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An Evaluation for Modification (Shorter) of the Kirby-Bauer Susceptibility Test

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AN EVALUATION FOR MODIFICATION (SHORTER) OF THE KIRBY-BAUER SUSCEPTIBILITY TEST

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AN EVALUATION FOR MODIFICATION (SHORTER) OF THE

KIRBY-BAUER SUSCEPTIBILITY TEST

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Walter K. Norris, Jr. April 1982 41 pages

Directed by: Paul Tanner, Robert Baum, and David Dunn

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The Kirby-Bauer test for determining antibiotic effectiveness is widely used in laboratories. The 10 to 20 hour incubation time needed to obtain useful results is a disadvantage of that test. This experimental research was developed to test a modification which could provide useful results in 5 hours.

The modification employed in this experimental technique used an increased inoculum at a 1.0 McFarland standard instead of the customary 0.5 standard. The 2 to 5 hour incubation period in the trypticase soy broth was deleted. The Mueller Hinton plates were incubated for 5 hours and then observed for resistant and/or sensitive patterns.

Controls for this experimental study were the results of the standard Kirby-Bauer test as recorded by the day and night shift personnel of the Medical Center at Bowling Green, Bowling Green, Kentucky. Tested were 33 cultures of Escherichia coli, 33 cultures of Pseudomonas aeruginosa, and 33 cultures of Staphylococcus aureus. The same cultures of each organism were tested using the 5 hour experimental procedure. A pure culture was inoculated in a tube of trypticase soy broth to a final turbidity equal to a 1.0 McFarland standard. A portion of this inoculum was swabbed onto the entire surface of a Mueller Hinton plate. Antibiotic discs were placed on the agar surface and tapped gently to

insure contact. The plates were put into a 37°C incubator for 5 hours then removed to observe zones of no growth. Results were classified as either "resistant" or "sensitive"; "intermediate" was deleted. If a zone of no growth was closer to the sensitive reading than the resistant reading for an antibiotic, the bacterium was considered sensitive to that antibiotic. The same was true for resistant readings. Measurements were taken with a caliper dial.

For the two procedures, identical results occurred 99.7% of the time for Pseudomonas aeruginosa. For Escherichia coli 96.8% of the tests were identical, and with Staphylococcus aureus 93.2% of the tests were identical. Strains of Staphylococcus aureus that were sensitive to penicillin G and ampicillin with the standard Kirby-Bauer test were resistant with the 5 hour test. It occurred 10 times with a quality control stock culture and 1 time with a clinical isolate for ampicillin. It occurred 9 times with a quality control stock culture and 1 time with a clinical isolate for penicillin G. It is likely that the differences with Staphylococcus aureus for ampicillin and penicillin G are due to the interaction between the organism and the two antibiotics. Further studies are needed to determine whether or not a 1 to 2 hour extension of the incubation time could alleviate this problem.

Chapter 1

INTRODUCTION

This experimental research is intended to determine whether or not a standard test procedure (Kirby-Bauer) can be modified to yield quicker results. Such a procedure would permit physicians to initiate appropriate antibiotic treatment sooner, thereby favorably influencing the prognosis for a serious infection. The procedure tested in this research could provide such results 5 to 18 hours earlier than the standard procedure.

Background

Antibiotics are fungal metabolites which have been shown to be useful in controlling bacteria populations (Jarett and Sonnenwirth, 1980). As the use of antibiotics became wide spread, an increase in bacterial strains resistant to antibiotics increased (Lorian, 1977). This increase in the numbers of resistant strains has become more evident within the past two decades (Lorian, 1977).

Before antibiotics are used to control a given infection, bacterial sensitivity to several antibiotics is tested (Lorian, 1977). This method for testing sensitivity is termed the Kirby-Bauer susceptibility method. The standard Kirby-Bauer susceptibility method, when performed and evaluated correctly, has been extremely useful as a guide in choosing the antibiotic suited for therapy of infections due to pathogenic bacteria (Boyle, 1973). Also, the Food and Drug Administra-

tion has recommended the Kirby-Bauer technique as a standardized procedure for the determination of antibiotic disk susceptibilities (U.S. Dept. HEW, 1970).

The general acceptance of this disk-susceptibility method has been aided by its simplicity and reproducibility (Boyle, 1973). The prolonged incubation interval required (10 to 20 hours) to determine susceptibility, which is the level at which a given bacterial strain is inhibited in growth or killed, has remained a notable disadvantage (Boyle, 1973).

Briefly, the standard Kirby-Bauer susceptibility test involves transferring a few colonies of a bacterial organism into 2 to 4 milliliters of broth. This inoculum contains about 1.5×10^8 organisms per milliliter. The broth tube is incubated 2 to 5 hours in a $37^{\circ}C$ air incubator or $37^{\circ}C$ water bath to produce a bacterial suspension with enough cloudiness to be equal in turbidity to a 0.5 McFarland standard. The organisms are then streaked over the entire surface of Mueller Hinton agar with a cotton swab. Dried filter-paper disks with a different antibiotic in each disk are placed on this agar and tapped gently to insure contact. After an 8 to 20 hour incubation, zones of inhibition are measured. From measured zone reactions the clinical pathogen's response to the antibiotic disks are recorded and placed into three categories: (1) susceptible, which means a given bacterial strain is inhibited in growth, (2) intermediate, which is of no clinical significance, and (3) resistant, a level of susceptibility beyond that normally achieved in the human body by the usual dose (Lennette, 1980).

During the 8 to 20 hour incubation period, processes of disk diffusion begin with the dried disks absorbing water from the agar medium, thus dissolving the drug (Lennette, 1980). The antimicrobic is then free to migrate through the adjacent agar medium, following the physical laws that govern diffusion of molecules through an agar gel. The end result is a gradually changing gradient of drug concentration in the surrounding area of each disk. As the antimicrobic diffusion progresses, microbial multiplication also proceeds. After an initial lag phase, a logarithmic growth phase is initiated. At that point, bacterial multiplication proceeds more rapidly than the drug can diffuse, and bacterial cells which are not inhibited by the antimicrobic will continue to multiply until growth can be visualized. There will be a no growth area where the drug is present in inhibitory concentrations; the more susceptible the test organism, the larger the zone of inhibition. The position of the zone of inhibition for most bacterial organisms is determined during the first few hours of incubation (Lennette, 1980). With these mechanics in mind, doubling the amount of organisms that are usually put in the broth then reading the plates at 5 hours would be a possible way to shorten the incubation time for the Kirby-Bauer susceptibility test.

Chapter 2

REVIEW OF LITERATURE

The Kirby-Bauer susceptibility test has been used by clinical laboratories since its develpoment in 1966 by Kirby, Bauer, and associates (Bauer et al., 1966). One disadvantage of this test is the time required by the incubation period (10 to 20 hours) to obtain results. Since 1966 considerable research has been conducted to improve and shorten this standard susceptibility test. Some of the research leading up to this experimental test are discussed in this chapter.

Hemoglobin Reduction-Pour Plate Technic

Melia and associates (Melia et al, 1971) developed a modification of the Kirby-Bauer method using 10% whole sheep blood in Mueller Hinton agar as a base layer. A measured amount of organism was placed in a tube of melted overlay agar composed of Mueller Hinton agar which contained 0.1% yeast extract and 0.2% glucose. The melted overlay agar was then poured over the 10% whole sheep blood-Mueller Hinton agar base and allowed to solidify. Antibiotic disks were then added by pressing them onto the agar surface. The plates were incubated at 4 hours, and zones of inhibition appeared as bright red zones of unreduced hemoglobin against a background of dark reduced hemoglobin. Melia (1971) reported this hemoglobin-reduction method produced sharp zones of inhibition which were often distinguishable at 3 hours and almost without fail at 4 hours. Using isolates of Escherichia coli for com-

parison, Melia (1971) recorded 99.4% agreement with the standard sensitivity test. There was 98.1% agreement established for Pseudomonas aeruginosa and 99.2% agreement for Staphylococcus aureus. Overall agreement was established at 98.7% with the various isolates tested. However, additional tests (Barry et al, 1973) failed to confirm these findings declared by Melia (1971). Barry (1973) found that use of the hemoglobin reduction-pour plate technique for sensitivity testing required establishment of new interpretative zone standards. Barry (1973) also found that with certain drugs and some bacterial strains the cell population did not grow rapidly enough for detection during the early hours of incubation.

Tetrazolium-Dye-Reduction

Boyle and his colleagues (1973) reported a rapid (6 to 7 hour) modified Kirby-Bauer test using derviatives of tetrazolium dyes to speed up the readability of the zones of inhibition in the Kirby-Bauer test. Their results were reproducible and proved accurate in comparison with the standard Kirby-Bauer method for the organisms that were tested.

However, this method calls for the use of several inconvenient procedures to be employed (Kluge, 1975). These were (1) the necessity for duplicate Kirby-Bauer tests, (2) an extra step of applying the tetrazolium dye, and (3) the need for technicians to read plates at 8:00 p.m. (Kluge, 1975).

Reduced Incubation

Barry (1973) examined the possibility of obtaining early readings by direct plating of clinical specimens that were read at 18 hours

and then the use of direct suspension of colonies without broth subculture, read at 5 to 6 hours. Pure cultures were not used, causing varying results. Unreliable readings were obtained with the direct plated specimens. Early readings after plating a direct suspension of colonies resulted in agreement in 90% of tests that were run.

Kluge (1975) used 100-mm petri plates instead of the 150-mm plates and read sensitivities at 4, 8, and 12 hours incubation and compared these findings with readings at 18 to 20 hours. There was an overall agreement of early and standard readings of 87% at 4 hours, 94% at 8 hours, and 96% at 12 hours. These results were comparable to Barry's (1973) overall 90% accuracy at 5 to 6 hours.

Liberman and Robertson (1975) ran comparison tests utilizing the Kirby-Bauer procedure. Comparisons were made of the test results at 7 to 8 hours and 18 to 20 hours utilizing 100% clinical isolates. Essentially this was a reinvestigation of the research by Barry (1973). The data tabulated by Liberman and Robertson (1975) indicated that zone sizes can be interpreted with reasonable accuracy, and the results can be available 10 to 14 hours sooner than obtained by the standard Kirby-Bauer test.

Dr. Victor Lorian and associates (1977) introduced a simple method for obtaining sensitivity values using only the ordinary diagnostic bacteriology equipment used in the Kirby-Bauer method. Lorian (1977) claimed this method furnished antibiotic susceptibility data within 5 hours of isolation of bacteria in pure culture. Lorian (1977) deviated from the standard Kirby-Bauer test by using a bacterial suspension at a turbidity equal to a 1.0 McFarland standard. A McFarland

standard shows the proper density that the trypticase soy broth with the added bacteria should have after the broth has been incubated and before the organisms are swabbed on the Mueller Hinton plates. This inoculum was not preincubated as in the standard Kirby-Bauer test and was twice the turbidity recommended by the Kirby-Bauer procedure (Bauer et al, 1966). Mueller Hinton agar was used for gram-negative organisms, and gram-positive organisms were plated on Mueller Hinton with blood. Classification as sensitive or resistant after 5 hours was the same after 24 hours in 98.9% of the tests for Enterobacteriaceae, 98.7% of the tests for gram-positive cocci, and 97.9% of the tests for Pseudomonas aeruginosa. Overall accuracy was 98%.

There is another procedure (Autobac) which will give results in a period of 3 hours. However, the Autobac equipment is expensive and therefore found only in large laboratories (Stubbs and Wicher, 1976).

The accuracy of a shortened Kirby-Bauer test has been brought within 98% comparability to the standardized Kirby-Bauer susceptibility test. From the literature cited one may act upon the thought that an increased inoculum with no preincubation will cause susceptibility reactions to occur faster, thereby shortening the incubation time so that sensitivity testing may be accomplished in a shorter time. This research project will partially replicate the Lorian study.

Chapter 3

METHODS AND PROCEDURES

The purpose of this experimental research is to determine whether using a higher concentration of bacterial inoculum combined with a shorter incubation period will give results as valid as those obtained by a longer incubation period using the standard Kirby-Bauer procedure.

Organisms

The organisms used in this study are three genera of pathogenic bacteria isolated in the clinical laboratory in the Medical Center at Bowling Green, Kentucky. These organisms are a gram-positive cocci, Staphylococcus aureus and gram-negative rods, Escherichia coli and Pseudomonas aeruginosa. The three organisms mentioned are the most often measured for drug susceptibility by the Kirby-Bauer method.

Procedure

As described in Chapter 1 the standard Kirby-Bauer susceptibility test involves transferring a few colonies of a bacterial organism into 2 to 4 milliliters of broth. This inoculum contains about 1.5 X 10^8 organisms per milliliter. The broth tube is incubated 2 to 5 hours in a 37°C air incubator or 37°C water bath to produce a bacterial suspension equal in turbidity to a 0.5 McFarland standard. The organisms are then streaked over the entire surface of Mueller Hinton agar with a

cotton swab. Dried filter-paper disks with a different antibiotic in each disk are placed on this agar and tapped gently to insure contact. After an 8 to 20 hour incubation period zones of inhibition are measured. The clinical pathogen's response to the antibiotic disks is determined by measuring the zone of inhibition around each disk with a caliper. The results are recorded and placed into three categories: (1) susceptible, which means a given bacterial strain is inhibited in growth, (2) intermediate, which is partially sensitive but not enough to be clinically optimum, and (3) resistant, a level of resistance beyond that assumed to occur in the human body by the usual dose (Lennette, 1980).

The 5 hour susceptibility test described by Lorian (1977) and used in this study requires the same equipment as the standard Kirby-Bauer procedure. With the 5 hour procedure an inoculum containing approximately 3.0×10^8 organisms (instead of 1.5×10^8 organisms) was transferred to a trypticase soy broth tube and compared to a 1.0 McFarland standard instead of a 0.5 McFarland standard. The organisms were then immediately streaked on Mueller Hinton agar rather than waiting for the 2 to 5 hour incubation period in the broth. After the plates were incubated for 5 hours, zones of inhibition were measured. From the measured zones, reactions were placed into two categories: susceptible and resistant. If patterns of zone sizes were closer to the sensitive reading than to the resistant reading, the organism was considered sensitive to that antibiotic. If zone sizes were closer to the resistant pattern reading than to the sensitive pattern, the organism was categorized as being resistant to that particular antibiotic. Difco resistant-susceptible patterns for each antimicrobial disk were

used as a guide in placing zone sizes in resistant or susceptible ranges. The zone sizes were measured with a caliper. Table 3.1 lists the antibiotics and their classification used in the standard and the 5 hour experimental procedure for both the gram-positive and gram-negative organisms.

Quality control organisms of each genera of pathogen isolated were utilized in this research and are listed in Table 3.2. Escherichia coli ATCC (American Type Culture Collection) 25922 is sensitive to all 12 antibiotics tested for gram-negative organisms. Staphylococcus aureus ATCC 25923 is sensitive to all 11 antibiotics tested for gram-positive organisms. Pseudomonas aeruginosa, a gram-negative organism, ATCC 27853 is resistant to 9 antibiotics tested for gram-negative organisms except three antibiotics: carbenicillin, gentamicin, and tobramycin. These quality control organisms are run twice each week at the Medical Center to insure uniform results with the media and antibiotic disks used with the Kirby-Bauer method. Table 3.3 list the source of each of the thirty-three organisms that were used in the Kirby-Bauer test for Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus.

The susceptibility studies were conducted in the clinical laboratory at the Medical Center at Bowling Green, Kentucky, under normal laboratory conditions and settings. The organisms isolated and used in the regular sensitivity studies by the Medical Center personnel were subsequently used in this 5 hour procedure and the results were compared. The control group for this study is the tests conducted by the standard Kirby-Bauer procedure.

Table 3.1

Sensitivity Test Antibiotics

Antibiotics Tested	Gram +	Gram -	Type Drug
Ampicillin	+	+	Penicillin
Carbenicillin	-	+	Penicillin
Cefamandole	+	+	Cephalosporin
Cephalothin	+	+	Cephalosporin
Chloromycetin	+	+	
Clindamycin	+	-	
Erythromycin	+	-	
Furadantin	-	+	Urinary Tract
Gantrisin	-	+	Urinary Tract
Gentamicin	+	+	Aminoglycoside
Nalidixic Acid	-	+	Urinary Tract
Oxacillin	+	-	Penicillin
Penicillin G	+	-	Penicillin
Sulfateimethoprin	-	+	Urinary Tract
Tetracycline	+	+	
Tobramycin	+	+	Aminoglycoside

Tested + Not Tested -

Table 3.2

Quality Control Organisms

Escherichia coli ATCC 25922
Pseudomonas aeruginosa ATCC 27853
Staphylococcus aureus ATCC 25923

The results for the control group were read by the day and night shift staff employed in the microbiology section in the Medical Center. This writer used the same organisms to accomplish the 5 hour experimental procedure and these experimental tests were read by the writer early in the morning before the day shift of the Medical Center began work. Table 3.3 list the source of each of the thirty-three organisms that were used in the Kirby-Bauer test using *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Table 3.3

Source of Tested Isolates

Gram-positive Source of Isolate		Gram-negative Source of Isolate	
Staphylococcus aureus		Escherichia coli	
	Quality Control-9		Urine----------16
	Wound----------7		Quality Control--6
	Throat----------4		Wound----------4
	Blood----------3		Blood----------3
	Nasopharygeal---3		Peritineum-------2
	Vaginal---------2		Spinal Fluid-----1
	Elbow aspirate--2		Sputum----------1
	Knee aspirate---1	Pseudomonas aeruginosa	
	Sputum----------1		Quality Control-14
	Urine----------1		Urine----------7
			Wound----------5
			Sputum----------4
			Decubitus--------1
			Ear--------------1
			Eye--------------1

Chapter 4

RESULTS AND DICUSSION

The purpose of this research was to determine whether a modified Kirby-Bauer antibiotic sensitivity test, using an increased inoculum concentration with a shorter 5 hour incubation time, would give results as reliable as the standard Kirby-Bauer test. A shorter Kirby-Bauer test would mean earlier results for the physician thereby allowing him to initiate effective antibiotic therapy as soon as possible.

This modified Kirby-Bauer technique uses the same media, broth tubes, and antibiotic disks as those required by the standard Kirby-Bauer technique. The only departures from the standard Kirby-Bauer test are (1) an increased inoculum concentration is used, (2) elimination of the 2 to 5 hour incubation in the broth tube, and (3) a 5 hour incubation period rather than the standard incubation time of 10 to 20 hours.

The procedure for the 5 hour modified version of the Kirby-Bauer sensitivity test used pure cultures of the three following organisms: Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus. Thirty-three Kirby-Bauer tests were run on each organism. Colonies from a pure culture were suspended in trypticase soy broth, and this suspension was diluted to a final turbidity of a 1.0 McFarland

standard rather than the 0.5 as in the standard procedure. This suspension was inoculated by streaking on Mueller Hinton agar plates. Sensitivity disks were placed on the Mueller Hinton plates, and the plates were incubated at 37°C for 5 hours. Measurement of zones of inhibition were accomplished by a caliper. From measured zones, reactions to the antibiotic disks were placed into two categories: susceptible and resistant. If sensitivity patterns of zone sizes were closer to the sensitive reading than the resistant reading, the organism was considered sensitive to that antibiotic. If sensitivity patterns of zone sizes were closer to the resistant reading than the sensitive reading, the organism was placed in the resistant category for that antibiotic.

Results

This section includes the results of the reactions using each antibiotic for the three organisms: *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. These results were subjected to a Chi square analysis by computer at Western Kentucky University using the Yates correction factor. However, these inferential statistics are not included. The nature of the data did not lend itself to an inferential analysis, since many of the cells in the two-by-two tables had an N of zero.

Each table in this section contains the test reaction which occurred with the standard Kirby-Bauer test and the experimental procedure. The top two squares of each table represent the number of organisms sensitive to the antibiotic listed in that table for the standard Kirby-Bauer test and the 5 hour modified test. The two bottom squares

of each table are the number of organisms resistant to the listed antibiotic in that table for the two test procedures. The results for the two Kirby-Bauer tests are identical when both numbers for the sensitive and resistant readings are the same. For Escherichia coli (gram-negative) the following results are shown in tables 4.1 through 4.12.

Table 4.1

Escherichia coli: Ampicillin

	Standard	5 Hour
Sensitive	27	29
Resistant	6	4

Table 4.2

Escherichia coli: Carbenicillin

	Standard	5 Hour
Sensitive	28	28
Resistant	4	4

Table 4.3

Escherichia coli: Cefamandole

	Standard	5 Hour
Sensitive	33	33
Resistant	0	0

Table 4.4

Escherichia coli: Cephalothin

	Standard	5 Hour
Sensitive	32	30
Resistant	0	2

Table 4.5

Escherichia coli: **Chloromycetin**

	Standard	5 Hour
Sensitive	33	33
Resistant	0	0

Table 4.6

Escherichia coli: Furadantin

	Standard	5 Hour
Sensitive	28	28
Resistant	0	0

Table 4.7

Escherichia coli: Gantrisin

	Standard	5 Hour
Sensitive	26	22
Resistant	2	6

Table 4.8

Escherichia coli: Gentamicin

	Standard	5 Hour
Sensitive	33	33
Resistant	0	0

Table 4.9

Escherichia coli: Nalidixic acid

	Standard	5 Hour
Sensitive	28	28
Resistant	0	0

Table 4.10

Escherichia coli: Sulfatrimethoprin

	Standard	5 Hour
Sensitive	28	28
Resistant	0	0

Table 4.11

Escherichia coli: Tetracycline

	Standard	5 Hour
Sensitive	29	30
Resistant	4	3

Table 4.12

Escherichia coli: Tobramycin

	Standard	5 Hour
Sensitive	32	33
Resistant	1	0

With Escherichia coli there were identical results 96.8% of the time for both tests. The number of results that did not compare between the two tests totaled 3.2%. For Pseudomonas aeruginosa (gram-negative) the following results are shown in tables 4.13 through 4.24.

Table 4.13

Pseudomonas aeruginosa: Ampicillin

	Standard	5 Hour
Sensitive	0	0
Resistant	33	33

Table 4.14

Pseudomonas aeruginosa: Carbenicillin

	Standard	5 Hour
Sensitive	32	32
Resistant	0	0

Table 4.15

Pseudomonas aeruginosa: Cefamandole

	Standard	5 Hour
Sensitive	0	0
Resistant	33	33

Table 4.16

Pseudomonas aeruginosa: Cephalothin

	Standard	5 Hour
Sensitive	0	0
Resistant	33	33

Table 4.17

Pseudomonas aeruginosa: Chloromycetin

	Standard	5 Hour
Sensitive	0	0
Resistant	32	32

Table 4.18

Pseudomonas aeruginosa: Furadantin

	Standard	5 Hour
Sensitive	0	0
Resistant	26	26

Table 4.19

Pseudomonas aeruginosa: Gantrisin

	Standard	5 Hour
Sensitive	0	0
Resistant	26	26

Table 4.20

Pseudomonas aeruginosa: Gentamicin

	Standard	5 Hour
Sensitive	31	32
Resistant	1	0

Table 4.21

Pseudomonas aeruginosa: Nalidixic Acid

	Standard	5 Hour
Sensitive	0	0
Resistant	26	26

Table 4.22

Pseudomonas aeruginosa: Sulfatrimethoprin

	Standard	5 Hour
Sensitive	0	0
Resistant	26	26

Table 4.23

Pseudomonas aeruginosa: Tetracycline

	Standard	5 Hour
Sensitive	0	0
Resistant	33	33

Table 4.24

Pseudomonas aeruginosa: Tobramycin

	Standard	5 Hour
Sensitive	33	33
Resistant	0	0

With Pseudomonas aeruginosa there were identical results 99.7% for both standard and 5 hour Kirby-Bauer tests. A 0.3 % differences was noted in results when comparing both tests. For Staphylococcus aureus (gram-positive) the following results are shown in tables 4.25 through 4.35.

Table 4.25

Staphylococcus aureus: Ampicillin

	Standard	5 Hour
Sensitive	11	0
Resistant	18	29

The results for ampicillin are totally different than results from previous tables. The standard test found 11 cultures of Staphylococcus aureus sensitive to ampicillin and 18 resistant. The 5 hour test had no organisms that were sensitive to ampicillin and 29 that were resistant to ampicillin. This may mean ampicillin takes longer to be effective. This will be discussed in more detail in Chapter 5.

Table 4.26

Staphylococcus aureus: Cefemandole

	Standard	5 Hour
Sensitive	32	32
Resistant	0	0

Table 4.27

Staphylococcus aureus: Cephalothin

	Standard	5 Hour
Sensitive	31	33
Resistant	2	0

Table 4.28

Staphlococcus aureus: Chloromycetin

	Standard	5 Hour
Sensitive	33	33
Resistant	0	0

Table 4.29

Staphylococcus aureus: Clindamycin

	Standard	5 Hour
Sensitive	33	33
Resistant	0	0

Table 4.30

Staphylococcus aureus: Erythromycin

	Standard	5 Hour
Sensitive	33	33
Resistant	0	0

Table 4.31

Staphylococcus aureus: Gentamicin

	Standard	5 Hour
Sensitive	32	32
Resistant	1	1

Table 4. 32

Staphylococcus aureus: Oxacillin

	Standard	5 Hour
Sensitive	33	31
Resistant	0	2

Table 4.33

Staphylococcus aureus: Penicillin G

	Standard	5 Hour
Sensitive	10	1
Resistant	22	31

These results for penicillin G are totally different as were those of ampicillin. For the standard Kirby-Bauer test there were 10 Staphylococcus aureus that were sensitive to penicillin G and 22 that were resistant to this antibiotic. For the 5 hour test 1 organism for Staphylococcus aureus was sensitive to penicillin G and 31 organisms were resistant. These results will also be dicussed later in Chapter 5.

Table 4.34

Staphylococcus aureus: Tetracycline

	Standard	5 Hour
Sensitive	33	33
Resistant	0	0

Table 4.35

Staphylococcus aureus: Tobramycin

	Standard	5 Hour
Sensitive	28	28
Resistant	1	1

With Staphylococcus aureus there were identical results 93.3% of the time when comparing the standard and 5 hour Kirby-Bauer tests. There was a 6.7% difference between these same tests. Table 4.36 displays the congruence of the two procedures for all the organisms tested.

Table 4.36

Congruence for Results

Organism	Total Isolates	Total Test	Congruence of Two Procedures
Escherichia coli	33	374	96.8%
Pseudomonas aeruginosa	33	363	99.7%
Staphylococcus aureus	33	353	93.3%

Summary

The standard Kirby-Bauer test results used in this study were

set up and read by the day and night shift technologists at the Medical Center at Bowling Green laboratory. The 5 hour Kirby-Bauer test was set up after the standard Kirby-Bauer test results had been recorded. The results of the experimental test were then read and recorded five hours later. A total of 374 antibiotic tests were utilized with 33 isolates of Escherichia coli. When comparing the final sensitivity readings of the standard Kirby-Bauer test and the 5 hour Kirby-Bauer test, there were identical readings 96.8% of the time for both tests. A total of 363 antibiotic tests were used with 33 isolates of Pseudomonas aeruginosa. When comparing the final sensitivity readings of the standard Kirby-Bauer test and the 5 hour Kirby-Bauer test, there were identical readings 99.7% of the time for both tests. A total of 353 antibiotics were tested with 33 isolates of Staphylococcus aureus. Comparison of the final sensitivity readings of the standard Kirby-Bauer test and the 5 hour Kirby-Bauer test showed identical readings 93.3% of the time for both tests.

With Staphylococcus aureus, the standard Kirby-Bauer test gave a sensitive reading for ampicillin and penicillin G; the 5 hour test gave a resistant reading except for one test result. This phenomenon was observed 11 times with ampicillin and 10 times with penicillin G.

Overall, a total of 1090 antibiotic tests were used to compare the standard Kirby-Bauer test and the experimental 5 hour procedure. The same results were obtained by both standard and experimental procedures 96.6% of the time. It should be noted that when the standard Kirby-Bauer procedure showed an intermediate reading on any of the antibiotics for the three tested organisms no comparison was made

using the 5 hour technique. For that reason some of the antibiotics were tested less than 33 times.

Chapter 5

SUMMARY AND CONCLUSIONS

The purpose of this thesis study was to evaluate a shorter procedure for determining the sensitivity of bacteria to various antibiotics and to determine if this procedure would be practical for use in the clinical laboratory. The standard test requires a total incubation time of 10 to 20 hours, whereas a modified test used in this research requires an incubation period of 5 hours. The 5 hour procedure uses an increased inoculum (1.0 McFarland standard instead of a 0.5 McFarland standard) based upon work by Lorian (1977). This experimental procedure would provide useful results to a physician 5 or more hours sooner than would the standard procedure.

Results

A total of 1090 antibiotic tests were used in comparing the standard Kirby-Bauer test and the experimental 5 hour procedure. The same results were obtained by both standard and experimental procedures 96.6% of the time. For Escherichia coli a total of 374 antibiotic tests were utilized with 33 isolates. Comparison of the sensitivity readings of the standard Kirby-Bauer test and the 5 hour Kirby-Bauer test gave similar readings 96.8% of the time. For Pseudomonas aeruginosa a total of 363 antibiotic test were used with 33 isolates. Comparison of the final sensitivity readings for Pseudomonas aeruginosa with the standard Kirby-Bauer test and the 5 hour Kirby-Bauer test exhibited similar

readings 99.7% of the time for both tests. For Staphylococcus aureus a total of 353 antibiotics were tested with 33 isolates. Comparison of the final sensitivity readings of the standard Kirby-Bauer test and the 5 hour Kirby-Bauer procedure indicated similar readings 93.2% of the time.

A major finding of this research was that the standard and experimental results were very similar, with one striking exception in the case of penicillin G and ampicillin for Staphylococcus aureus. It was observed that when the standard Kirby-Bauer test gave a sensitive reading for ampicillin (11 times) and penicillin G (10 times) the 5 hour test consistently gave a resistant reading except for one test result for penicillin G. This occurred 10 times with a quality control stock culture and one time with a clinical isolate for ampicillin. It occurred 9 times with a quality control stock culture and one time with a clinical isolate for penicillin G. Due to uniformity of results for all the other tests (98.4%) and because with Staphylococcus aureus ampicillin and penicillin G differed for 20 of 21 readings, it is assumed by this researcher that the exceptions for these two were not due to procedural factors. Rather, it is more likely that the differences with Staphylococcus aureus for ampicillin and penicillin G are due to the interaction between the organism and the two antibiotics. In a recent study by Furtado and Harris (1982), comparing the standard Kirby-Bauer test with a 3 hour incubation (Autobac), this same phenomenon was observed with penicillin also using a Staphylococcus aureus organism. They hypothesized that a shorter incubation period could cause unreliable results due to the delayed onset of the bactericidal

effectiveness of penicillin (Furtado and Harris, 1982). Both penicillin and ampicillin work by interfering with active cell wall synthesis of bacteria. Since ampicillin is also a form of penicillin, a shorter incubation period could be the reason for the different results with the standard Kirby-Bauer test and the 5 hour procedure in this research. The Furtado-Harris study (1982) suggested a possible way to avoid the discrepancies also found by this writer. This procedure would involve incubating for an additional 1 to 2 hours for the tests which were read as resistant at 5 hours. Increased incubation time of an additional 1 to 2 hours would still mean a shorter time period as compared to the standard Kirby-Bauer test.

Recommendations

Since discrepancies occurred in the 5 hour experimental procedure as compared with the standard Kirby-Bauer procedure for Staphylococcus aureus with ampicillin and penicillin G, an increase in the incubation time of 1 to 2 hours may allow for more reliable results. Additional tests should be conducted to confirm these findings. The purpose of such tests would be to determine if ampicillin and penicillin G required more than 5 hours to act against the Staphylococcus aureus used. Perhaps the shorter procedure could be used in instances where early results become especially critical. However, the traditional Kirby-Bauer procedure should also be run to provide a check in the cases where Staphylococcus aureus is suspected and penicillin G or penicillin-like compounds are being considered. Also, the 5 hour experimental procedure should be conducted for microorganisms other than the three

tested in this study to determine the effectiveness of this experimental procedure over the standard Kirby-Bauer test.

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