CHAPTER 1

INTRODUCTION

According to Isenberg (1), the value of the clinical laboratory can be measured only by the significance of the guidance it gives the practicing physician in the treatment of his patients. In no other area of clinical microbiology does this statement become more pertinent than in testing of clinical isolates for their susceptibility to antimicrobial agents. With the increasing number of these agents at the physicians' disposal, the changing pattern of resistance and susceptibility among bacteria particularly the Enterobacteriaceae which have acquired multiple antibiotic resistant factor (2,3) the proliferation of new antibiotics and cogeners with differing characteristics and also the increasing difficult therapeutic problems. For all these reasons, the clinician must rely more and more upon sensitivity testing to guide his selection of appropriate drugs or alter an already imposed regimen. Therefore, to a large extent, a laboratory report showing susceptibility or resistance to a particular antimicrobial agent becomes an endorsement of its usefullness or withdrawal (4).

Generally, the principal methods presently used by the laboratory to evaluate the efficacy of antimicrobial agents can be performed by 3 techniques (5,6,7,8).

1. Agar Diffusion or Disk Diffusion Methods, utilizing antibiotic-impregnated disks.

2. MIC Antibiotic Susceptibility Tests or Serial Dilution Methods.

   2.1 The Broth Dilution Method

   2.2 The Agar Dilution Method

   2.3 The Microtube Broth Dilution Method

3. Test of Therapeutic Effect. A direct method applied only for determining the antibacterial potency in serum or other body fluid during antibacterial therapy. This method of which first described by Schlichter and associates(9, 10)is used in case of acute infections (particularly subacute bacterial endocarditis) and can recommend it as a valuable and practical guide to the antibiotic therapy of severe or complicated bacterial infections, in patient of renal failure and patients who fail to respond to the normal dosage of antibiotic or sensitive to get renal toxicity from antibacterial therapy. This test of therapeutic effect can be achieved by

   3.1 Serum Antimicrobial Activity (Schlichter Serum Killing Power Test)

   3.2 Serum Drug Assay

However, in the interpretation of any in vitro susceptibility tests, it is wise to remember that they are essentially artificial measurement.

The only absolute criterian of microbial response to antibiotics is the clinical response of the patient when adequate dosage of the appropriate antibacterial agent is administered.

The three major categories of in vitro susceptibility tests commonly used in practice include disk diffusion test and dilution tests (broth tube and agar plate dilution procedures).

Each method has different advantages and limitations, and these must be understood and appreciated in order to obtain maximum usefullness of the results. Since there is a place for all of these methods in different clinical laboratory the choice of the technique will be depend upon the application of the results.

Early systematic, at the time Penicillin became available as a prescription drug to treat all types of infections, observations of the effect of antimicrobial agent on specific microorganisms involved incorporation of antimicrobial directly into liquid growth media (11,12). Quantitative estimates of the degree of activity of the antimicrobic against the microorganism was then attempted by inoculating a suspension of organisms into the media with serial dilutions of agents. The serial dilution techniques whether performed in an agar or broth media, first came into popular use in the year 1940s as the only tests available and were the precursors of the disk diffusion test. In the serial dilution methods (broth or agar dilution methods) specific amounts of the antimicrobic prepared in decreasing concentration (2-fold) in liquid (broth) or solid (agar) media by the serial dilution technique, are inoculated with a

culture of the bacterium to be tested. The susceptibility of the organisms is determined, after a suitable period of overnight incubation, by the observation of the presence or absence of growth in the varying concentrations of the antimicrobial agent. The lowest concentration of drug which prevented visible growth was defined as the minimum inhibitory concentration or MIC. This serial dilution method is generally recognized as being the most satisfactory method to determine MIC (13, 14) and is reasonably precise (15) and gives a fairly accurate determination of susceptibility to measured amounts (either units or micrograms) of the antibiotic. It is a complicated, expensive and time consuming method (2a, 3). However, especially when the clinician wants to know the susceptibility of an organism to a number of antimicrobics, because one dilution test determine only the action of one antibiotic on a single bacterial strain, so it is confined to very few clinical laboratories and to research purpose (14, 15, 16) or to some specific cases when quantitative results may be of value (8). However, it is sometimes used in practice where the antibiotic dose has to be calculated on MIC as in endocarditis or when slow growing bacteria to be tested or when the Minimum Bactericidal Concentration (MBC), the lowest concentration of drug require to kill all the sensitive organisms, is required in testing the immunocompromised patients where the antibacterial dosage has to be reach its MBC value. The MBC value can be obtained by subcultured all the tubes from the broth dilution technique, with no growth on the antibacterial free medium, those which show no growth demonstrate bactericidal activity (15). The determination of MIC or MBC is not required in the majority of invitro antibacterial susceptibility tests. However,

with certain infectious disease caused by bacteria which must be treated with antimicrobic whose dosage must be monitored, the laboratory should be prepared to offer an appropriate broth or agar dilution test to determine the MIC and/or MBC (2). The serial dilution method may be recommended for determining the susceptibility of organisms isolated in the following instances: blood cultures, patients who fail to respond to apparently adequate therapy, and from patients who relapse while undergoing such therapy (8). A number of factors (25) must be considered in establishing the procedures and in evaluating the results of these tests. They include the following : medium in which the tests are performed, the stability of antibiotics, inoculum size, growth rate of organism and the period of incubation of the tests. Any variation in one or more of these factors may influence the tests, and the results obtained by one procedure may not agree with those arrived at by a slightly different method (17, 48) Nevertheless, when uniform procedures were followed, the MIC endpoints have been fairly reproducible over the years at least within a given laboratory. Thus, for nearly three decades the overnight 2-fold dilution MIC has shown remarkable persistance in medical microbiology and is the most reliable means to determine antimicrobial susceptibility in vitro despite the many variables and despite recognized problems of interpretation (3, 4a). It has become the basis for comparison and evaluation of other susceptibility testing methods. Eventhough, the MIC end point actually represents a range somewhere between consecutive 2-fold dilutions, it has been used as the reference basis for accuracy comparisons of other techniques (2). For example, the discontinuous MIC endpoints have long been used to calculated regression equations for evaluating a procedure

such as the disk diffusion test, eventhough the latter is quantitated with a continum of measurement values (zone diameter in millimeters) rather than discontinuous endpoints.

With the serial dilution methods, the agar dilution technique has four major advantages over broth dilution method (2).

1. By using an inoculum replicating apparatus, a fairly large number of strains may be tested at the same time.
2. Microbial heterogeneity or contamination can be detected readily by observing the nature of bacterial growth on the surface of the agar plates as compared to that in a broth medium.
3. The medium may be supplemented with whole blood or blood products to permit testing of some of the nutritionally fastidious microorganisms that cannot be tested satisfactory in a clear broth media.
4. The standard procedure may be modified in a number of ways in order to permit testing of a particular type of microorganism and this is quite acceptable as long as appropriate controls are included to demonstrated that the modification does not affect the end result.

The major disadvantage to the agar dilution procedure for susceptibility testing is the fact that test plates cannot be subcultured easily in order to determine the bactericidal activity of the antimicrobial agents. For that purpose, broth disk procedure is more ideally suited (2, 16).

A major consideration which has contributed to limit the application of dilution method (2) in most clinical laboratories is the controlling the

preparation and maintenance of standard stock solution of antimicrobial agents. Most laboratories have not been feasible to obtain standardized reference antimicrobial agents and to maintain adequate control procedures to ensure consistent potency. After the decision to use a dilution method, whether broth or agar dilution, involves further consideration. Again, should MBCs be required, broth dilution would be a satisfactory method either macrotube or microtiter trays. For more extensive projects or for regular reference laboratory use, involving testing of large numbers of strains and multiple agents, most laboratories use either agar dilution or broth microdilution procedures.

Perhaps the most useful, and certainly the most used, laboratory test for antibacterial susceptibility is the disk diffusion procedure (13,25). Its simplicity, speed of performance, economy, convenience and reproducibility (under standardized conditions) (8, 13, 18) make it ideally suitable for the busy diagnostic laboratory when the more laborious serial dilution methods may not be practiced. In the agar or disk diffusion method, as originally described by Bondi and associates (19), filter paper disks impregnated with antimicrobial agents of specific concentration are carefully placed on an agar culture plate inoculated with a tested organism. After the overnight incubation, the susceptibility is determined by observing the size of the resulting zone of inhibition around the disk containing the agent to which the organism is susceptible, whereas a resistant organism will grow up to (and under) the periphery of the disk. This method is recognized as the most suitable and successful method for routine testing of antimicrobial susceptibility of rapid growing pathogenic bacteria (13, 20, 21). However, this procedure is restricted to interprete systems

comprised of two or three categories, such as susceptible, intermediate and resistant (22) and also for a given antibacterial disk, the size of zone of inhibition is markedly influenced by a number of variables of which may contribute to large discrepacies (2, 8, 19, 23, 24). Among those which have been identified are the following, 1) selection and concentrations of antimicrobial disks; 2) selection, volume, age and composition of plating medium; 3) storage and handing of disks; 4) methodology of testing; 5) inoculum density; 6) growth characteristics of tested strains; 7) temperature and time of incubation; 8) presence of serum protein or other drug interactions in the reservoir and criteria used for interpreting results. Failing to adequately control and define the above factors can easily lead to erroneous interpretation of susceptibility or resistant. However, results of routine susceptibility testing may unreliable in some tests (13, 25). Numerous attempts have been made to standardize the disk diffusion procedure, including the work of Bauer, Kirby and co-workers (2, 49). Ericcson (57), the World Health Organization (WHO), the Food Drug Administration (27) and most recently the National Committee for Clinical Laboratory Standard (NCCLS). The Kirby-Bauer disk diffusion method which the American Food and Drug Administration has recommended as a standardized procedure for the determination of antimicrobial disk susceptibility, when having all other variables held constant and performed or evaluated correctly has been extremely useful as a guide in choosing antimicrobial agent best suited for in vivo therapy (28). The size of the inhibitation zone reflects the degree of susceptibility of the test strain, the larger the zone of inhibition, the more susceptible the best organism(2).

However, all the recommendations involve such rigid control of the test conditions that the nature of the simplicity of the disk diffusion test is to some extent lost and also many laboratory may be unable to adopt these recommendations in full. The disk diffusion test is not recommended to test the slow growing bacterias or anaerobes which are much more likely to be subjected to lethal doses of oxygen when they are spread on to the agar surface (29).

In selecting of any particular procedure to be used, several criteria must be taken into consideration. Type of laboratory, a different problem is involved in the choice of method for a clinical microbiology laboratory as compared to a research or reference laboratory. For the research or reference laboratory, especially one involved in clinical-pharmacological studies, fully quantitated MIC determinations are required, so that broth or agar dilution often involve in such studies. A laboratory which has accumulated a body of regression data from carefully standardized disk agar diffusion assays in comparison with dilution end-points can utilize the measured zone diameter from the disk diffusion assay to determine MIC quite accurately (30). However, for certain antibacterial agents and certain organisms disk diffusion zone diameter versus dilution method end-points do not correlate with sufficient quantitative accuracy to allow more than estimation of the MIC range (31). The particular species to be tested in each laboratory may be another factor. Slow growing, fastidious, non-fermentative gram-negative bacilli, Neisseria and a few strains of Streptococci may be easier to reproduce in liquid media rather than in agar.

As the three common methods have the different advantages and limitations individually. Different laboratories will select particular method of which can serve their purposes. However, the district disadvantage in all these methods is the prolonged incubation period require (18-20 hrs.) which affords considerable delay in getting necessary information to the clinician (32). The clinician who is faced with the management of a patient presenting symptoms of an infection always require a rapid identification of the species of offending organisms and the determination of those antimicrobial agents to which the offending microorganism is susceptible and which are relevant to the successful management of the patient. The ability to determine antimicrobial susceptibility patterns early could influence favourably the prognosis in all infections especially the serious infections (32). Any modification for a rapid detection of susceptibility results have to be based on the established routine procedures.

General acceptance of the in vitro disk diffusion method has been supported by its simplicity and rapidity (28). The broth dilution method has stood the test of time as the most reliable mean to determine antimicrobial susceptibility in vitro (24) and remained the basic concept in a large body of infectious diseases and clinical pharmacology literature (2). However, the prolonged incubation interval required (18-20 hrs.) of these methods between the determination of susceptibility in vitro and the utilization of the antimicrobial agent in vivo has remained a distinct disadvantage. Many research works have been invested to modify the broth dilution method in order to reduce the expensed prolonged incubation period, so that the categorization would be even more practical for the clinical

laboratories. Following Schneiersen's method (33), Stalon and Thornsberry (29) suggested the introduction of paper disk in broth dilution method to deliver the antibiotic to the broth. This method has been used by Isenberg et al (34) and Thornsberry et al (35) for automated or mechanized susceptibility tested on aerobic and facultative organisms, and by Wilkins and Thiel (36) for anaerobic bacteria, also by Kriangsag Punsuk and Kriangsag Saithanu (37) in comparison with disk diffusion method.

In the course of this study, the potential application of Modified Broth Disk technique to in vitro susceptibility determination was considered.

In Thailand, the routine antimicrobial susceptibility tests are carried out mostly against the enteric bacteria isolated from patients sufferred from acute infectious diarrhoea of which, according to the Ministry of Public Health, is one of the most frequent infectious diseases here.

Diarrhoea is still one of the major public health and clinical problem, especially in developing country like Thailand. It is estimated that there are about five hundred million episodes in children each year in Africa, Asia and Latin America resulting in 5-18 million deaths p.a. (38). Diarrhoea also kills adults but in millions more the chronic ill health from dysentery, intestinal parasites and malabsorption syndromes is considerable.

The frequent diarrhoeal causative bacteria in Thailand are Salmonella sp, Shigella sp, pathogenic E. coli and Vibrio sp. Cholera, the causative bacteria of which is Vibrio cholera is the quarantinable disease here especially during summer. After year 1969, Cholera was found to be caused by Vibrio cholerae El Tor Biotype with 3 different serotypes as Ogawa, Inaba and Hikojiwa (39).

In Thailand, about 80% of the strain isolated since 1950 are Ogawa serotype (39) and the least are Inaba. The incidence of cases and percentage of death caused by Cholera are shown in Table 1. In Asia, the majority of food poisoning diarrhoea are caused by Vibrio parahaemolyticus (4, 38). Also in Thailand the report from the CDC (39) showed that more than half of the food poisoning cases are caused by Vibrio parahaemolyticus, and Salmonella typhimurium in paediatric group. Apart from the Salmonella typhimurium, the diarrhoea in paediatric group can be frequently caused by pathogenic E. coli (40). It is found from various isolation from the tested specimen in hospitals within the Bangkok municipality that around 21% of diarrhoeal cases are caused by Salmonella and Shigella groups and lesser percentage by Vibrio parahaemolyticus (4). The choice of chemotherapeutic therapy for specific diarrhoeal causative organisms are shown in Table 2.

Table 1 Data of cholera epidemic in Thailand and percentage of death (26)

NO	YEAR OF EPIDEMIC	NO. OF PATIENT	NO. OF DEATH	PERCENTAGE OF DEATH
1	1918 - 1920	19,413	13,918	71.69
2	1925 - 1929	21,591	14,902	69.02
3	1935 - 1937	15,557	10,005	64.31
4	1943 - 1947	19,169	13,036	68.01
5	1958 - 1959	19,359	2,372	12.25
6	1963 - 1965	3,672	204	5.50
7	1977 - 1978	4,085	135	3.30

Table 2 Current Use of Antimicrobial Agents and The Therapy of Infections (38, 39, 41, 42, 43)

Bacteria	Diseases	Drug order of choice		
		1st	2nd	3rd
gram negative				
Salmonella	Typhoid fever Paratyphoid fever Bacteremia Acute gastroenteritis	Ampicillin Chloramphenicol	TMP/SMX	-
Shigella	Acute gastroentiritis Bacillary dysentery Shigellosis	Ampicillin Chloramphenicol Neomycin	Kanamycin Polymyxin-B Tetracycline	TMP/SMX
Escherichia coli	Other infectious eg. Gastroenteritis	Ampicillin Gentamicin	Cephalosporin Chloramphenicol Tetracycline	Kanamycin Polymycin B
Vibrio cholera	Cholera	Tetracycline	Chloramphenicol Furazolidone	TMP/SMX Erythromycin
Vibrio parahaemolyticus	Food poisoning	Tetracycline	-	-

Although the most important criterion in treating diarrhoea is adequate hydration to maintain the electrolyte and water balance of the patients. However, antibacterial agent is still important in shortening the duration of symptom and to prevent the spread of infection within the community (38) and also to reduce the incidence of infectious carriers (44). Ideally, when treating any bacterial infection, the choice of chemotherapeutic agent is made on the result of sensitivity tests which should be carried out on specimen obtained from patients. Results should be interpreted with reference to the response achieved with the indicated treatment in similar condition. When it is necessary to treat patients before sensitivity tests have been carried out, the known local patterns of bacterial resistance should be taken into account before deciding initial antibiotic treatment. Sensitivity tests should still be done as results can confirm the choice or indicate a change (45). However, in practice, most patients with suspected bacterial infections are frequently given potent broad spectrum or combination chemotherapy until culture and susceptibility data are available.

Although warranted, such therapy is expensive, toxic and finally cause drug resistant problem (46). In Thailand, the administration of ineffective antibacterial agent, inadequate dosing, insufficient dosage interval or any improper usage are the frequent problems which cause more drug resistance (47). The report from one of the teaching hospital, Ramathibodi, as in Table 3 will show the decrease antimicrobial sensitivity of various common strains isolated during 1978-1979.

Table 3 ANTIMICROBIAL SUSCEPTIBILITY REPORT 1979
DIVISION OF CLINICAL MICROBIOLOGY
DEPARTMENT OF PATHOLOGY
RAMATHIBODI HOSPITAL (55)

PERCENTAGE OF SUSCEPTIBLE ORGANISMS

ANTIBIOTICS	A. antiratus (Herelles)	A. lwoffi (Mima polymorpha)	Citrobacter	Enterobacter	E. coli	H. influenzae	Klebsiella	N. gonorrhoeae	Pseudomonas aeruginosa	Proteus mirabilis	Proteus morganii	Salmonella typhi	Shigella flexneri	Shigella sonnei	Staph aureus	Staph epidermidis
AMIKACIN*	75 (91)	– –	– –	99 (97)	94 (97)	– –	96 (96)	– –	87 (78)	– –	– –	– –	– –	– –	– –	– –
AMPICILLIN	10 (9)	72 (50)	2 (9)	14 (8)	25 (25)	64 (61)	5 (5)	69 (94)	– –	45 (40)	6 (6)	85 (79)	13 (28)	76 (60)	– –	– –
CARBENICILLIN	43 (51)	90 (79)	17 (39)	47 (35)	27 (25)	– –	18 (14)	– –	68 (65)	65 (58)	69 (51)	88 (87)	25 (27)	87 (74)	– –	– –
CEPHALOTHIN	7 (7)	65 (48)	25 (7)	21 (14)	73 (61)	69 (61)	66 (52)	81 (73)	– –	67 (56)	2 4	100 (99)	98 (97)	100 (79)	100 (99)	99 (98)
CHLORAMPHENICOL	12 (8)	67 (65)	32 (32)	50 (39)	30 (29)	89 (91)	43 (38)	99 (98)	– –	43 (37)	36 (28)	78 (60)	31 (21)	9 (3)	83 (83)	44 (47)
COTRIMOXAZOLE	48 (42)	71 (53)	20 (28)	52 (39)	64 (66)	47 (44)	43 (44)	44 (30)	– –	46 (42)	45 (52)	98 (97)	70 (80)	83 (100)	90 (91)	59 (72)
ERYTHROMYCIN	72 (79)	86 (78)	– –	– –	– –	93 (85)	– –	99 (99)	– –	– –	– –	– –	– –	– –	98 (98)	83 (87)
GENTAMICIN	66 (76)	85 (85)	37 (53)	65 (65)	89 (91)	81 (63)	68 (69)	79 (77)	56 (66)	80 (74)	83 (82)	100 (100)	94 (97)	100 (96)	91 (94)	89 (92)
KANAMYCIN	56 (48)	77 (60)	21 (23)	50 (40)	53 (54)	92 (82)	53 (48)	93 (80)	– –	54 (46)	49 (58)	98 (99)	94 (91)	88 (68)	86 (88)	60 (63)
LINCOMYCIN	– –	– –	– –	– –	– –	5 (27)	– –	(–)	– –	– –	– –	– –	– –	– –	95 (88)	83 (79)
METHICILLIN	– –	– –	– –	– –	– –	14 (54)	– –	19 (20)	– –	– –	– –	– –	– –	– –	94 (96)	75 (79)
NALIDICIC ACID	– –	– –	94 (84)	61 (69)	96 (96)	– –	36 (81)	– –	– –	89 (86)	100 (100)	– –	– –	– –	– –	– –
NITROFURANTOEN	– –	72 (33)	94 (95)	60 (57)	95 (94)	– –	75 (73)	– –	– –	24 (29)	61 (72)	– –	– –	– –	– –	– –
PENICILLIN	– –	– –	– –	– –	– –	36 (32)	– –	55 (68)	– –	– –	– –	– –	– –	– –	6 (6)	23 (29)
POLYMYXIN B	93 (96)	80 (95)	95 (94)	95 (90)	96 (94)	– –	95 (96)	– –	96 (96)	6 (3)	4 (1)	100 (100)	100 (100)	100 (92)	– –	– –
STREPTOMYCIN	46 (39)	61 (45)	26 (28)	52 (41)	35 (34)	98 (79)	53 (45)	24 (13)	– –	53 (48)	50 (49)	79 (59)	8 (7)	27 (17)	– –	– –
SULBENICILLIN*	22 (–)	73 (–)	– –	18 (–)	13 (–)	– –	4 (–)	– –	72 (–)	– –	– –	– –	– –	– –	– –	– –
TETRACYCLINE	56 (31)	74 (58)	33 (21)	46 (27)	19 (16)	90 (83)	43 (30)	93 (90)	– –	5 (5)	27 (24)	78 (55)	6 (6)	25 (15)	41 (43)	23 (26)
TOBRAMYCIN*	44 (66)	71 (–)	– –	32 (18)	65 (63)	– –	41 (41)	– –	74 (75)	– –	– –	– –	– –	– –	– –	– –

( ) Result of 1978.
* Organisms which resisted to majority of antibiotics (approx 10 %) and all Pseudomonas aeruginosa were tested.

The selection of antibacterial agents for the clinical management of the patients involves judgement predic ted upon the clinical status of patient and the infectious nature or extent. Although the offending microorganism is proven susceptible to a large number of antibiotics, the clinician is recommended to employ only those antibiotics which are least toxic, least costly and equally effective drug. Hence, the in vitro sensitivity assay influences the therapeutic management of the patient as well as the general pattern of antibacterial usage. With extensive use of antibacterials, selection of resistant strains whose susceptibility profiles are different from their parent strains will pose serious problem. One of the means available for controlling the rate of development of resistance to specific antibacterial agent is to limit the gamut of antibacterial employed. However, even adequate control of antibacterial administration, spontaneous alternations in susceptibility profiles of common pathogens will still occur and must therefore be identified (2).

In treating infectious diarrhoeal cases here in Thailand, the increase resistance of bacteria to the routine antibiotic regimen is also the problem. Since 1969 (45), the enteropathogenic bacteria isolated from diarrhoeal patients frequently resist to multiple antibacterial agents i.e. Ampicillin, Chloramphenicol, Kanamycin, Streptomycin and Tetracycline. The resistance is often transferable, for it is the infectious drug resistance. Thus, the infectious drug resistance is now a significant local problem. The ineffective treatment will help to spread drug resistance in the environment. A more rational approach to combating infection by multiple resistant enteropathogens depends on administering

Table 4 list of antibiotics and attainable MIC blood and urine levels that can be expected for differ dosages via several routes of administration (7)

Antibiotic	Route of Administration	Therapeutic Dose (Adults)	Dose Schedule	Attainable Blood Levels µg/ml	Attainable Urine Levels µg/ml
Ampicillin	Oral	250 mg	q6h	1.5-2.5	50-100
	I.M.	500 mg	q6h	2.5-4.0	200-400
	I.V.	1.0-1.5 g	q4h	18-20	200-400
Carbenicillin	Oral	1.0 g	q6h	8-10	250-1400
	I.V.	4.0-5.0 g	q4h	100-150	1000-7000
Cephalexin	Oral	0.25 g	q6h	2-6	300-400
	Oral	1.0 g	q6h	20-80	1000-2000
Cephalothin	I.M.	0.5-1.0 g	q6h	10-20	1000-2000
	I.V.	1.0-2.0 g	q6h	30-80	1000-2000
Chloramphenicol	Oral	250-500 mg	q6h	2-5	200-800
	I.V.	500 mg-1.0 g	q6h	10-20	500-1400
Clindamycin	Oral	150-300 mg	q6h	2-3.5	30-90
	I.V.	300-600 mg	q6h	4-8	45-240
Erythromycin	Oral	500 mg	q6h	3-5	Very low
Gentamicin	I.M. or I.V.	1.0-2.0 mg/kg	q6-8h	5-10	Up to 250
Kanamycin	I.M. or I.V.	500 mg	q8-12h	15-20	100-600
Methicillin	I.M.	1.0 g	q4h	10-15	700-1000
	I.V.	2.0 g	q4h	20-25	1000-2000
Nitrofurantoin	Oral	50-100 mg	q6h	Insignificant	200+
Oxacillin	Oral	0.25-1.0 g	q6h	1-8	100-700
	I.M.	1.0 g	q4h	10-15	700-1000
	I.V.	2.0 g	q4h	20-25	1000-2000
Penicillin	Oral	250 mg	q6h	1.0-1.5	
	I.V.	1.0 Mil. Units	q4h	15-20	1000-3000
Tetracycline	Oral	250-500 mg	q6h	2-5	600-1200
T/S (trimethoprim (TMP), sulfamethoxatole (SMX)	Oral	160 mg TMP 800 mg SMX	1-2 tab. q12h	1-3 TMP 40-60 SMX	100-200 TMP 200-300 SMX
Tobramycin	I.M. or I.V.	3.5 mg/kg	q8h	4-8	50-150

* If Colistin or Poly B susceptibility tests are desired, please notify laboratory

only those drugs to which the bacteria are sensitive.

In the individual patient, this approach necessitates cultures of the causative organisms and determination of its pattern of drug resistance and sensitivity as a guide to the selection of the therapeutic agent...

There is a general agreement that, the in vitro susceptibility testing provides clinically useful, and often essential information for specific antimicrobial therapy. Mirrett S. and Reller B. (46) have shown that physicians did change from initial emperical therapy to more appropriate antimicrobial therapy in 71% of the episodes confirmed bacteremia after susceptibility results were reported. How then can susceptibility results be obtained faster, and what are the pitfalls?

The present study describe I The use of modified broth disk method (36) as a system for testing the antibacterial sensitivity of enteropathogenic bacteria. The modified broth disk method is adapted by using only one concentration of antibacterial agents. The final concentration of specific antibacterial agent in tube, the source of which is derived from the commercially available disks, will approximately equal to that achievable in blood via the normal route of administration (36) (Table 4). The results of which will compared with the disk diffusion method. II The local disks produced by the Medical Scientific Research Department, are tested in comparison with the imported BBL disks using modified broth disk and disk diffusion methods. III The agar dilution and broth dilution tests are also performed, using the

commercially available antibacterial powders. The potential utilization of the modified broth disk method as a guideline for rapid in vivo therapy is discussed.