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A THREE-YEAR SURVEY OF ANTIMICROBIAL AGENTS

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The bacteriologist and clinician should maintain a constant vigilance on the susceptibility patterns of both old and new antimicrobial agents. This can be accomplished by a monthly and/or yearly surveillance of the antibacterial spectra of the chemotherapeutic agents, using the data within one's own institution and comparing this information with the reports of other investigators in the current literature. The reports of at least the fluctuations should be made available to any individual concerned with antimicrobial testing and/or therapy. It should be recognized that the time lapse between the collection of the data and the publication may well render the information obsolete by the time of issue. Therefore, it is suggested that the bacteriologist and practicing physicians be informed by reports at the time of periodic meetings such as grand rounds or by means of either bulletins or journals.

Since a great many variables are involved in determining the relative *in vitro* as well as the *in vivo* efficacy of the different antimicrobial agents, it was deemed most desirable to conduct a three-year survey in order to have sufficient number of susceptibility tests for each group of organisms and the respective drug under study. The relative efficacy of each agent must be compared under similar testing procedures for a period sufficiently long to eliminate temporary variations due to occasional changes in methodology based on such factors as technique or a substitute technologists. The responsibility for the compilation of laboratory data should be accepted by the clinical bacteriologist who is constantly in touch with the bacteriological testing methods.

This study includes a survey of the majority of clinical isolates obtained from the routine bacteriological services at this hospital in 1962, 1963 and 1964. Both the Gram-positive and Gram-negative group of organisms which occur most frequently have been evaluated.

The value of tube-dilution studies is discussed and compared to the routinely empolyed disk susceptibility test procedure. The latter method is much more practical for the average hospital bacteriological service but the value of the tube dilution susceptibility technique is in determining Minimum Inhibitory Concentrations in select instances.

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Another aspect of susceptibility testing, namely the *in vitro* sulfonamide procedure will be considered. It is well known that the methodology for conducting these tests is somewhat different than the method used for the other antimicrobial agents. This is particularly due to competitive actions of para-amino-benzoic acid (PABA) which is present in many of the bacteriological media and is also present in large inocula.

Materials and Methods

The study was based on a separate evaluation of the isolates obtained from (1) the "urine service" and (2) the "routine service". The inoculation procedure for the susceptibility tests and the choice of antimicrobial discs was also slightly different, depending upon the group of organisms (Gram-positive or Gram-negative) obtained from each service. Thus the detailed procedure will be presented separately, based on the service, organism and types of antimicrobial agents (ie. sulfonamides).

The testing procedures within one's own laboratory must be evaluated critically in order to avoid wide variations[1]. Furthermore, it should be noted that the data obtained by microbiologists from different hospitals may show even greater variation due to the differences in the choice of media, quantity of inoculum, possible pre-incubation of inoculated plates or variations due to the drying time previous to the addition of discs.

There is also some controversy with regards to the disc concentrations (ie. low and/or high potency) which should be employed. However, all aspects having been considered, there has been greater uniformity in susceptibility tests since 1958 when the United States Food and Drug Administration[2] required the manufacturers of discs to produce a more uniform product. Other suggestions to improve the performance and interpretation of disc susceptibility testing has been reported by this author[3] and other investigators.[4-8]

A. *Procedure for susceptibility tests performed on the isolates obtained from Routine Service.*

I. The method of picking colonies and inoculating plates for susceptibility testing was the same for both the Gram-positive and Gram-negative organisms. The inoculum in 1.0 ml of broth was *undiluted* in contrast to that described in part B for the *Urine Service* which was *diluted* for the Gram-negative. *Procedure* for both Gram-negative and Gram-positive organisms:

1. Two to five colonies of the organism were picked from the primary or secondary plate with a sterile cotton swab and emulsified in 1.0 ml of sterile Trypticase-Soy Broth (Baltimore Biological Laboratory — BBL).
2. Excess liquid is removed from the swab by pressing it against the side of the tube.
3. The appropriate susceptibility test medium (i.e., 5 per cent Sheep Blood Agar — SBA) was inoculated by streaking the swab, which was previously saturated with organisms, over the surface of the plate, taking care to spread the inoculum evenly.
4. The sulfonamide discs were placed on Sulfonamide Resistant (SR) agar (Difco) which had been previously inoculated as described above.
5. Chocolate Agar Plates (Difco) were inoculated when the more fastidious organisms, such as *Haemophilus sp., Neisseria meningitidis,* etc. were to be tested.

II. Antimicrobial test discs and their concentrations. The following discs were dispensed onto the plates according to the classification shown below.

A. Gram-positive organisms.

	Potency	
	Low	*High*
1. Penicillin (P)	2 units	10 units
2. Chloramphenicol (C)	5 mcg.	30 mcg.
3. Tetracycline (Te)	5 mcg.	30 mcg.
4. Erythromycin (E)	2 mcg.	15 mcg.
5. Ampicillin (PC)	2 mcg.	10 mcg.

The staphylococci were also tested with the following synthetic penicillins:

	One Concentration Only
1. Methicillin (SC) (DP)	5 mcg.
2. Oxacillin (OX) (PS)	1 mcg.

B. (i) Gram-negative organisms — SBA plate.

	Low	*High*
1. Chloramphenicol (C)	5 mcg.	30 mcg.
2. Tetracycline (Te)	5 mcg.	30 mcg.
3. Dihydrostreptomycin (DS)	2 mcg.	10 mcg.
4. Kanamycin (K)	5 mcg.	30 mcg.

(ii) Gram-negative organisms — SR plate

1. Colistin (Cl)	2 mcg.	10 mcg.
2. Ampicillin (PC)	2 mcg.	10 mcg.
3. Triple Sulfa (SSS) — Sulfadiazine — Sulfamethazine — Sulfamerazine	.25 mg.	1.0 mg.
4. Choice of other Sulfonamides: — sulfisoxazole, sulfamethoxypyridazine, sulfadimethoxine	.25 mg.	1.0 mg.

Other antimicrobial discs were added or substituted whenever the need arose. Among these agents were the following: Novobiocin, Ristocetin, Vancomycin, etc.

C. Other new Antimicrobial Agents were included on special request.

1. Cephalothin (KF) Broad Spectrum, one concentration, 30 mcg.
2. Lincomycin (L) Gram-positive Spectrum — one concentration, 2 mcg.
3. Naldixic Acid (NA) Gram-Negative Spectrum — high and low concentrations, 30 mcg. and 5 mcg.

The inoculated agar-disc plates were incubated at 37° C. under aerobic, anaerobic or CO_2 atmosphere, as deemed necessary.

III. Interpretation of Antimicrobial Susceptibility Patterns. The zone sizes below are only approximate values (+ or —2 mm. or more) since caliper readinsg were *not* routinely used.

Five degrees of susceptibility were reported.

1. Very sensitive (VS) — Large zones of inhibition around both high and low potency discs.
   High = greater than 20 mm. in diameter
   Low = 15 — 18 mm. in diameter
2. Moderately sensitive (MS) — Large zone of inhibition around high concentration disc and a medium to slight zone around the low disc.
   High = 16 — 20 mm. diameter
   Low = 10 — 15 mm. diameter
3. Slightly sensitive (SS) — Fair size zone around high disc and no zone around low disc.
   High = 10-20 mm. in diameter
   Low = no zone
4. Sensitive (S) — When only one disc concentration is used, the organism is reported as being either sensitive or resistant to the antibiotic, according to the presence or absence of an inhibition zone around the single disc.
5. Resistant (R) — Organisms are said to be resistant to the antibiotic when there is no zone of inhibition around both the high and low concentration disc.

B. *Procedure for susceptibility tests performed on the isolates obtained from the Urine Service. The inoculum of Gram-negative organisms was diluted because the PABA, in high concentrations would antagonize the action of the sulfonamides.*

I. (i) Procedure for the inoculation of Gram-negative organisms:

1. Two to five colonies of the organism are picked from the plate with a sterile cotton swab and emulsified in 1.0 ml. of trypticase Soy Broth.
2. Surplus liquid is expressed against the sides of the trypticase Soy Broth.
3. The swab is then emulsified in 9 ml. of sterile saline.
4. Surplus liquid is again removed by expressing against the side of the tube containg the saline.
5. The same swab is emulsified in a second tube containing 9 cc. of sterile saline.
6. Excess liquid is again removed by depressing the swab against the sides of the tube. The swab is then used to inoculate the respective plate (SBA or SR media).

(ii) Procedure for the inoculation of Gram-positive organisms:

This was essentially the same as that described in part A, section 1. That is the inoculum was usually undiluted for the Gram-positives since overgrowth in the zone of inhibition was usually not a problem.

II. The choice of antimicrobial test discs was somewhat different for the isolates from the *Urine* specimens because the treatment programs were frequently somewhat different.

A. Antimicrobial agents for Gram-Negative Organisms (SBA plate).

	Low	*High*
1. Kanamycin (K)	5 mcg.	30 mcg.
2. Chloramphenicol (C)	5 mcg.	30 mcg.
3. Tetracycline (Te)	5 mcg.	30 mcg.
4. Dihydrostreptomycin (DS)	2 mcg.	10 mcg.
5. Ampicillin (PC)	2 mcg.	10 mcg.
6. Colistin (CL) or	2 mcg.	10 mcg.
7. Polymyxin-B (PB)	50 mcg.	300 mcg.
	One Concentration	
8. Nitrofurantoin	100 mcg.	
9. Methenamine Mandelate (M)	3 mg.	

B. Antimicrobial Agents for Gram-Negative Organisms (SR plate).

	Low	*High*
1. Sulfisoxazole (G)	0.25 mg.	1.0 mg.
2. Sulfamethoxypyridazine (KY)	0.25 mg.	1.0 mg.
3. Triple Sulfa (SSS)	0.25 mg.	1.0 mg.
4. Sulfadimethoxine (Md)	0.25 mg.	1.0 mg.

C. Antimicrobial Agents for the Gram-Positive Organisms.

	Low	*High*
1. Penicillin	2 mcg.	10 mcg.
2. Chloramphenicol	5 mcg.	30 mcg.
3. Tetracycline	5 mcg.	30 mcg.
4. Erythromycin	2 mcg.	15 mcg.
5. Novobiocin	5 mcg.	30 mcg.
6. Vancomycin	5 mcg.	30 mcg.
	One Concentration	
7. Methicillin	5 mcg.	
8. Oxacillin	1 mcg.	

III. Interpretation of the Susceptibility Patterns. The readings were performed as discussed in detail in part A, section III dealing with the isolates from the *Routine Service*.

This survey consisted of the antibacterial spectra of 23 agents which were tested against 20 different groups of organisms. Studies of this type require the effort and patience of many bacteriologists as well as the personnel (see acknowledgement) in one of our computer areas. The project consisted of the (1) collection of data from the original bacteriological ledgers for 1962, 1963 and 1964 was undertaken by recording the daily antibacterial spectra for each group of organisms. (2) then this formation was summarized on a monthly and yearly basis for each of the three years. (3) this data was then rechecked by a second technologist before transferring it to punch cards in preparation for processing by the #1620 IBM computer. Furthermore, the yearly totals and percentages were processed by the computer in two ways. Firstly — the antibacterial spectrum of 23 agents was listed according to the individual group of organisms and secondly — the 20 organisms was evaluated according to their susceptibility to each antibacterial agent.

Table I

LEGEND FOR ABREVIATIONS APPEARING IN TABLES

Codes For Antibiotics:

Cl = Colistin
C = Chloramphenicol
DS = Dihydrostreptomycin
Te = Tetracycline
K = Kanamycin
AM = Ampicillin

Codes For Sulfonamides:

G = Sulfisoxazole
Ky = Sulfamethoxypyridazine
SD = Sulfadiazine
SSS = Triple Sulfa — combination of Sulfadiazine, Sulfamethazine and Sulfamerazine

Codes For Chemicals:

Fd = Nitrofurantoin
M = Methenamine Mendelate
NA = Naldixic Acid

Others:

VS = Very Sensitive
MS = Moderately Sensitive
SS = Slightly Sensitive
S = Sensitive
R = Resistant
T = Total Number of Organisms.

Results

The data reported herein will consist of the antimicrobial spectra of the more commonly isolated organisms. The latter are divided into two categories according to the type of specimen from which they were isolated — namely, from either (1) 'routine' specimens (i.e., exudates, fluids, sputa, throats, tissues, etc.), or (2) 'urine' specimens. The reader can evaluate and interpret the data more readily by first examining Table I, which defines the abbreviations appearing in subsequent tables. The data on each of the gram-negative and gram-positive group of organisms will be presented separately.

Summary of the Bacterial Susceptibility Patterns.

A. *Gram-negative organisms.*

1. *Coliforms.* This group consisted chiefly of the members of the genus *Escherichia,* but included strains belonging to the *Aerobacter* group, as well as organisms which had an intermediate type of IMViC reaction. The data in Table II shows the *in vitro* effectiveness of four antibiotics against these organisms. Note the inhibitory effect of kanamycin and colistin sulfate against a very large percentage of all strains which were tested during the three year period. Chloramphenicol usually inhibited more than 90 per cent of the specimens, as compared to 70 to 80 per cent susceptibility to ampicillin, dihydrostreptomycin and tetracycline.

Table II

EFFECTIVENESS OF FOUR ANTIBIOTICS AGAINST
MEMBERS OF THE COLIFORM GROUP ISOLATED FROM THE "URINE SERVICE".

	1962								1963								1964							
	VS		MS		SS		R		VS		MS		SS		R		VS		MS		SS		R	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
K	216	80.5	28	10.4	13	4.8	11	4.1	997	94.9	23	2.1	14	1.3	16	1.5	2103	97.2	10	0.4	12	0.5	38	1.7
Cl	24	77.4	2	6.4	2	6.4	3	9.6	423	84.2	14	2.7	48	9.5	17	3.3	1985	93.3	44	2.0	58	2.7	39	1.8
C	1350	77.4	101	5.8	72	4.1	213	12.2	1970	85.8	73	3.1	63	2.7	188	8.1	2291	86.6	73	2.7	107	4.0	172	6.5
Te	366	21.5	334	14.0	343	20.1	659	38.7	1044	47.0	319	14.3	255	11.3	601	27.0	1176	44.5	339	12.8	400	15.1	726	27.4

Table III

EFFECTIVENESS OF FOUR SULFA DRUGS AGAINST
MEMBERS OF THE COLIFORM GROUP ISOLATED FROM THE "URINE SERVICE".

	1962								1963								1964							
	VS		MS		SS		R		VS		MS		SS		R		VS		MS		SS		R	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
G	480	64.3	10	1.3	2	0.2	254	34.0	258	70.8	2	0.5	3	0.8	101	27.7	1591	75.1	14	0.6	2	0	510	24.0
SSS	468	63.5	11	1.4	6	0.8	252	34.1	241	71.7	4	1.1	1	0.2	90	26.7	2	66.6	0	0.0	0	0	1	33.3
SD	480	63.4	10	1.3	6	0.7	261	34.4	258	70.8	4	1.0	2	0.5	100	27.4	37	67.2	0	0.0	0	0	18	32.7
Ky	467	63.1	11	1.4	9	1.2	252	34.1	240	72.5	1	0.3	6	1.8	84	25.3	0	0.0	0	0.0	0	0	0	0.0

The sulfonamides, which are so widely used in the treatment of urinary tract infections, usually inhibited between 65 to 75 per cent of the coliform strains tested (see Table III). The data in this table shows the three sulfonamides, as well as triple sulfa, equally effective in terms of per cent of inhibitory activity.

The results in Table IV show the effectiveness of methenamine mandelate and nitrofurantoin against the coliforms. The greater effectiveness of methenamine mandelate, in 1963 and 1964, may be due to a difference in performance of the discs. This aspect of the data is under study. The effect of the newer chemical, known as naldixic acid, has not been investigated in sufficient detail as yet, and, thus, the percentages cannot be presented in this study.

It was of some interest to compare the inhibitory effect of two high molecular weight compounds — namely, colistin sulfate and polymyxin-B, against both 'routine' and 'urine' isolates. It will be noted that more than 90 per cent of the coliform strains were susceptible to either drug, irregardless of the source (see Table V).

2. *Pseudomonas.* Members of the *Pseudomonas* are frequently resistant to most antimicrobial agents, but very susceptible to either colistin sulfate or polymyxin-B (see Table V). A careful review of the susceptibility patterns for the coliforms against these two drugs shows no significant difference in their effectiveness during the three year period.

The effectiveness of colistin sulfate against the *Pseudomonas* is compared with three other antibiotics in Table VI. It can be seen that chloramphenicol and kanamycin usually inhibit approximately 50 per cent of the strains, as was the case for dihydrostreptomycin, whereas ampicillin and tetracycline are frequently ineffective.

The sulfonamides usually inhibited 50 per cent of the strains of *Pseudomonas,* as is shown in Table VII. Again, as was the case with the coliforms, the four sulfonamides usually demonstrated comparable effectiveness against the *Pseudomonas.*

Methenamine mandelate inhibited 83 to 94 per cent of the *Pseudomonas* strains, whereas nitrofurantoin was rarely effective against these organisms (see Table VIII). Sufficient experience with naldixic acid has not been accumulated at this stage to quote percentages.

Table IV

EFFECTIVENESS OF TWO CHEMICALS AGAINST
MEMBERS OF THE COLIFORM GROUP ISOLATED FROM THE "URINE SERVICE".

	1962				1963				1964			
	S		R		S		R		S		R	
	T	%	T	%	T	%	T	%	T	%	T	%
M	1535	89.2	193	10.7	2170	95.9	91	4.0	2370	98.8	28	1.1
Fd	1501	88.6	184	11.3	2143	95.3	104	4.6	2530	96.3	97	3.6

Table V

EFFECTIVENESS OF COLISTIN AND POLYMYXIN B ON COLIFORM AND *PSEUDOMONAS*

Organism	Source	Year	COLISTIN				POLYMYXIN B			
			Sens.	% Sens.	Res.	% Res.	Sens.	% Sens.	Res.	% Res.
Coliforms	Urines	1962	28	90.4	3	9.6	235	95.6	11	4.4
		1963	485	96.7	17	3.3	848	98.2	16	1.8
		1964	2087	98.2	39	1.8	2099	98.4	35	1.6
	Routines	1962	2	100.0	0	0.0	401	89.6	47	10.4
		1963	86	96.7	3	3.3	306	92.8	24	7.2
		1964	603	94.7	34	5.3	3	100.0	0	0.0
Pseudomonas	Urines	1962	19	95.0	1	5.0	118	93.7	8	6.3
		1963	53	89.9	6	10.1	114	100.0	0	0.0
		1964	185	97.4	5	2.6	186	96.9	6	3.1
	Routines	1962	11	84.7	2	15.3	249	98.1	5	1.9
		1963	150	97.5	4	2.5	196	99.0	2	1.0
		1964	415	97.7	10	2.3	18	94.8	1	5.2

Table VI

EFFECTIVENESS OF FOUR ANTIBIOTICS AGAINST
MEMBERS OF THE *PSEUDOMONAS* GROUP ISOLATED FROM THE "URINE SERVICE".

	1962								1963								1964							
	VS		MS		SS		R		VS		MS		SS		R		VS		MS		SS		R	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
Cl	16	80.0	1	5.0	2	10.0	1	5.0	24	40.6	2	3.3	27	45.7	6	10.1	135	71.0	5	2.6	45	23.6	5	2.6
C	8	5.4	15	10.2	53	36.3	70	47.9	9	5.1	12	7.4	61	34.8	92	52.5	12	6.4	5	2.6	75	40.1	93	50.8
K	18	12.4	11	8.0	49	35.7	60	43.7	4	2.6	9	5.9	73	48.0	66	43.4	8	4.3	6	3.2	105	57.6	63	34.6
Te	8	5.5	5	3.4	10	6.8	122	84.1	2	1.1	5	2.8	29	16.6	138	79.3	8	4.2	2	1.0	34	18.1	143	76.4

Table VII

EFFECTIVENESS OF FOUR SULFA DRUGS AGAINST
MEMBERS OF THE PSEUDOMONAS GROUP ISOLATED FROM THE "URINE SERVICE".

	1962								1963								1964							
	VS		MS		SS		R		VS		MS		SS		R		VS		MS		SS		R	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
SD	42	51.2	5	6.0	4	4.8	31	37.8	24	44.4	1	1.8	4	7.4	25	46.2	7	63.6	1	9.0	0	0.0	3	27.2
SSS	31	37.8	11	13.4	8	9.7	32	39.0	21	47.7	1	2.2	3	6.8	19	43.1	2	18.1	0	0.0	0	0.0	9	81.8
Ky	25	30.4	10	12.1	8	9.7	34	47.5	16	37.2	1	2.3	2	4.6	24	55.8	0	0.0	0	0.0	0	0.0	0	0.0
G	26	32.0	8	9.8	9	11.1	38	46.9	16	29.0	1	1.8	7	12.7	31	56.3	36	25.8	7	5.0	14	10.0	82	58.9

Table VIII

EFFECTIVENESS OF TWO CHEMICALS AGAINST MEMBERS OF THE PSEUDOMONAS GROUP ISOLATED FROM THE "URINE SERVICE".

	1962				1963				1964			
	S		R		S		R		S		R	
	T	%	T	%	T	%	T	%	T	%	T	%
M	117	82.9	24	17.0	152	88.9	19	11.0	179	93.6	12	6.2
Fd	14	10.6	117	89.3	4	2.5	150	97.4	6	3.1	183	96.8

3. *Proteus*. A summary of the antimicrobial spectra for the *Proteus* group is given in Tables IX, X and XI. The data in Table IX shows the great *in vitro* effectiveness (98 per cent susceptibility) of kanamycin against these organisms. Chloramphenicol inhibited from 85 to 90 per cent of the *Proteus* strains, but not as effectively as kanamycin. One of the newer synthetic penicillins — ampicillin, which has only been used during the past year, has proven to be effective against *Proteus*, but it has not been tabulated because only one year of data is available. Tetracycline and colistin sulfate were relatively ineffective against these organisms. Inspection of Tables X and XI shows that the sulfonamides, methenamine mandelate and nitrofurantoin may be inhibitory against many strains of *Proteus* and, thus, should be considered for *in vitro* and *in vivo* testing wherever the circumstances so require.

4. *Paracolobactrum*. The three year summary of the antibacterial spectrum against the late lactose fermenters, frequently grouped by some workers in the genus Paracolobactrum (or as *Escherichia freundii* or *Citrobacter* by others), is shown in Table XII. The data demonstrates the variability in antibacterial spectra. Chloramphenicol, triple sulfa and Furadantin were frequently very effective. The unpublished data on *Paracolobactrum* strains isolated from the urine service showed that colistin sulfate, dihydrostreptomycin, kanamycin, methenamine mandelate and sulfisoxazole were, also, very inhibitory to these organisms. Ampicillin and the tetracyclines inhibited from 50 to 75 per cent of the strains.

5. *Haemophilus*. The susceptibility pattern of the *Haemophilus* species is shown in Table XIII. Chloramphenicol was the most effective drug when large numbers were tested. Penicillin, tetracycline and the sulfonamides were, also, frequently effective. Ampicillin, erythromycin and kanamycin showed considerable inhibitory effect against this group of organisms. No experience has been gained with methenamine mandelate or nitrofurantoin against these isolates.

B. *Gram-positive organisms.*

1. *Diplococcus pneumoniae*. The antibacterial spectra of the pneumococci is presented in Table XIV. These organisms were routinely susceptible to ampicillin, chloramphenicol, erythromycin and penicillin. Many other antimicrobial agents, such

Table IX
EFFECTIVENESS OF FOUR ANTIBIOTICS AGAINST
MEMBERS OF THE *PROTEUS* GROUP ISOLATED FROM THE "URINE SERVICE".

	1962								1963								1964							
	VS		MS		SS		R		VS		MS		SS		R		VS		MS		SS		R	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
K	204	85.7	24	10.0	6	2.5	4	1.6	409	97.6	4	0.9	5	1.1	1	0.2	606	94.6	11	1.7	10	1.5	13	2.0
C	231	53.4	89	20.6	59	13.6	53	12.2	386	65.2	61	10.3	86	14.5	59	9.9	435	57.0	86	11.2	152	19.9	89	11.6
Te	39	9.1	12	2.8	8	1.8	365	86.0	34	5.8	7	1.1	20	3.4	523	89.5	56	7.8	13	1.8	21	2.9	621	87.3
Cl	4	40.0	0	0.0	0	0.0	6	60.0	12	9.0	3	2.2	3	2.2	115	86.4	33	4.8	4	0.5	4	0.5	645	94.0

Table X
EFFECTIVENESS OF FOUR SULFA DRUGS AGAINST
MEMBERS OF THE PROTEUS GROUP ISOLATED FROM THE "URINE SERVICE".

	1962								1963								1964							
	VS		MS		SS		R		VS		MS		SS		R		VS		MS		SS		R	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
SSS	134	75.7	5	2.8	1	0.5	37	20.9	67	77.9	0	0.0	0	0.0	19	22.0	0	0.0	0	0.0	0	0.0	0	0.0
Ky	131	75.2	5	2.8	1	0.5	37	21.2	76	79.1	0	0.0	0	0.0	20	20.8	1	33.3	0	0.0	0	0.0	2	66.6
G	132	75.0	5	2.8	3	1.7	36	20.4	75	75.0	0	1.0	0	0.0	24	24.0	511	82.6	5	0.8	3	0.4	99	16.0
SD	134	75.7	5	2.8	1	0.5	37	20.9	74	74.7	0	0.0	1	1.0	24	24.2	9	64.2	0	0.0	0	0.0	5	35.7

Table XI
EFFECTIVENESS OF TWO CHEMICALS AGAINST
MEMBERS OF THE PROTEUS GROUP ISOLATED FROM THE "URINE SERVICE".

	1962								1963								1964							
	VS		MS		SS		R		VS		MS		SS		R		VS		MS		SS		R	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
M	0	0	251	59.3	0	0	172	40.6	292	50.4	160	27.6	0	0	127	21.9	206	80.3	0	0	0	0	147	19.6
Fd	0	0	102	24.3	0	0	317	75.6	176	30.8	76	13.3	0	0	319	55.8	436	57.7	0	0	0	0	319	42.2

Table XII

EFFECTIVENESS* OF NINE ANTIBACTERIAL AGENTS AGAINST MEMBERS OF THE PARACOLON GROUP, ISOLATED FROM THE "ROUTINE SERVICE"

1962

	Chl		Te		P		Fd		M		G		SSS		SD		Ky	
	T	%*	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
VS	51	86.4	22	37.2	1	1.7	0	0.0	0	0	2	100	2	100	2	100	2	100
MS	3	5.0	17	28.8	2	3.4	47	81.0	0	0	0	0	0	0	0	0	0	0
SS	2	3.3	8	13.5	4	6.8	0	0.0	0	0	0	0	0	0	0	0	0	0
R	3	5.0	12	20.3	51	87.9	11	18.9	0	0	0	0	0	0	0	0	0	0
T	59	—	59	—	58	—	58	—	0	—	2	—	2	—	2	—	2	—

1963

	Chl		Te		P		Fd		M		G		SSS		SD		Ky	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
VS	44	86.2	23	46.0	1	4.1	0	0	0	0	0	0	0	0	0	0	0	0
MS	1	1.9	9	18.0	0	0.0	14	70	0	0	0	0	0	0	0	0	0	0
SS	5	9.8	14	28.0	1	4.1	0	0	0	0	0	0	0	0	0	0	0	0
R	1	1.9	4	8.0	22	91.6	6	30	0	0	0	0	0	0	0	0	0	0
T	51	—	50	—	24	—	20	—	0	—	0	—	0	—	0	—	0	—

1964

	Chl		Te		P		Fd		M		G		SSS		SD		Ky	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
VS	110	74.3	46	31.2	0	0	0	0	0	0	0	0	81	84.3	0	0	0	0
MS	12	8.1	26	17.6	0	0	0	0	0	0	0	0	1	1.0	0	0	0	0
SS	10	6.7	30	20.4	0	0	0	0	0	0	0	0	5	5.2	0	0	0	0
R	16	10.8	45	30.6	0	0	0	0	0	0	0	0	9	9.3	0	0	0	0
T	148	—	147	—	0	0	0	—	0	—	0	—	96	—	0	—	0	—

* % = per cent susceptible of the total (T) as indicated for each antimicrobial agent listed above.

Table XIII

EFFECTIVENESS OF NINE ANTIBACTERIAL AGENTS AGAINST MEMBERS OF THE HAEMOPHILUS GROUP, ISOLATED FROM THE "ROUTINE SERVICE"

1962

	Chl		Te		P		Fd		M		G		SSS		SD		Ky	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
VS	48	97.9	41	85.4	41	83.6	0	0	0	0	1	100	1	100	1	100	1	100
MS	1	2.0	5	10.4	4	8.1	0	0	0	0	0	0	0	0	0	0	0	0
SS	0	0.0	0	0.0	2	4.0	0	0	0	0	0	0	0	0	0	0	0	0
R	0	0.0	2	4.1	2	4.0	0	0	0	0	0	0	0	0	0	0	0	0
T	49	—	48	—	49	—	0	—	0	—	1	—	1	—	1	—	1	—

1964

	Chl		Te		P		Fd		M		G		SSS		SD		Ky	
	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%	T	%
VS	149	98.6	138	91.3	107	71.3	0	0	0	0	0	0	12	60.0	0	0	0	0
MS	0	0.0	3	1.9	12	8.0	0	0	0	0	0	0	0	0.0	0	0	0	0
SS	0	0.0	7	4.6	24	16.0	0	0	0	0	0	0	0	0.0	0	0	0	0
R	2	1.3	3	1.9	7	4.6	0	0	0	0	1	100	8	40.0	1	100	1	100
T	151	—	151	—	150	—	0	—	0	—	1	—	20	—	1	—	1	—

as oxacillin, prostaphlin and the sulfonamides were frequently inhibitory to these organisms. Thus, the pneumococci have remained susceptible to a great many agents.

2. *Staphylococcus pyogenes.*

(a) *Var. albus.* The data in Table XV summarizes the inhibitory effect of four commonly used antimicrobial agents against *S. albus.* Approximately 90 per cent of the *S. albus* strains were susceptible to chloramphenicol and erythromycin. Penicillin G was slightly more effective (60 to 77 per cent of strains) than tetracycline (53 to 61 per cent of strains). Two synthetic penicillins (not listed in this Table) — methicillin and oxacillin — were inhibitory to some 98 per cent of these organisms. Ampicillin inhibited between 80 to 90 per cent of the *S. albus* strains.

Table XIV
EFFECTIVENESS OF FIVE ANTIBACTERIAL AGENTS AGAINST MEMBERS OF THE PNEUMOCOCCI GROUP ISOLATED FROM THE "ROUTINE SERVICE"

1962

	P		C		Te		AM		E	
	T	%	T	%	T	%	T	%	T	%
VS	60	98.3	61	100	59	96.7	0	0	44	97.7
MS	1	1.6	0	0	1	1.6	0	0	1	2.2
SS	0	0.0	0	0	1	1.6	0	0	0	0.0
R	0	0.0	0	0	0	0.0	0	0	0	0.0
T	61	—	61	—	61	—	0	—	45	—

1963

	P		C		Te		AM		E	
	T	%	T	%	T	%	T	%	T	%
VS	36	100	34	100	33	100	0	0	33	100
MS	0	0	0	0	0	0	0	0	0	0
SS	0	0	0	0	0	0	0	0	0	0
R	0	0	0	0	0	0	0	0	0	0
T	36	—	34	—	33	—	0	—		

1964

	P		C		Te		AM		E	
	T	%	T	%	T	%	T	%	T	%
VS	116	99.1	117	99.1	111	94.0	54	100	117	100
MS	0	0.0	0	0.0	1	0.8	0	0	0	0
SS	1	0.8	0	0.0	4	3.3	0	0	0	0
R	0	0.0	1	0.8	2	1.6	0	0	0	0
T	117	—	118	—	118	—	54	—	117	—

Table XV

SENSITIVITY OF *STAPHYLOCOCCUS ALBUS* TO TETRACYCLINE, ERYTHROMYCIN, PENICILLIN AND CHLORAMPHENICOL.

Source	Year	Tetracycline					Erythromycin				
		Total No.	No. Sens.	% Sens.	No. Res.	% Res.	Total No.	No. Sens.	% Sens.	No. Res.	% Res.
Urines	1962	171	94	54.9	77	45.1	128	102	79.6	26	20.4
	1963	208	126	60.5	82	39.5	173	150	86.7	23	13.3
	1964	245	131	53.4	114	46.6	267	230	86.1	37	13.9
Routines	1962	280	191	68.2	89	31.8	202	190	94.0	12	6.0
	1963	349	217	62.1	132	37.9	335	307	91.3	28	8.7
	1964	443	273	61.6	170	38.4	442	397	89.8	45	10.2
		Penicillin					Chloramphenicol				
Urines	1962	169	103	60.8	66	39.2	168	148	88.0	20	12.0
	1963	208	161	77.4	47	22.6	214	197	92.0	17	8.0
	1964	243	171	70.3	72	29.7	245	222	90.6	23	9.4
Routines	1962	281	207	73.6	74	26.4	283	260	91.8	23	8.2
	1963	349	234	67.2	115	32.8	349	326	93.4	23	6.6
	1964	434	294	67.7	140	32.3	444	410	92.3	34	7.7

(b) *Var. aureus.* Approximately 50 per cent of the *S. aureus* strains were susceptible to penicillin G and ampicillin. Tetracycline inhibited some 70 per cent of the strains, as compared to 83 to 90 per cent susceptibility to erythromycin (see Table XVI). The majority of strains were inhibited by one of the following chloramphenicol, methicillin and oxacillin.

3. *Streptococcus.*

(a) *Beta streptococcus.* The large majority of the strains reported in Table XVII belong to the Lancefield Group A. The strains of this group were routinely susceptible to penicillin G and usually susceptible to erythromycin. However, the reader should note that from 6 to 18 per cent may be resistant to tetracycline (see Table XVII). The *in vitro* experiences demonstrated the marked inhibitory effect which ampicillin and chloramphenicol have against these organisms.

(b) *Enterococcus.* Penicillin G inhibited 87 to 98 per cent of the enterococcal strains, as compared to 92 to 99 per cent effectiveness for chloramphenicol and erythromycin (see Table XVIII). Ampicillin was, also, very effective against the enterococci. Some 48 to 62 per cent of the enterococcal strains were resistant to tetracycline.

Discussion

This study was directed primarily at the susceptibility and resistance patterns of the more pathogenic strains isolated in the routine diagnostic bacteriology laboratory during the past three years. The data shows that the greatest number of resistant organisms were those whose natural habitat in humans is the gastrointestinal tract. These consist most often of the gram-negative genera, such as *Aerobacter, Escherichia, Paracolonbactrum (E. freundi* or *Citrobacter), Proteus* and *Pseudomonas.* The data shows that some antibacterial agents which should be considered for *in vitro* testing of the gram-negatives are the following: Ampicillin, chloramphenicol, colistin sulfate, dihydrostreptomycin, kanamycin, polymyxin-B, sulfonamides, etc. The choices can be further reduced by knowing the general types of antibacterial spectrum to expect for each genus of micro-organism. For instance, both clinicians and clinical microbiologist should know that colistin sulfate and polymyxin-B are usually effective against the *Pseudomonas,* but not the *Proteus* group.

The response of the more common gram-positive organisms to the antibiotics has been uniformly good except for the members of the genus *Staphylococcus.* The susceptibility of *Diplococcus pneumoniae* and *Streptococcus pyogenes* (Lancefield group A) has been very high to penicillin, erythromycin and chloramphenicol, but more resistant to tetracycline. The clinical import of these matters have been discussed by Isenberg.[8] It is important for the physician to obtain an antibiogram on the above organisms, if they are responsible for disease in a penicillin-sensitive patient. The enterococci, also, demonstrated considerable resistance to penicillin and tetracycline, thus requiring antibiograms before deciding on a therapeutic program.

Table XVI

SENSITIVITY OF *STAPHYLOCOCCUS AUREUS* TO TETRACYCLINE, ERYTHROMYCIN, PENICILLIN AND CHLORAMPHENICOL.

Source	Year	Tetracycline					Erythromycin				
		Total No.	No. Sens.	% Sens.	No. Res.	% Res.	Total No.	No. Sens.	% Sens.	No. Res.	% Res.
Routines	1962	1246	881	70.7	365	29.3	1218	1093	89.7	125	10.3
	1963	1142	760	66.5	382	33.5	1158	951	82.1	207	17.9
	1964	1357	928	68.3	429	31.7	1376	1194	86.7	182	13.3
		Penicillin					Chloramphenicol				
Routines	1962	1242	640	51.5	602	48.5	1250	1214	97.1	36	2.9
	1963	1144	572	50.0	572	50.0	1148	1134	98.7	14	1.3
	1964	1378	631	45.7	747	54.3	1379	1344	97.4	35	2.6

Table XVII

SENSITIVITY OF BETA STREPTOCOCCI TO TETRACYCLINE AND ERYTHROMYCIN FROM 1962 TO 1964

Source	Year	Tetracycline					Erythromycin				
		Total No.	No. Sens.	% Sens.	No. Res.	% Res.	Total No.	No. Sens.	% Sens.	No. Res.	% Res.
Urines	1962	43	35	81.4	8	18.6	37	37	100	0	0
	1963	69	65	94.2	4	5.8	68	66	97.1	2	2.9
	1964	32	29	90.7	3	9.3	32	32	100	0	0
Routines	1962	328	308	94.0	20	6.0	274	272	99.3	2	0.7
	1963	275	257	93.0	18	7.0	268	267	99.3	1	0.7
	1964	446	414	91.0	41	9.0	448	448	100	0	0

The synthetic penicillins (i.e. methicillin, oxacillin), as well as chloramphenicol were highly effective against *S. aureus* and *S. albus*. The lower order (50 per cent of the strains) of staphylococcal susceptibility to penicillin G was expected because a large percentage of the cultures were obtained from "in-patients" as well as "out-patients".

The role of the clinical bacteriologist and/or the specialist of infectious diseases is two-fold. They should understand the general response of a particular bacterium to specific agents.[8] This can guide the physician to use the best therapeutic agent. The second and very important function of the bacteriologist, in relation to antibiotic

Table XVIII

EFFECTIVENESS* OF FIVE ANTIBACTERIAL AGENTS AGAINST MEMBERS OF THE ENTEROCOCCI GROUP ISOLATED FROM THE "URINE SERVICE"

1962

	P		C		Te		AM		E	
	T	%*	T	%	T	%	T	%	T	%
VS	113	77.3	110	75.8	50	34.4	0	0	89	88.1
MS	7	4.7	17	11.7	6	4.1	0	0	3	2.9
SS	6	4.1	6	4.1	18	12.4	0	0	5	4.9
R	20	13.6	12	8.2	71	48.9	0	0	4	3.9
T	146	—	145	—	145	—	0	—	101	—

1963

	P		C		Te		AM		E	
	T	%	T	%	T	%	T	%	T	%
VS	140	81.3	126	72.4	61	35.4	0	0	145	87.3
MS	7	4.0	11	6.3	4	2.3	0	0	7	4.2
SS	17	9.8	28	16.0	16	9.3	0	0	6	3.6
R	8	4.6	9	5.1	91	52.9	0	0	8	4.8
T	172	—	174	—	172	—	0	—	166	—

1964

	P		C		Te		AM		E	
	T	%	T	%	T	%	T	%	T	%
VS	189	86.3	159	72.6	51	23.2	165	91.6	197	90.7
MS	8	3.6	24	10.9	3	1.3	2	1.1	5	2.3
SS	18	8.2	34	15.5	28	12.7	11	6.1	4	1.8
R	4	1.8	2	0.9	137	62.5	2	1.1	11	5.0
T	219	—	219	—	219	—	180	—	217	—

* % = per cent susceptible of the total listed in each column.

therapy, is his role in predicting the most likely causative agent by use of the Gram smear technique. Both physician and microbiologist should be fully aware that subsequent cultural findings may temper his original information.

It should, also, be emphasized that frequently the only "constant" of the bacteriologist is variability due to differences in technique, materials and/or the occurrence of persistors or mutants. Since the microbiologist deals with large populations of mirco-organisms, it is necessary in the more serious clinical conditions to perform replicate tests, such as performing both the disk and tube dilution procedures.

In conclusion, the author would like to stress the fact that *in vitro* susceptibility tests are a *guide* to therapy. If properly used, the *in vitro* antibacterial spectra of drugs can be extremely valuable in guiding the physician in his chemotherapeutic management of the 'infected patient'. The physician's general knowledge of the clinical effectiveness of certain drugs for specific microorganisms is also very helpful, as outlined in this paper.

Summary

A three year survey of the antibacterial spectra of 23 agents against 20 genera of micro-organisms has been processed on the #1620 IBM computer.

The data shows that the greater number of resistant organisms belong to the gram-negative group, such as *Aerobacter, Escherichia, Paracolobactrum (E. freundii* or *Citrobacter), Proteus* and *Pseudomonas. In vitro* antibacterial testing should be based on clinical and laboratory experience with the type of organism and the site of isolation. A survey of the data presented herein might serve as a guide to interested parties.

It is especially important to obtain antibiograms on *D. pneumoniae* and *Str. pyogenes* (Lancefield Group A) if the patient is penicillin-sensitive, because some of these strains are resistant to such antibiotics as tetracycline. The synthetic penicillins were highly effective against strains of both *S. aureus* and *S. albus.*

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