RELIABLE AND REALISTIC APPROACH TO SENSITIVITY TESTING

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S. Hafiz, N. Lyall, S. Punjwani, Shahida Q. Zaidi (Department of Microbiology, The Aga Khan University and University Hospital, Karachi.)

Abstract

Antibiotic testing by Disc was compared with Break-Point method using 535 clinical isolates; Disc sensitivity method was carried out in the routine way using the disc provided by pharmaceutical company. Break-point sensitivity was done by incorporating the substrate into solid medium, the results obtained showed greater resistance by Break-point which appears to be more realistic in the light of indisz criminate usage of antibiotic. The method is simple, cost effective, reliable and realistic (JPMA 39 ;94, 1989).

INTRODUCTION

Since the introduction of antibiotics in 1929, clinicians, for some time, were impressed by their success in the therapy of previously fatal infections but treatment failures later prompted the need for susceptibility testing and development of new antibiotics. The need for skilled laboratory control and testing of antimicrobial agents have never been greater than in the present day. The resistance pattern of common bacteria vary considerably usually in response to varying drug usage, made more complex by the introduction of semi-synthetic derivatives of an increasing number of antibiotics, among some of which cross resistance is no longer a foregone conclusion. The laboratory influences drug usage by the sensitivity tests it reports and, therefore, must see that these tests are applied to appropriate organisms and suitable drugs and by standard and reliable methods[1]. The disc diffusion tests most widely used for sensitivity testing by all laboratories in our country include Kirby Bauer Method (Federal Register, 1972) [2]. the ICS method[3] or the Stokes method. [4] All the methods have limitations and require control organisms, storage conditions. inoculum size, conditions for the storage of discs and methods of applications. Break-point method offers a solution to most of the problems and utilizes agar dilution method for antibiotic seasitivity testing, with sensitivity reported merely by observing growth or non growths[5] - This method is now popular. We have compared the routinely used disc sensitivity testing method with the Break-point method on local routine isolates.

MATERIAL AND METHODS

Clinical specimens received in the laboratory were processed according to standard recommended methods for the isolation of pathogen and routine sensitivity tests carried out. These were not compared until the end of the study. Discs: The discs used were of Penicillin 10 units, Ampicillin 25, Augmentin 30, MethicilIn 5, carbenicillin 100, Amoxacillin 25, Erythromycin 15, Chloramphenicol 30, Cotrimoxa-zole 25, Cephalothin 30, Cefotaxime 30, Gentamicin 10, Amikacin 30, Nitrofurantoin 300, Nalidixic acid 30, Pipimedic acid 50, Ofloxacin 30, Enoxacin 30 mcg per disc respectively, mostly supplied by the pharmaceutical companies.

Break-Point Sensitivity Test

Media: The basal medium used for antibiotic sensitivity testing was oxoid sensitest agar in most cases, and in other specific media recommended for the growth of organisms were used. Agar concentration

was increased to 2% in order to stop swarming of organisms. Antibiotic dilutions were made in ug/ml Penicillin 0.25, Ampidillin 32,8,1, Augmentin 32, 8, 1, Methicillin 8, Carbenicillin 256, 64, Arnoxacillin 32, 8, 1, Erythromycin 1, 0.25, Chloramphenicol 16, 4, Cotrimoxazole (40z8, 10:2, 25:0.5), Cephalothin 32, 8, 2, Cefotaxime 32, 8, 2, Gentamicin 8, 2, Amikacin 16, 4, Nitrofurantoin 32, Nalidixic acid 32, Pipimedic acid 32, Afloxacin and Enoxacin 10, 2, 025. Antibiotic solutions were made and diluted according to the method recommended by Anhalt and Washington If - Plates were packed and sealed in plastic bags, stored at 4°C and used within 2 weeks. Methicillin plates also contained 5% Sodium Chloride.

Culture: Clinical isolates were grown on optimum medium under optimum conditions, growth harvested and emulsified in Trypticase soya broth to give a colony count of 10^6 orgs/ml. 2 ml of suspension was transferred to curette used for multipoint inoculator (Denley). On each plate 20 organisms were inoculated including a control organisms of known sensitivity. Two plates containing no antibiotics were used as controls, one control plate inoculated before inoculations to antibiotic plates and the other at the end. Plates were incubated and results recorded after 24 hours of incubation in most cases for the presence of growth and no growth. Growth of all test organisms was checked on the two control plates before recording the sensitivity results.

RESULTS

TABLE I. Showing% Sensitive Pattern of Urinary Tract Infection Isolates.

Genus	Escherichia			Pseudomonas			Klebsiella			Proteus			Enterobacter			Salmonella			Citrobacter			Staphy-lococcus			Streptococcus		
ANTIBIOTIC	D	BP		D	BP		D	BP		D	BP		D	BP		D	BP		D	BP		D	BP		D	BP	
	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR
Penicillin	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	25	NIL	–	87.5	50	–
Ampicillin	27	9.25	9.25	NT	NIL	NIL	NIL	NIL	NIL	33.3	NIL	33.3	33.3	NIL	13.3	100	NIL	100	NIL	NIL	NIL	25	NIL	25	87.5	37.5	25
Augmentin	NT	NIL	16.6	NT	NIL	NIL	NT	NIL	NIL	NT	33.3	NIL	NT	7.14	NIL	NT	50	50	NT	NIL	NIL	100	50	25	100	62.5	–
Methicillin	NT	9.25	–	60	NIL	–	NT	4.16	–	NT	NIL	–	NT	NIL	–	NT	NIL	–	NT	33.3	–	100	50	–	50	57.1	–
Carbenicillin	NIL	21.1	1.9	33.3	30	NIL	NT	NIL	NIL	NT	66.6	NIL	NT	13.3	NIL	NT	100	–	NT	50	NIL	NT	75	NIL	NT	62.5	12.5
Amoxacillin	NT	13.2	5.6	NT	NIL	NIL	NT	NIL	NIL	NT	33.3	NIL	NT	14.2	NIL	NT	100	–	NT	NIL	NIL	NIL	75	NIL	NT	62.5	NIL
Erythromycin	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NIL	NT	NIL	NIL	66.6	50	–	100	42.8	14.2
Chloramphenicol	NT	1.8	18.5	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	33.3	NT	NIL	20	NT	NIL	100	NT	NIL	NIL	NT	NIL	NIL	NT	37.5	12.5
Cotrimoxazole	29.6	11.1	NIL	NT	NT	NT	12.5	NIL	NIL	66.6	NT	NT	42.5	NIL	NIL	100	100	NT	50	NIL	NIL	NIL	31	3.4	NT	NT	NT
Cephalothin	NT	46.2	27.7	NT	11.1	–	NT	18.1	9.09	NT	66.6	NIL	NT	40	20	NT	100	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	62.5	25
Cefotaxime	98.2	81.4	9.2	77.7	22.2	33.3	100	52.1	21.7	100	66.6	33.3	100	66.6	13.3	100	100	–	100	33.3	NIL	NT	75	25	NT	44.4	NIL
Gentamicin	96	50	33.3	90	40	NIL	43.4	31.8	13.6	100	NIL	66.6	80	40	20	100	100	–	NIL	NIL	NIL	66.6	50	25	55.5	NIL	50
Amikacin	97	45.6	44	100	33.3	44.4	100	34.7	47.8	100	33.3	33.3	100	25	62.5	100	100	–	NT	NIL	33.3	NT	50	50	NT	22.2	11.1
Nitrofurantoin	98.1	NIL	NIL	NIL	NIL	NIL	79	NIL	NIL	100	NIL	NIL	76.9	NIL	NIL	100	NIL	NIL	50	NIL	NIL	NT	NIL	NIL	NT	12.5	NIL
Nalidixic Acid	94.3	61.5	–	50	22.2	–	62.5	43.4	–	100	33.3	–	80	73.3	–	NT	100	–	50	50	–	NT	25	–	NT	NIL	–
Pipimedic Acid	93.3	81.4	–	100	33.3	–	91.6	50	–	NT	33.3	–	75	73.3	–	100	100	–	NT	NIL	–	NT	75	–	NT	NIL	–
Ofloxacin	NT	100	–	100	75	25	NT	75	25	NT	100	–	NT	100	–	NT	100	–	NT	100	–	100	100	–	100	85.7	14.3
Enoxacin	NT	84	16	NT	57.1	42.9	NT	66.6	33.3	NT	100	–	NT	86.6	13.4	NT	100	–	NT	100	–	NT	66.6	33.3	NT	14.2	57.1

KEY = D = Disc Sensitivity; BP = Break-Point Sensitivity; S = Sensitive; MR = Moderately Sensitive; NT = Not Tested; NIL = None Sensitive

Table 1 shows the results of common urinary tract pathogens and gives the percent sensitive to Discs

and Break-point to different antibiotics. In all 135 isolates were compared.

Isolates from various Sources Excluding U.T.I.

Acinetobacter			Shigella			Legionella			Branhamella			Neisseria			Staphylococcus			Streptococcus			Norcardia		
D	BP		D	BP		D	BP		D	BP		D	BP		D	BP		D	BP		D	BP	
S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR
NT	NIL	–	NT	NIL	–	NT	NIL	–	83.3	62.5	–	NT	50	–	13.8	13.0	–	78	76.1	–	NT	NT	–
25	NIL	–	NIL	NIL	NIL	NT	NIL	–	100	NIL	–	NT	50	–	16.4	12.2	10.5	92.8	47.6	14.2	NT	NT	–
NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	37.5	62.5	NT	50	NIL	94.3	20	20	100	52.9	41.1	NT	66.6	NIL
NT	60	–	NT	100	–	NT	100	–	NT	60	–	NT	56.6	–	79.4	56.6	–	62.5	85.7	–	NT	NT	–
NT	100	–	100	NIL	100	NT	75	25	NT	77.7	–	NT	100	–	100	68.8	1.1	NT	50	NIL	NT	50	NIL
NT	NIL	–	NT	NIL	NIL	NT	NIL	–	NT	37.5	62.5	NT	56.6	33.3	NIL	37.7	26.5	NT	29.4	29.4	NT	NT	–
NT	NIL	NIL	NT	NIL	NIL	100	100	–	100	60	10	NT	33	NIL	51.7	39.3	2.1	85	60	–	NT	66.6	–
20	NIL	20	NT	NIL	NIL	NIL	NIL	–	NT	20	20	NT	100	–	NIL	9.4	33.7	NT	38	14.2	NT	33.3	66.6
33.3	NIL	NIL	NIL	NIL	NIL	NT	NT	NT	NT	56.6	NIL	NT	NT	NT	NIL	11.3	11.3	NT	16.6	NIL	NT	NT	–
NT	20	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	60	20	NT	66.6	NIL	NT	55.4	9.7	NT	21	NIL	NT	33.3	33.3
60	NIL	60	100	50	NIL	NT	40	60	NT	70	NIL	NT	66.6	NIL	66.6	45.3	31.3	NT	76.1	4.7	NT	66.6	33.3
100	33.3	NIL	100	100	–	NT	NIL	NIL	NT	60	NIL	NT	50	50	78.5	52.5	18.5	60	61.9	9.5	NT	75	NIL
100	60	NIL	100	NIL	100	NT	NIL	NIL	NT	70	10	NT	100	–	100	37.5	35.2	NT	52.1	4.3	NT	100	–
NT	NIL	–	NT	NIL	–	NT	NIL	–	NT	20	–	NT	33.3	–	NT	2.2	–	NT	28.5	–	NT	50	–
NT	20	–	NT	50	–	NT	100	–	NT	70	–	NT	100	–	NT	18.8	–	NT	47.6	–	NT	50	–
NT	40	–	NT	50	–	NT	20	–	NT	80	–	NT	66.6	–	NT	22.9	–	NT	42.1	–	NT	50	–
NT	40	20	100	100	–	NT	NIL	75	NT	88.8	11.2	NT	75	NIL	100	48.3	38.2	100	38.7	48.3	NT	50	50
100	40	NIL	100	100	–	100	NIL	75	NT	38.8	NIL	NT	75	NIL	100	38.2	32.5	100	35.4	29	NT	100	–

In general it seems that disc sensitivity tests gave a higher percent sensitive to the antibiotics.

TABLE II. Showing % Sensitive Pattern of Clinical

GENUS	Escherichia			Pseudomonas			Klebsiella			Proteus			Enterobacter			Salmonella			Citrobacter		
	D	BP		D	BP		D	BP		D	BP		D	BP		D	BP		D	BP	
Antibiotics	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR	S	S	MR
Penicillin	NT	NIL	–	NT	NIL	–	NIL	NIL	NIL	NT	NIL	–	NT	NIL	–	NT	6.2	–	NT	NIL	–
Ampicillin	58.3	NIL	13.6	60	NIL	6.2	4.3	NIL	3	28.5	NIL	–	23	NIL	6	70	28.1	21.8	50	NIL	50
Augmentin	NT	NIL	2.3	NT	NIL	3.2	NT	NIL	17.6	NT	NIL	NIL	NT	NIL	5.8	NT	16.6	36.6	NT	NIL	NIL
Methicillin	NT	19	–	66.6	26.4	–	50	11.7	–	NT	NIL	–	NT	13.3	–	100	45	–	NT	NIL	–
Carbenicillin	44.4	65	17.5	72.2	59.3	15.6	100	21.8	18.7	NT	12.5	37.5	NT	28.5	21.4	NT	71.8	–	NT	NIL	–
Amoxacillin	NT	2.3	16.6	NT	NIL	13	NT	NIL	15.3	NT	NIL	NIL	NT	NIL	17.6	NIL	13.3	46.6	NT	NIL	33.3
Erythromycin	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL	NT	NIL	NIL
Chloram phenicol	45	NIL	15.5	42.8	NIL	9.6	82.3	2.9	26.4	NIL	NIL	NIL	64.2	NIL	29.4	75	48.6	18.1	50	NIL	NIL
Cotrimoxazole	29.1	16	NIL	71	27.2	NIL	80	28.5	28.5	NIL	NIL	NIL	66.6	NIL	NIL	61.2	50	NIL	50	NIL	NIL
Cephalothin	NT	26.6	26.6	NT	36.5	2.4	NT	21.4	17.8	NT	NIL	28.5	NT	5.8	17.6	NT	60.6	12.1	NT	NIL	NIL
Cefotaxime	96.2	71.7	5.1	76.6	13.3	10	88.4	64.7	11.7	100	66.6	NIL	100	50	NIL	100	NIL	NIL	100	76.9	11.5
Gentamycin	92	58	34.8	82.7	38.8	36.1	81.4	70.5	NIL	100	12.5	62.5	84.6	50	14.2	96.7	84.3	9.3	NT	NT	NT
Amikacin	100	55.5	37.7	100	57.6	42.3	100	67.6	17.6	100	28.5	57.1	100	56.2	31.2	100	90.3	6.4	100	NIL	NIL
Nitrofurantoin	NT	4.8	–	NT	NIL	–	NT	NIL	–	NT	NIL	–	NT	NIL	–	NT	5	–	NT	NIL	–
Nalidixic Acid	100	85.7	–	NT	33	–	NT	67.6	–	NT	57.1	–	NT	75	–	NT	92.5	–	NT	60	–
Pipimedic Acid	NT	93	–	NT	80.6	–	NT	89.6	–	NT	35.7	–	NT	62.5	–	NT	93.7	–	NT	NIL	–
Ofloxacin	100	84	16	100	43.5	46.1	100	47	35.8	100	80	10	100	78	21.8	100	94	2.9	100	NIL	100
Enoxacin	100	84	7	100	56.4	33.3	100	54.7	20.7	100	80	10	100	71.8	15.6	100	94.1	5.9	100	NIL	50

KEY: D = Disc Sensitivity; BP = Break-Point Sensitivity; S = Sensitive; MR = Moderately Resistant; NT = Not Tested; NIL = None Sensitive

Table II records the results of 400 clinical isolates from sources other than urinary tract.. The Disc sensitivity methods give considerably higher percentage of organisms sensitive to the antibiotics tested as opposed to the Break-point sensitivity.

DISCUSSION

In vitro,, antibiotic sensitivity testing plays a major role in the management of patients. It provides a guideline for initiation of therapy hence it is extremely important that sensitivity tests are carried out accurately. One of the points to take into consideration is that achievable levels of antibiotics in various body fluids differ hence as a guide the suggested concentrations of antibiotics disc for urinary tract isolates are of higher potency as compared to isolates from other sources. For example, the suitable disc concentrations for Ampicillin, Streptomycin and Tetracycline are 25, .25 and 30 ug/ml respectively. The recommended disc concentrations for isolates from all other sources are Amikacin, Gentamicin, Carbenicillin, Cefuroxime, Chloramphenicol, Erythromycin, Tetracycline 10 ug/ml, Methicfflin 5 ug/ml and Penicillin 1 unit, while in practice. discs of much higher concentrations are used and at the same time the recommended procedures are not followed resulting in inconsistent results. The advantage of Break-point sensitivity is that it takes into consideration the level of concentrations achieved in body fluids and dependent on the levels, plates are prepared giving results as sensitive,

moderately resistant and resistant. Sensitive means the antibiotic would eliminate the pathogens almost certainly, moderately resistant means the organism requires higher levels of antibiotic to achieve desired effect and suggest a case where fluid levels should be done specially in treatment failure cases. The antibiotic dilutions are prepared and shelf life is known, control organism is used on each and every plate and hence the quality is always checked. The results obtained by the two methods clearly highlight the points of difference in sensitivity. We are of the opinion that Break-point sensitivity method gives a more realistic approach to in vitro testing. It is cheaper and time-saving and should be adopted by the laboratories.

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