

Comparison of E. Test and Disk Diffusion Agar in Antibiotic Susceptibility of *E. coli* Isolated from Patients with Urinary Tract Infection in Shariati Hospital (Iran)

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Abstract: Urinary Tract Infection (UTI) is one of the most common bacterial infections and *E. coli* is known to be as an important cause of UTIs. Because bacterial resistances to antibiotics are increasing, therefore a reliable method of antimicrobial resistance detection is important in treatment of UTIs. The objective of the present study is to evaluate and compare the performance of disk diffusion agar with E.test for antimicrobial susceptibility testing of *E. coli* isolated from UTI. This study was carried out on 250 isolates of *E. coli* from patients with UTI in Shariati Hospital of Tehran University of Medical Sciences in year 2005. Antibiotic susceptibility testing was performed by disk diffusion method (Iranian and Italian) for Trimetoprim sulfamethoxazole, Gentamycin, Ceftazidim, Nitrofurantoin and Ciprofloxacin and Minimum Inhibitory Concentration (MIC) determination was performed by E.test for the same set of antimicrobial. All tests were performed on Muller Hinton agar. The results of this study show that disk diffusion agar may be used as a preliminary screen for antibiotic susceptibility testing of *E. coli* and is less sensitive than Italian disk diffusion and E.test. By comparison of 3 above mentioned methods E.test is the most sensitive and shows the effective dose of antibiotic for treatment and prevention of antibiotic resistance. Comparison of E.test with Iranian disk diffusion agar showed paramount differences in antibiotic agreement (max 37.8 %) these differences in case of Ceftazidim and Gentamycin were, respectively 76.8 and 62.2% whereas comparison of E.test with Italian disk diffusion agar showed less difference of antibiotics agreement (max 11.2%).

Key words: UTI, *E. coli*, antibiogram, disk diffusion agar, E.test

INTRODUCTION

The prevalence of antibiotic resistance in bacteria isolated from nosocomial infections and patients is increasing. This is important in preventing the appearance and spread of resistant strains. Gram negative bacteria cause urinary tract infections, intra-abdominal infections, bactremia and infections in other sites. Most strains isolated from these infections are: *Escherichia coli*, *Klebsiella*, *Proteus*, *Enterobacteriaceae* and ... (Dargo *et al.*, 2000). The life-threatening infections such as septicemia and endocarditis different antibiotics are used together in order to achieve a wide spectrum effect and increase the effect of these antibiotics *in vivo*. This can cause antibiotic resistance (Kocazeybek *et al.*, 2002). Urinary tract infections (UTIs) are highly prevalent in the community. It is considered a

costly infection in medicine. Its most common etiologic factor is *Escherichia coli* (*E. coli*) (Ejraes, 2004, Mims *et al.*, 2004). In order to determine bacterial sensitivity to a certain antibiotic, disc diffusion agar test is an appropriate method for first screening. Thus it is necessary to recheck antibiotic resistance in resistant bacteria with a Minimum Inhibitory Concentration (MIC) method (Manoharan *et al.*, 2003). Epsilometer test (E. test) is a new method for this purpose (Sanchez *et al.*, 1999).

Urinary tract infections due to gram negative bacteria are highly prevalent and a high percentage of these infections are due to *E. coli*. Recently the number of reports upon the resistance to antibiotic discs is rising (Kocazeybek, 2001). Thus the choice of an effective and appropriate drug after diagnosis becomes of utmost importance.

The routine method to measure antibiotic sensitivity in bacteria is based on two principles: 1- dilution and 2- diffusion. E. test is based on both these principles. It determines the antibiotic sensitivity directly and quantitatively, just like the MIC method. Since MIC with a predefined E. test method uses a continuous and consistent antibiotic gradient it can be more precise than the MIC method.

The disc diffusion agar method is a method based on diffusion alone and can determine the sensitivity or resistance of the bacteria to a specific antibiotic. In the E. test method not only the sensitivity or resistance of the bacteria is detected, but also since an artificial concentration gradient of the antibiotic is used on a strip, the effective dose of the antibiotic can also be found.

E. test is an accurate, sensitive and quantitative method, especially in samples resistant to antibiotic discs (Hanberger *et al.*, 1999a). In this study, the comparison of disc diffusion agar and E. test methods, for the antibiotic sensitivity of *E. coli* were evaluated.

MATERIALS AND METHODS

The study carried out throughout 2005 in the microbiology research center of Tehran medical university. Two hundred and fifty patients suffering urinary tract infections, which their urinary culture had proved the etiological factor to be *E. coli*, were selected. An antibiogram with some antibiotics was carried out using both methods. These antibiotics were: Bactrim, Gentamicin, Nitrofurantoin, Ciprofloxacin, Piperacine and Cefazidime. The first method used was the common and routine method is the disc diffusion agar. In this method the bacteria was selected and bacterial suspensions were prepared using the 0.5 McFarland method. Afterwards, the bacteria were transferred on Muller Hinton agar plates. Iranian and Italian made antibiotic discs were placed on the surface of the plate using sterile forceps. The degree of resistance or sensitivity was determined by measuring the inhibited growth areas around the disc after 24 h of incubating the plates in 37°C. E. test was the second method used. Here also a bacterial suspension using the 0.5 McFarland methods was prepared and bacteria transferred to Muller Hinton agar plates. The E. test strips for each antibiotic was placed on the surface of the plate. The triangle inhibited growth areas (Fig. 1) were also studied after 24 h incubation in 37°C and the sensitivity of *E. coli* was determined using the reference table provided by the producer of the E. test strips (AB. Biodisc, Solna Sweden). In each of the 2 methods used, the sensitivity, resistance and borderline situations for one of the above-mentioned antibiotics were determined and the results were compared.

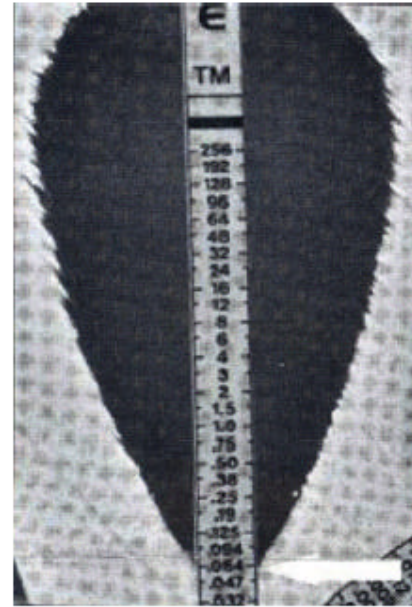


Fig. 1: formation of triangularly growth inhibition zone for increasing Antibiotic concentration near E. test Tape

RESULTS

The aim of the study was to compare the E. test method (Swedish) and disc diffusion agar method (Iranian and Italian) for the antibiotic sensitivity in *E. coli* bacteria isolated from urinary cultures of UTI patients. First the number of sensitivity to the antibiotics was measured and compared (Table 1-3). The highest sensitivity rate was for Nitrofurantoin. The figures in E. test, Italian and Iranian disc diffusion agars were 91.6, 92 and 68.3%, respectively. The lowest sensitivity figures for the 3 methods were 40, 40 and 39.1%, respectively. The highest resistance in all 3 methods was for Bactrim with the figures being 56.0% in E. test method, 60% in Italian disc diffusion agar method and 60.1% in Iranian disc diffusion agar method. Due to the significant difference seen in sensitivity results obtained from Iranian antibiotic discs and foreign ones an overall agreement percentage for Iranian and foreign discs were calculated (Table 1-3). The results obtained were as follows:

- In the Bactrim disc the conformity between foreign products is high with a 6% difference. The difference between the Iranian and Italian product is about 9.6% and with the Swedish product this figure reaches 12.4%.
- The difference of the foreign Gentamicin products is 7%. The Iranian Gentamicin disc has a difference of 31.6% and 37.8% with Italian and Swedish products, respectively.

Table 1: The comparison of Italian disk diffusion agar and E. test Sweden and calculation of overall agreement

Antibiotic	Italian disk diffusion agar			E.test Sweden			Overall agreement
	S	R	I	S	R	I	
SXT	(40%) 100	(60%) 150	-	(40%) 100	(56.6%) 149	(0.4%) 1	94
GM	(74%) 185	(22.8%) 57	(3.2%) 8	(72%) 180	(20.4%) 51	(7.6%) 19	2.93
FM	(92%) 230	(6.4%) 16	(1.6%) 4	(91.6%) 229	(4%) 10	(4.4%) 11	94
Cip	(54.8%) 137	(43.6%) 109	(1.6%) 4	(55.4%) 138	(43.8%) 109	(0.8%) 2	2.95
CAZ	(66%) 165	(33.2%) 83	(0.8%) 2	(66.8%) 167	(28.4%) 18	(4.8%) 12	8.88

Table 2: The comparison of Italian disk diffusion agar and Iranian disk diffusion agar and calculation of overall agreement

Antibiotic	Italian disk diffusion agar			Iranian disk diffusion agar			Overall agreement
	S	R	I	S	R	I	
SXT	(40%) 100	(60%) 150	-	(39.1%) 97	(60.1%) 151	(0.8%) 2	4.90
GM	(74%) 185	(22.8%) 57	(3.2%) 8	(48.2%) 121	(32.5%) 81	(19.3%) 48	4.68
FM	(92%) 230	(6.4%) 16	(1.6%) 4	(68.3%) 171	(16.5%) 41	(15.2%) 38	8.70
Cip	(54.8%) 137	(43.6%) 109	(1.6%) 4	(51.8%) 130	(45.8%) 114	(2.4%) 6	2.91
CAZ	(66%) 165	(33.2%) 83	(0.8%) 2	(58.5%) 146	(36.7%) 92	(4.8%) 12	8.82

Table3: The comparison of Iranian disk diffusion agar and E. test Sweden and calculation of overall agreement

Antibiotic	Iranian disk diffusion agar			E.test Sweden			Overall agreement
	S	R	I	S	R	I	
SXT	(39.1%) 97	(60.1%) 151	(0.8%) 2	(40%) 100	(56.6%) 149	(0.4%) 1	6.87
GM	(48.2%) 121	(32.5%) 81	(19.3%) 48	(72%) 180	(20.4%) 51	(7.6%) 19	2.62
FM	(68.3%) 171	(16.5%) 41	(15.2%) 38	(91.6%) 229	(4%) 10	(4.4%) 11	4.68
Cip	(51.8%) 130	(45.8%) 114	(2.4%) 6	(55.4%) 138	(43.8%) 109	(0.8%) 2	4.88
CAZ	(58.5%) 146	(36.7%) 92	(4.8%) 12	(66.8%) 167	(28.4%) 18	(4.8%) 12	8.76

S: Sensitive, R: Resistance, I: Intermediate, SXT: Bactrim, GM: Gentamicin, FM: Nitrofurantoin, Cip: Ciprofloxacin, CAZ: Ceftazidime

- The Nitrofurantoin foreign discs had a difference of 6%. The difference for the Iranian disc with the Italian disc and Swedish E. test strips was 29.2 and 32.6%, respectively.
- Ceftazidime disc had a difference of 11.2% for foreign products. The Iranian disc differed from Italian and Swedish products by 17.2 and 23.2%.

DISCUSSION

Based on a lot of studies carried out *E. coli* is the most bacterial factor causing UTI (Ejraes *et al.*, 2004, Mims *et al.*, 2004; Manoharan *et al.*, 2003; Nir *et al.*, 2005). Pervious the Swedish E. test and disc diffusion agar test (Iranian and Italian) were used to determine antibiotic sensitivity of the bacteria *E. coli* isolated from UTI patients. The results showed that the highest rate of sensitivity was to Nitrofurantoin. The results of this study are in correlation with results of Noemia and Goldraich (2002) study. We also shown that the highest resistance level was towards Bactrim, which this had also been shown in the experiment carried out by Marcus (Hanberger *et al.*, 1999b).

Using the E. test method as a sensitive and precise method in determining the sensitivity of bacteria to antibiotics was confirmed in previous studies (Manoharan *et al.*, 2003; Sanchez *et al.*, 1999; Hanberger *et al.*, 1999b).

In this study we compared the Swedish E. test with Italian diffusion test agar method and the Iranian diffusion agar test method for the first time. Based on our results the highest resistance was seen towards Bactrim in all three methods; the percentage being 56.6% using the E. test method lower than the two other methods used. This shows the higher sensitivity and accuracy of the E. test method in determining bacterial antibiotic resistance.

The highest sensitivity rate was for Nitrofurantoin, with a rate of 91.6% in E. test, 92% in the Italian diffusion disc agar and 68.3% in the Iranian diffusion disc agar methods. As it is evident there is a difference of about 22-23% between the Iranian methods and foreign methods. This can be concluded as the Iranian Nitrofurantoin discs do not have the sensitivity of foreign products.

After calculating the overall agreement percentage for all antibiotics in the 3 methods it was evident that the results obtained from Iranian diffusion disc agar and the Swedish E. test methods were not concordant and these differences were significant. These differences were as follows: Bactrim 12.4%, Gentamicin 37.8%, Nitrofurantoin 31.6%, Ciprofloxacin 11.6% and Ceftazidime 23.2%. The results obtained from the Italian disc diffusion agar method and the Swedish E. test method was more concordant, with the differences observed being: 6% for Bactrim, 6.8% for Gentamicin, 6% for Nitrofurantoin, 4.8% for Ciprofloxacin and 11.2% for Ceftazidime.

CONCLUSION

According to the results obtained it can be concluded that E. test is the most sensitive and the most accurate method amongst the 3 methods used in this survey and it is recommended in bacteria reported to be resistance in ordinary methods. It can also determine the effective antibiotic dose for treatment and also prevent the appearance of antibiotic resistance.

The Iranian disc diffusion agar method can be used as a primary screening method to determine antibiotic resistance levels in *E. coli*. The Italian diffusion disc agar method is more accurate than the former and the Swedish E. test is much more precise than the Iranian method. Thus the Iranian disc diffusion agar test should not be used alone in determining antibiotic sensitivity. At least in antibiotic resistant bacteria the results should be confirmed using a more accurate method like E. test.

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