaxime** 5 mg/l gave a better bacteriocidal effect than each drug alone . Where one strain was killed after 4 hrs. and 5 strains after 6 hrs. All strains were killed

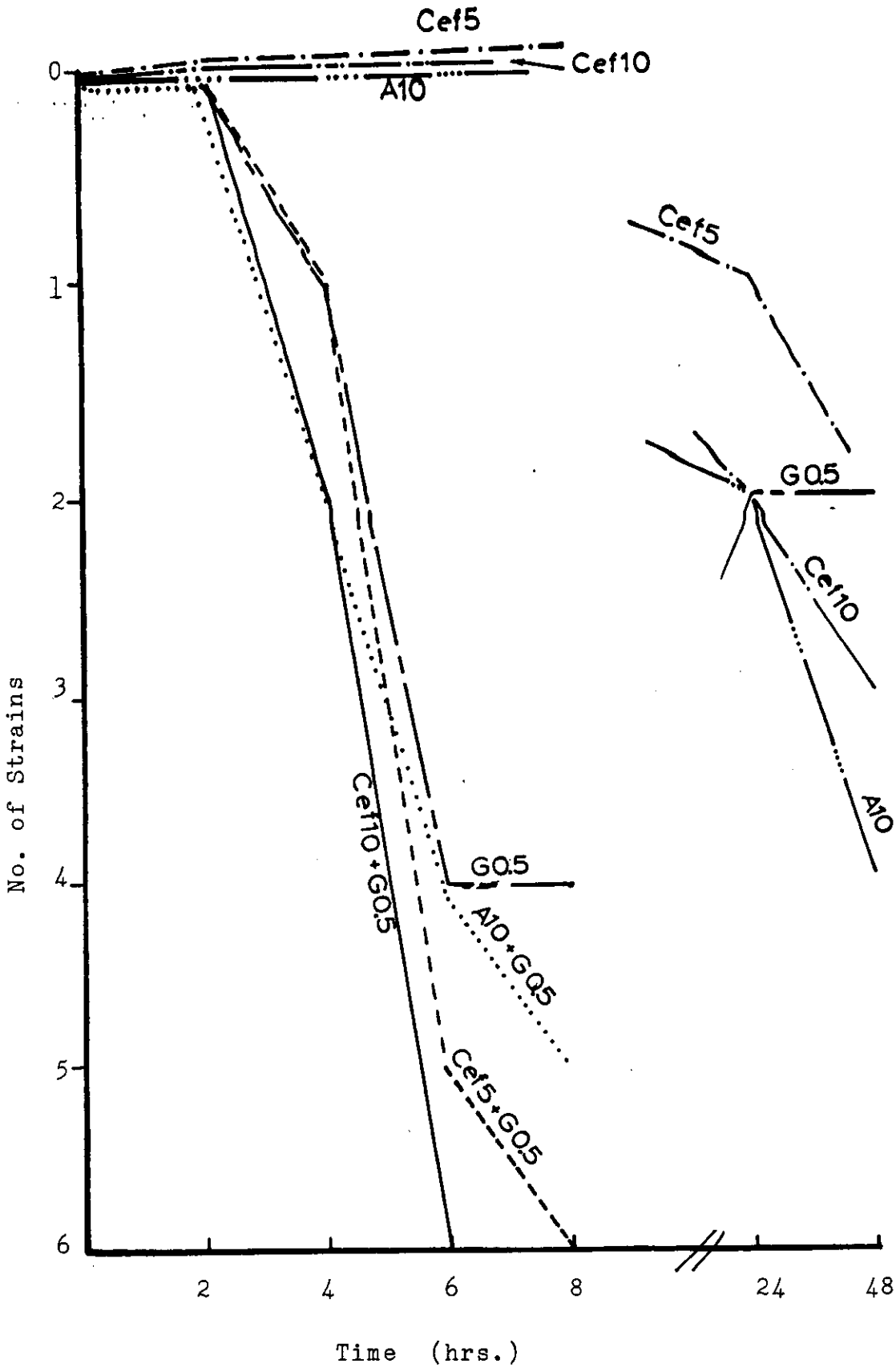

Fig. 4.5 Kill Curves of the Six Strains of L. Monocytogenes (the Inoculum was Prepared from 18 h. Culture) With Single Gentamicin 0.5 µg/l and Cefotaxime 5 & 10 µg/l .

after 24 hrs. **Fig. 4.5** . When **Gentamicin** 0.5 mg/l was combined with **Cefotaxime** 10 mg/l all the strains were killed after 6 hrs. only . **Figure 4.6** shows the kill curves of the six strains of L. monocytogenes with **Gentamicin** , **Cefotaxime** and **Ampicillin** when each was studied alone in different concentrations .

As regards **Gentamicin** in a concentration of 1 mg/l , 5 strains were killed after 6 hrs. while 1 strain remained resistant . When the concentration of **Gentamicin** was 0.5 mg/l one strain was killed after 4 hrs. , 4 strains after 6 hrs. and two strains were resistant. After 24 hrs. the bacteriocidal effect decreased and resistant strains emerged so that only two strains were killed . When the concentration of **Gentamicin** was decreased to 0.25 mg/l the bacteriocidal effect decreased so that only two strains were killed after 10 hrs. while there was no bacteriocidal action for that concentration after 24 hrs.
It was unsurprising to find that **Gentamicin** , when used in low concentration of 0.12 mg/l , had no cidal effect on the six strains even after 48 hrs.

Figure 4.7 shows the kinetics of the bacteriocidal activities of **Ampicillin + Gentamicin** against a strain of L. monocytogenes according to time and antibiotic concentrations . The drug was considered bacteriocidal when the bacterial count was < 0.01% .

With **Ampicillin** 10 mg/l used alone after 4 hrs. the bacterial count was 10% (survivals of organism) while after 8 hrs. it decreased to 1-10% . After 24 hrs. more decrease was found in the bacterial count to be 0.1-

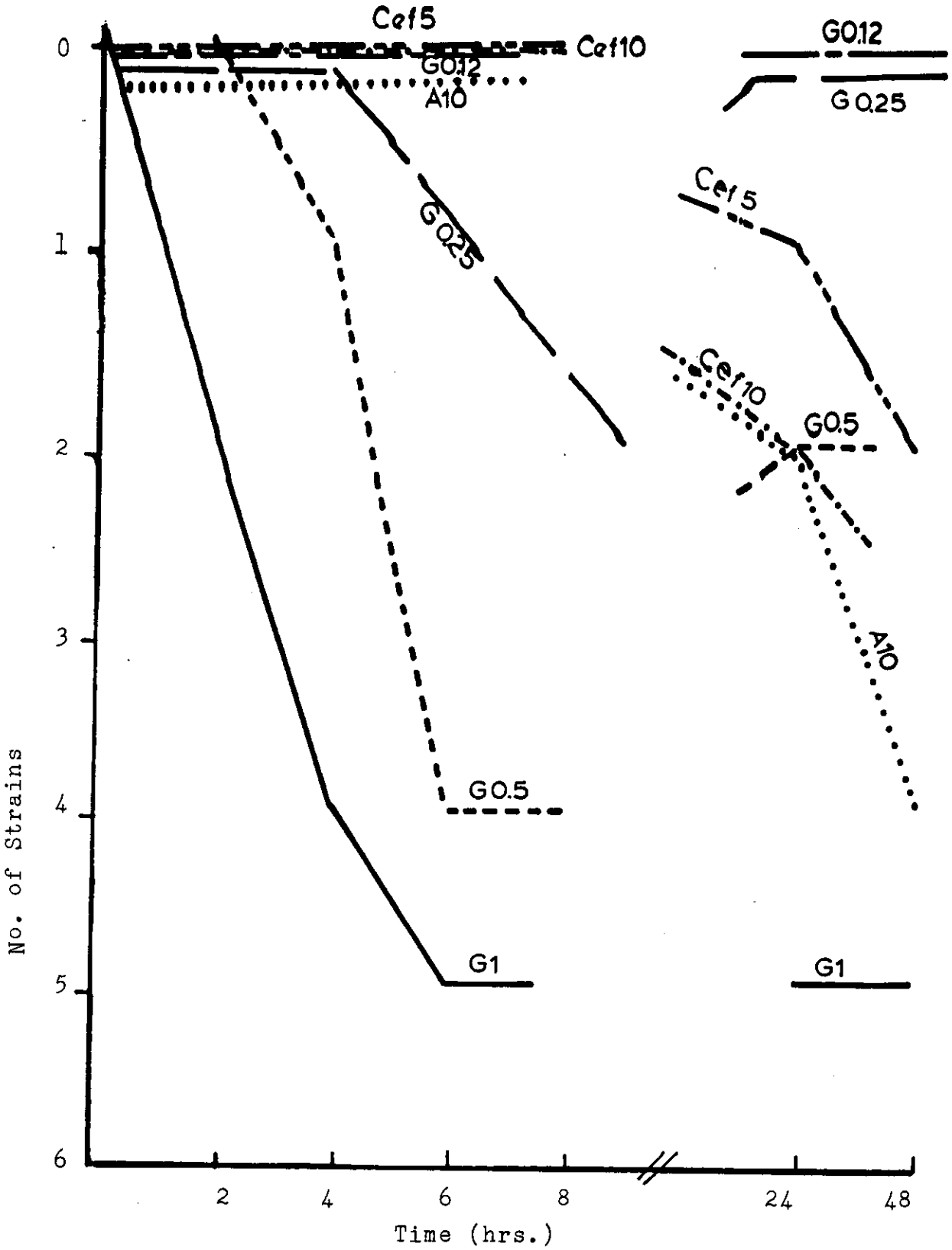

Fig. 4.6 Kill Curves of the Six Strains of L. Monocytogenes (the Inoculum was Prepared from 18 h. Culture) with : Gentamicin(0.12, 0.25, 0.5 , 1 µg/l) , Cefotaxime (5 & 10 µg/l) and Ampicillin (10 µg/l)

1% and after 48 hrs. 0.01-0.1% .

As regards **Gentamicin** when its concentration was 1 mg/l a bacteriocidal effect was found after 4 hrs. When its concentration decreased to 0.5 mg/l there was a delay of 2 hrs. so that the bacteriocidal action occurred after 6 hrs. , when the effect of a small dose of **Gentamicin** , 0.25 mg/l and 0.12 mg/l was tested it was noticed that although there was a decrease in the bacterial count , yet no bacteriocidal action occurred , even an increase of the bacterial count occurred after 24 hrs.

V- Results of the Kinetics of the Bacteriocidal Activities of the Antibiotics Studied :

The study of the kinetics of the bacteriocidal activities (kill curve) of **Ampicillin** 10 mg/l when combined with **Gentamicin** in different concentrations 0.5 , 0.25 and 0.12 mg/l revealed the following :

- When **Ampicillin** 10 mg/l was combined with **Gentamicin** 1 mg/l , a bacteriocidal effect occurred after 4 hrs. only ; when combined with **Gentamicin** 0.5 mg/l or 0.25 mg/l the bacteriocidal action occurred after 6 hrs. ; and when combined with **Gentamicin** 0.12 mg/l the bacteriocidal action occurred after 8 hrs.

It is clear from **Fig. 4.7** that the bacteriocidal effect of **Ampicillin** 10 mg/l which was achieved after 48 hrs. or more was reached after 4 hrs. when combined with **Gentamicin** 1 mg/l , and after 6 hrs. when combined with **Gentamicin** 0.5 mg/l or even with 0.25 mg/l . Also the shoot in the bacterial count noticed with **Gentamicin** 0.25 mg/l and 0.12 mg/l after

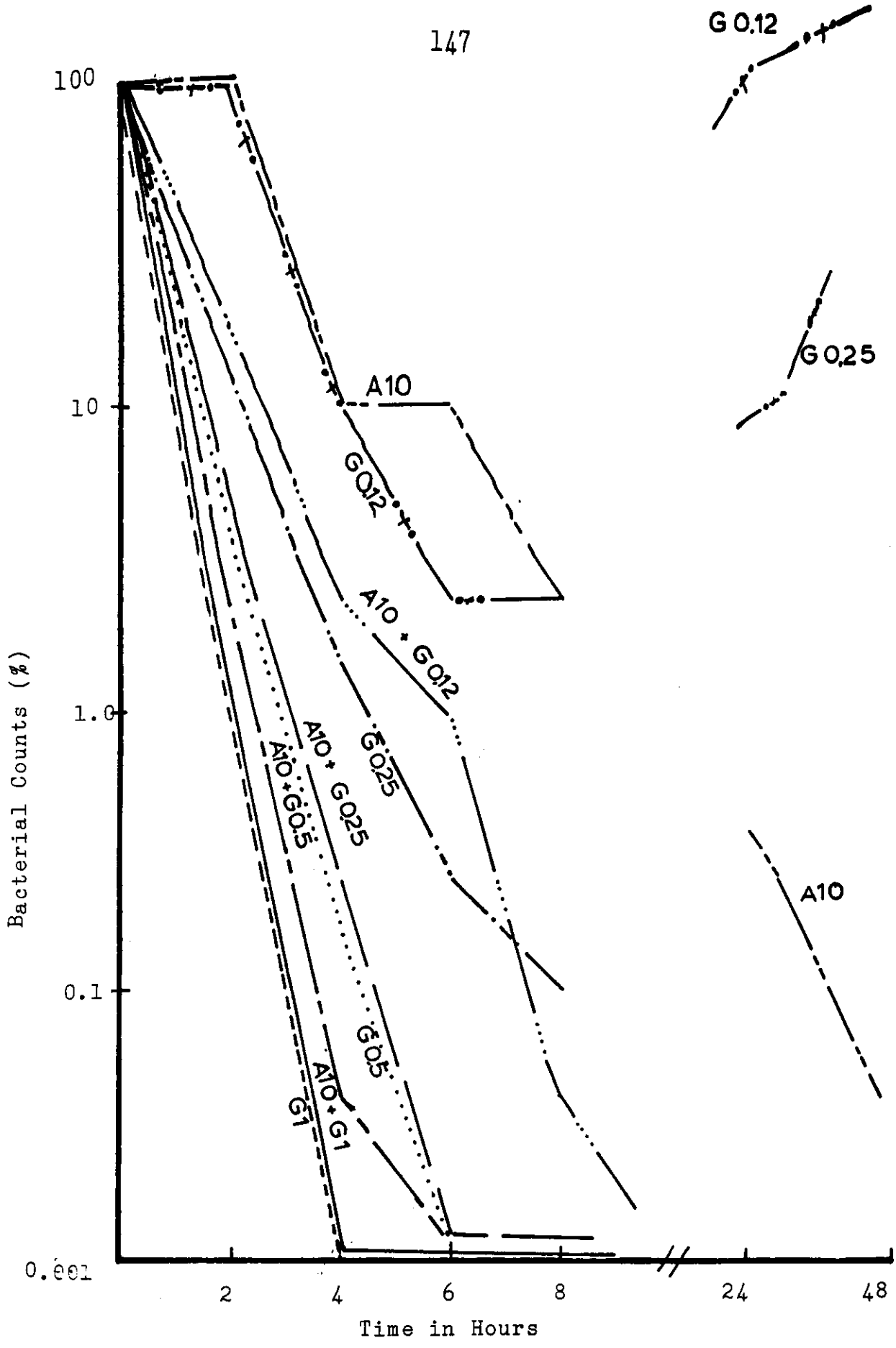

Fig. 4.7 Kinetics of the Bacteriocidal Activities (Kill Curve) of Ampicillin + Gentamicin against a Strain of L. Monocyt-

24-48 hrs. was abolished when combined with **Ampicillin** 10 mg/l .

Figure 4.8 shows the kinetics of the bacteriocidal activities (kill curve) of **Cefotaxime** + **Gentamicin** against a strain of L. monocytogenes according to time and antibiotic concentrations .

Regarding **Cefotaxime** when used alone in a concentration of 5 mg/l or 10 mg/l the bacteriocidal action occurred after 48 hrs.

When combination was made between **Gentamicin** 1 mg/l and **Cefotaxime** whether 10 mg/l or 5 mg/l , the bacteriocidal action occurred after 4 hrs. While when **Gentamicin** 0.5 mg/l was combined with **Cefotaxime** 10 mg/l or 5 mg/l the bacteriocidal action occurred after 6 hrs. When the **Gentamicin** concentration was decreased to 0.25 mg/l and combined with **Cefotaxime** 10 mg/l the bacteriocidal activity occurred after 24 hrs. , but when combined with **Cefotaxime** 5 mg/l the bacteriocidal action occurred after 48 hrs. No shooting was observed in the bacterial count with the **Gentamicin** 0.25 mg/l and **Gentamicin** 0.5 mg/l after 24 and 48 hrs. when they were combined with **Cefotaxime** whether 5 or 10 mg/l . Also the bacteriocidal effect of **Cefotaxime** was achieved earlier since it occurred after 24-48 hrs. when used alone , but after 4 hrs. only when combined with **Gentamicin** 1 mg/l , and after 6 hrs. when combined with **Gentamicin** 0.5 mg/l .

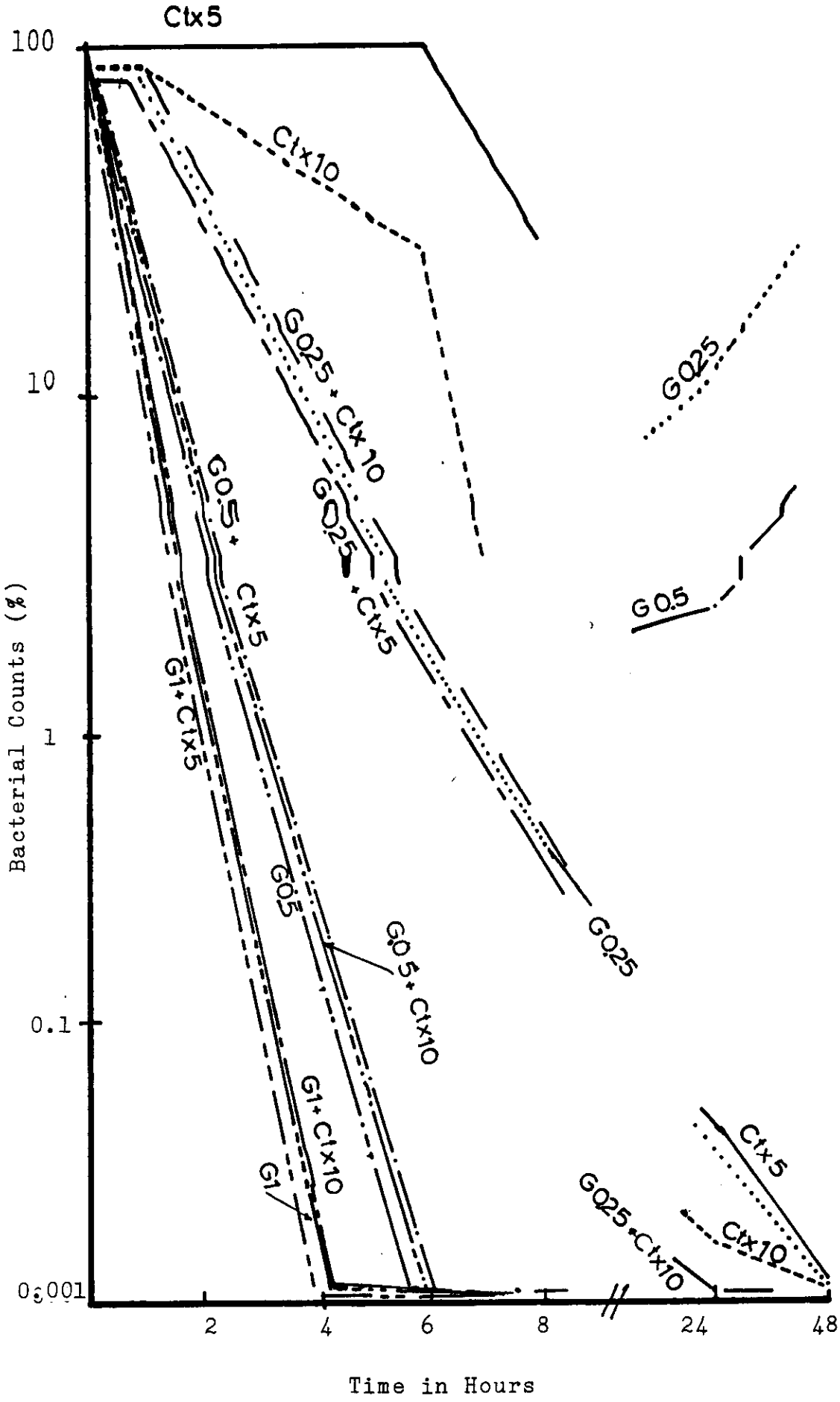

Fig. 4.8 Kinetics of the Bacteriocidal Activities (Kill Curve) of Cefotaxime + Gentamicin Against a Strain of L. Monocytogenes

CASE REPORT

In this study three strains of L. monocytogenes were isolated from three pregnant women , it was not possible to sample their neonates . Three other strains of L. monocytogenes were isolated from diseased neonates unfortunately we were unable to sample their mothers .

Maternal Case Report

The following data were collected from the three pregnant women from whom L.monocytogenes was isolated .

Case 1 :

The organism was isolated from the blood culture and vaginal swab of a primigravida , 26 weeks gestational age , feverish with 38.4° C , premature rupture fetal membranes , chest infection , urinary tract infection . The woman aborted after 24 hours . The organism was isolated from the placenta which showed pathological changes in the form of infarctions and clots . There was no history of contact with animals nor diabetes or other diseases or drug intake .

Case 2 :

The organism was isolated from the vagina of a diabetic pregnant woman , in the 20 weeks of gestation . She was complaining of symptoms of flu-like illness and sore throat twice during the pregnancy . She was P 2+8 with 1 A/W . The history of her previous abortions were between 12- 16 weeks . There was a history of intrauterine fetal death two years ago . She was treated with Ampicillin for 10 days and had a normal vaginal delivery , the fetus was alife and normal .

Case 3 :

This woman lived in the village with positive history of contact with animals and working in the fields . She was P_{3+1} and a history of early neonatal death one year ago . She had fever 39° C during delivery which was done by caesarian section . The fetus was full term , born alife but died immediately after delivery . Listeria monocytogenes serotype 1a was isolated from the blood .

Maternal Case Report of Positive Sera Containing Antibodies to L. monocytogenes :

The following is the clinical data collected from the high risk pregnant women who had positive antibodies to L. monocytogenes in their sera with titer ≥ 40 .

Case A :

Diabetic pregnant woman , P_{1+3} , 2 A/W , all previous abortions occurred between 12 weeks and 20 weeks , the last abortion 10 monthes ago . The vaginal swab was negative for L. monocytogenes , and the serum contained antibodies to L. monocytogenes serotype 4b with a titer 1/40 . The outcome of the delivery was a normal full term with normal vaginal delivery .

Case B :

Diabetic pregnant woman , P_{3+1} , the abortion occurred at 12 weeks in 1987 , she was sampled in 10/5/1988 , while being 16 weeks pregnant . The serum had a positive titer 1/80 to L. monocytogenes an-

tibodies serotype 1a . The outcome of the pregnancy was a normal full term fetus delivered vaginaly .

Case C :

Diabetic pregnant woman P3+2 , 1 A/W , history of still birth at 8 monthes for 3 times , last 2 years ago in 1985 ; the two abortions occurred at 12 weeks and 20 weeks in 1977 and 1979 . The vaginal swab culture showed gram Positive bacilli which unfortunately we could not confirm to be Listeria .

Case D :

Diabetic pregnant , P1+3 , 2 A/W , history of early neonatal death twice , in 1985 and in August 1987 , The neonate lived for 3 days in the last situation. She was sampled in 18/4/1988 while 28 weeks pregnancy . The outcome of that pregnancy was a normal alife , full term fetus, delivered par vagina .

Case E :

Diabetic pregnant woman P2+1 . Twice intrauterine foetal death at 38 weeks . Last 1 year ago . Four times abortions occurred at 12 weeks,20 weeks , 16 weeks and 20 weeks respectively . The last abortion was 6 monthes ago . Unfortunately the outcome of the present pregnancy could not be known .

NEONATAL CASE REPORT

The following data were collected from the three cases of neonatal listeriosis:

These data are tabulated in **Table 4.6** .

Case I :

A male , preterm neonate , 36 weeks , weighing 1 kgm 600 , delivered vaginaly from a mother who had only one child alife , P1.0 . The condition was fatal within 8 days . It started with hepatomegaly 10 cm , splenomegaly 4 cm , respiratory distress syndrome . Then after 3 days meningitis occurred . It was possible to isolate the organism from the blood during life and the liver during necropsy (**Plate 2**) the Listeria monocytogenes isolated was serotype 4b .

Case II :

A male , full term neonate , forceps delivery 4 kg . Respiratory distress syndrome was present at delivery and one day later meningitis started the condition was fatal after 5 days . The Listeria monocytogenes was isolated from both the blood and C.S.F. and was serotype 4b .

Case III :

A male neonate , preterm 36 weeks , delivered vaginaly 1kgm 500 . The condition started one day after delivery with septicemia and hepatosplenamegaly . The organism was isolated from the blood . It was serotype 1a . The condition ended fataly within 4 days .

TABLE 4.6 DATA ON THE THREE CASES OF NEONATAL LISTERIOSIS

Case No.	Date of birth	Type of birth	Week of pregnancy	Birth weight (9)	Sex	Serotype of Lm	Site of Growth in infant	Site of Growth in mother	Conditon of mother	Neonatal manifest.	Outcome
1	1/2/88	Vaginal	36 w	1.6 Kgm	♂	4b	Necropsy (liver, spleen C.S.F.)	ND	Pg + 0 1 A/W	septicaemia meningitis marked generalised edema orsclerena hepatomyly 10 cm splenomyly 4 cm RDS	Fatal
2	2/4/88	Forceps	Full term	4 Kgm	♂	4b	C.S.F.	ND	ND	RDS meningitis	Fatal
3	1/2/88	Vaginal	36 w	1.5 Kgm	♂	1a	liver spleen blood	ND	ND	septicaemia hepatomyly splenomegaly	Fatal

Lm = Listeria Monocytogenes
RDS = Respiratory Distress Syndrome
C.S.F.= Cerebro-Spinal Fluid