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Evaluation the Accuracy of drug susceptibility testing by disk diffusion method in Medical diagnostic Laboratories of Hamedan University of Medical Sciences

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ABSTRACT

Susceptibility testing is one of the key tests in the therapeutic process of bacterial diseases, the accuracy in performance of the test and attention of physicians to the answer of this test can cause avoiding of bacterial resistance. In this study we evaluated the Accuracy of antibiotic susceptibility testing by disk diffusion method in Medical diagnostic Laboratories of Hamedan University of Medical Sciences in which the three Standards Bacteria; *Escherichia coli*, *Staphylococcus aureus* and *pseudomonas aeruginosa* were used. The disks of antibiotics from different companies in company with different disks from various laboratories for performance of susceptibility testing by disk diffusion agar method were prepared for Teaching Hospitals Laboratories of Hamadan. The accuracy of the laboratory work examined with a questionnaire with 39 questions. During this studying, 87.5% of laboratory staff didn't have any attention to the effect of the antibiotics before use, 50% of laboratory personnel did not comply the medium standard diameter (4-5 mm), 62.5% of laboratories personnel did not fulfill the standard temperature incubator (35 ° C), 37.5% of staff not reported the genus of bacteria before performance of antibiogram test, 37.5% of laboratories personnel not prepared suspension in accordance with the 0.5 McFarland turbidity. In conclusion, our results show that to better carry out the testing, the laboratory personnel should be spend more time, also it is recommended that an incubator with 35 ° C for susceptibility testing should be prepared in the laboratory. Health center evaluate periodically the accuracy of antibiogram with preparation of bacteria and proper antibiotics in the laboratories.

Key words: Accuracy, Antibiotic susceptibility testing, Disk diffusion agar

INTRODUCTION

With increasing antibiotic resistance among many pathogens, it is appearing that the accurate results to patients and public health is very important. In order to closely monitor drug resistance, physicians and public health officials are need accurate reporting of antimicrobial susceptibility test results in the laboratory. Disk diffusion method is one of the most common antibiotic susceptibility tests that are used in most laboratories [1-3].

Table1.Measurement of inhibition zone diameter based on studied laboratories

Antibiotics	Type of bacteria	Manufacturer	Inhibition zone diameter							
			reference	Sina	Fatemieh	Besat	Ekbatan	Shehid Beheshti	Health Center	School of Medicine
Ampicillin	<i>E.Coli</i>	<i>padtanteb</i>	16	15	20	17	14	18	15	19
		Himedia	17	14	20	17	14	19	14	17
		Mast	20	15	17	17	22	19	17	17
	<i>Staphylococcus Aureus</i>	<i>padtanteb</i>	35	28	28	17	29	40	35	30
		Himedia	44	27	28	17	32	39	33	28
		Mast	40	26	21	17	22	R	34	28
	<i>Pseudomonas aeruginosa</i>	<i>padtanteb</i>	R	R	R	R	R	R	R	R
		Himedia	R	R	R	R	R	R	R	R
		Mast	R	R	R	R	R	R	R	R
<i>E.Coli</i>	<i>padtanteb</i>		17	17	19	17	22	22	20	20
		Himedia	20	16	20	17	22	24	17	21
		Mast	22	14	18	17	22	24	18	21
	<i>Staphylococcus Aureus</i>	<i>padtanteb</i>	20	16	24	17	12	21	24	24
		Himedia	25	22	20	17	25	28	25	24
		Mast	24	19	20	17	27	R	23	23
	<i>Pseudomonas aeruginosa</i>	<i>padtanteb</i>	21	16	22	17	24	21	24	24
		Himedia	25	21	26	17	26	26	28	23
		Mast	21	15	24	17	22	21	26	22
Imipenem	<i>E.Coli</i>	<i>padtanteb</i>	30	25	25	16	20	32	30	28
		Himedia	30	14	27	16	26	30	25	26
		Mast	30	23	30	16	21	27	24	26
	<i>Staphylococcus Aureus</i>	<i>padtanteb</i>	21	19	33	16	22	21	30	31
		Himedia	44	32	34	16	29	38	36	35
		Mast	32	34	25	16	24	R	40	34
	<i>Pseudomonas aeruginosa</i>	<i>padtanteb</i>	27	16	28	16	28	23	30	28
		Himedia	26	22	R	16	25	27	28	27
		Mast	22	11	22	16	25	20	28	24
Cefixime	<i>E.Coli</i>	<i>padtanteb</i>	19	16	20	19	23	21	17	23
		Himedia	26	17	22	19	21	21	18	24
		Mast	20	14	18	19	22	22	17	23
	<i>Staphylococcus Aureus</i>	<i>padtanteb</i>	11	R	23	19	15	19	15	18
		Himedia	17	23	23	19	22	21	17	19
		Mast	12	16	15	19	15	R	12	13
	<i>Pseudomonas aeruginosa</i>	<i>padtanteb</i>	R	R	R	R	12	R	R	R
		Himedia	R	R	R	R	R	R	R	R
		Mast	R	R	R	R	R	R	R	R
Antibiotics	Inhibition zone diameter									
			reference	Sina	Fatemieh	Besat	Ekbaan	Shehid Beheshti	Health Center	School of Medicine
Ciprofloxacin	<i>E.Coli</i>	<i>padtanteb</i>	28	26	32	20	23	35	32	33
		Himedia	35	31	34	20	28	36	33	32
		Mast	33	21	32	20	28	32	30	30
	<i>Staphylococcus Aureus</i>	<i>padtanteb</i>	29	19	28	21	30	33	31	28
		Himedia	34	25	23	21	28	30	32	30
		Mast	27	16	27	21	31	R	25	26
	<i>Pseudomonas aeruginosa</i>	<i>padtanteb</i>	31	26	36	21	34	30	33	33
		Himedia	36	R	35	21	33	33	35	33
		Mast	30	21	34	21	31	26	35	32
Sulfamethoxazol/ Trimethoprim	<i>E.Coli</i>	<i>padtanteb</i>	27	22	25	16	24	28	25	26
		Himedia	27	23	28	16	25	29	24	24
		Mast	25	20	26	16	25	26	21	22
	<i>Staphylococcus Aureus</i>	<i>padtanteb</i>	27	21	27	16	31	35	25	26
		Himedia	30	22	25	16	30	32	25	25
		Mast	28	19	27	16	22	R	22	25
	<i>Pseudomonas aeruginosa</i>	<i>padtanteb</i>	R	R	R	R	R	R	R	R
		Himedia	R	R	R	R	R	R	R	R
		Mast	R	R	R	R	R	R	R	R

One of the key tests in treatment process of bacterial infection is susceptibility testing in which the accuracy of this test help the physician to provide the best treatment in use of the effective drugs. However, in most cases, and as a result of the patient's condition and in an emergency condition the physician does not even wait for the test answer and begins the use of several antibiotics simultaneously but need to the antibiogram answer which according to its needs revised in treatment of patients, but More laboratories do not pay so much attention to precision and accuracy of this test, so this is contributing to antibiotic resistance in the community and in some cases the physician insisted on recognition of bacteria and drug resistance, unfortunately, the test standards that listed in the CSLI in detail are not respected by the relevant laboratories personnel. The main reason for this test is not considered serious by personnel could be due to lack of time and workload which cannot be logical reason for this negligence[3, 4].

MATERIALS AND METHODS

Three standard strains of bacteria and six types of antibiotics disks from three different companies, as well as a questionnaire with 39 questions on the test method also were placed for laboratory personnel. This study was conducted at spring - 2014 in Microbiology Laboratories of Besat, Ekbatan, Sina, shehied Beheshti, Fatemiyeh Hospitals, Laboratory of Health Centre, Reference Laboratory, and Laboratory of School of Medicine. We did evaluating the accuracy of drug susceptibility testing by disk diffusion method in Medical Diagnostic Laboratories of Hamedan University of Medical Sciences in which the three standards bacteria; *Ecoli*, *Staphylococcus aureus* and *pseudomonas aeroginosa* were used. The profile ID of them from plate pack and only with the code number [1, 2, 5] which was marked on the plate with disks of antibiotics from different companies in company with different disks from various laboratories for performance of susceptibility testing by disk diffusion agar method were prepared for Teaching Hospitals Laboratories of Hamadan City. The microbiology department personnel were asked to do as routine susceptibility testing as well as examined the accuracy of the laboratory work with a questionnaire with 39 questions. After test performance, the results of medical microbiology and reference laboratories based on the health reference standard «CLSI» were compared and data analyzed using ANOVA and Chi Square tests and SPSS16 software.

Table 2. Distribution of laboratories evaluating the variables

Variable	%	Number
The diameter of the used medium		
2-4mm	12.5	1
4-5mm	50	4
5-6mm	25	2
6-7mm	12.5	1
Total	1	8
duration of the incubation medium		
24 h	75	6
20 h	0	0
16-18 h	12.5	1
≥24	12.5	1
Total	100	8
Duration time of placing of disks after inoculation with bacteria		
Immediately	25	2
After 5 minutes	37.5	3
After 10 minutes	12.5	1
After 15 minutes	25	2
Total	100	8
Temperature of incubator		
37	50	4
36	12.5	1
35	37.5	3
Total	100	8
Determination genus of bacteria before test performance		
yes	62.5	5
No	37.5	3
Total	100	8
PH of medium		
6-7	12.55	1
7-8	12.5	1
6.5-7.5	12.5	1
7.2-7.4(standard)	62.5	5
Total	100	8
Determination of PH of medium by laboratory		
yes	37.5	3
no	62.5	5
Total	100	8
Preparation of 0.5 McFarland		
Yes(standard)	37.5	3
no	62.5	5
Total	100	8
Use of wicker ham page		n
Yes(standard)	37.5	3
no	62.5	5
Total	100	8
Disk storage conditions		n
Refrigerator	12.5	1
Frizzier	0	0
Frizzier- Refrigerator(standard)	87.5	7
Total	100	8

RESULTS

78.5% of laboratories didn't have any effect on studied antibiotics before use of them; this means that the ampicillin based on CLSI guidelines should not be used for *Pseudomonas* susceptibility testing. 25% of the laboratories are applied one of three similar disks but from three different companies which were resistant to one bacterium. 12.5% of laboratories have reported similar results for inhibition zone diameter from three disks belonging to three companies.

50% of laboratories have provided the standard diameter of medium (4-5 mm), 37.5% of laboratories have provided standard duration after bacteria inoculation (5 minutes) to insert disks, 37.5% of laboratories have provided standard incubator temperature (35 C°), then 62.5% of laboratories have reported the determination of bacteria genus before antibiogram. 62.5% of laboratories have the correct information about the PH medium but in general, 37.5% of laboratories measured the PH of medium, finally 62.5% of laboratories prepared suspension in accordance with the 0.5 McFarland standards (table 2).

Most errors in the antibiogram testing process was related to the incubation time on Mueller Hinton Agar medium in incubator in which just 12.5% of selected laboratories have provided the mentioned period in CLSI.

Table 3. The results of evaluated the various parameters

The evaluated Parameters	Errors (%)	Accuracy (%)
Diameter size of medium	50	50
	12.5	87.5
Duration time of disk	37.5	62.2
Incubator temperature	37.5	62.2
Determination of bacterium genus	62.2	37.5
pH of medium	37.5	62.2
Preparation of 0.5 McFarland	37.5	62.2
Use of wicker hampage	37.5	62.5
Disk storage conditions	87.5	12.5

Among 54 disks were given to each laboratory after collecting the results of inhibition zone diameter, observed that the lowest error was related to the Microbiology Laboratory of University of Medical Sciences and the maximum error was associated to the Besat Hospital (data not shown).

Table 4. Result of falout of control range according to the CLSI for three antibiotics (Padtanteb, Himedia and Mast) for each bacterium in each laboratory

Antibiotics	Type of bacteria	Reference	Sina	Fatemieh	Besat	Ekbatan	Shahid Beheshti	Health Center	School of medicine
Ampicillin	<i>E. Coli</i>	0	3	0	0	2	0	2	0
	<i>Staphylococcus aureus</i>	2	1	1	2	1	3	0	0
	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-
Amikacin	<i>E. Coli</i>	0	3	1	3	0	0	2	0
	<i>Staphylococcus aureus</i>	0	2	0	3	2	2	0	0
	<i>Pseudomonas aeruginosa</i>	0	2	0	3	0	0	1	0
Imipenem	<i>E. Coli</i>	0	3	1	3	2	0	2	0
	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-
	<i>Pseudomonas aeruginosa</i>	0	2	1	3	0	0	1	0
Cefixime	<i>E. Coli</i>	2	3	3	3	2	3	3	0
	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-	-
	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-
Ciprofloxacin	<i>E. Coli</i>	1	2	0	3	3	0	0	0
	<i>Staphylococcus aureus</i>	1	2	0	3	1	2	2	0
	<i>Pseudomonas aeruginosa</i>	1	2	3	3	1	0	2	0
Sulfamethoxazol/Trimethoprim	<i>E. Coli</i>	0	2	0	3	0	0	1	1
	<i>Staphylococcus aureus</i>	0	3	0	3	1	2	1	0
	<i>Pseudomonas aeruginosa</i>	-	-	-	-	-	-	-	-

DISCUSSION

The present study was conducted on accuracy of susceptibility testing using agar disk diffusion method in clinical diagnostic laboratories of the educational hospitals of Hamadan University of Medical Sciences to identify strengths and weaknesses in performance of this test, improve and enhance the quality of services provided in the laboratories.

The study performed in the eight microbiology laboratories at the University of Medical Sciences. In total, full respect for labor standards consistent with the CLSI standards was performed only in a laboratory (Faculty of Medicine) and other laboratories have provided only some of mentioned points in the CLSI. Despite the sincere cooperation of laboratories, the reasons that personnel do not act in accordance with CLSI standards are including: workload and little time for responding that causes an error in reporting of antibiogram test. This study showed that the one of the most important tests in treatment of bacterial diseases considered unimportant the lack of precision in its performance have direct relationship with drug resistance in the community has considered unimportant and with proper planning and academic training laboratories must help the laboratories in better performance of this test. Among standards that have direct correlation with the test results and should be have adequate attention to these issues can be stated as follows:

- 1) Using pure colonies
- 2) Suspension preparation
- 3) Measure the pH of medium
- 4) Proper size of medium diameter
- 5) Proper time for placing of disks after bacterial inoculation
- 6) Appropriate temperature of incubator[6]

Similar to our study in study conducted by Ms. S. Abbasi and et al in Kerman in 2008 with title the accuracy of laboratory diagnosis and routinely antibiogram gram-negative bacteria compared with standard methods concluded that the detection of bacteria were in the genus level, and in some cases was different with real organisms, this possibly due to human error, Lack of personnel attention, careless staff, little time for responding, improper storage of materials, lack of standard strains which have been made the laboratory errors[7]. Sedighi and colleagues in a study in 2010 compared the effectiveness of Padanteb antibiotics with antibiotics from Mast Company on the *E.coli* strains isolated from children with urinary tract infection suggested that the quality of Iranian disks will be increase[8]. In another one carried out by Ashtiani et al in Tehran in 2008 on 77 strains of coagulase-negative *staphylococci*, in which have been compared agar disk diffusion method with E.test method for antibiotic susceptibility. The results showed that the E.test was more accurate [9]. Julia A and his colleagues in New York in 2000 performed a comprehensive study in 320 laboratories to assess the accuracy of antibiogram test by disk diffusion method. For this purpose, *Staphylococcus aureus* and *Enterococcus* was used and four parameter were considered in the experiment which these include: the preparation of 0.5 McFarland suspensions of bacteria, the selecting the proper medium for disk diffusion testing, the number of disks in each plate and conditions and the time and condition of incubation. The findings of this study have reported the greatest error in the disk diffusion test and the time of incubation at 37 ° C which exactly in line to present study[10].

CONCLUSION

in conclusion our results show that to better carry out the testing, the laboratory personnel should be spend more time, also it is recommended that an incubator with 35 ° C for susceptibility testing should be prepared in the laboratory. Health center evaluate periodically the accuracy of antibiogram with preparation of bacteria and proper antibiotics in the laboratories. Also the microbial resistance immediately should be notified to physicians and hospital infection control committee.

Conflict of Interests

The authors declare that there is no conflict of interests about the publication of this paper.

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