

Full Length Research Paper

Comparison of Iranian disc diffusion agar and epsilometer test (E-test) accuracy, in antibiotic susceptibility of *Escherichia coli* isolated from patients with urinary tract infection

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Here we aim at comparing the accuracy of Padtan Teb disc (Iranian DDA) with epsilometer test (E-test) for antibiotic susceptibility, in *Escherichia coli* isolated from patients with urinary tract infections (UTI) in Shariati University Hospital. Two hundred and fifty patients with UTIs, diagnosed with urinary culture which was confirmed to be infected with *E. coli* were recruited. The highest sensitivity was perceived for Nitrofurantoin (91.5% in E test and 61.3% in Iranian DDA). The lowest sensitivity in two methods was detected for TMP-SMX (37.9% in Iranian DDA, and 34.4 in E test). The highest resistance was for Trimetoprim sulfamethoxazole (Iranian DDA 53.2%, 61.6% in E test), while the lowest resistance in two methods were obtained for Nitrofurantoin (14.5% Iranian DDA and 3.5% in E test). We hence concluded that the differences between Iranian DDA and Swedish E test results are prominent, and the sensitivity of Iranian DDA is not enough to determine antibiotic sensitivity therefore the results of this method should be confirm by other reliable ones such as Swedish E test.

Key words: Epsilometer test, agar disc diffusion, urinary tract infections (UTI), *Escherichia coli*, antibiogram.

INTRODUCTION

There is universal concern about the manifestation and increase of bacterial resistance to commonly used antibiotics (Howard et al, 2003; Livermore, 2003). The main reason for this increase is assumed to be widespread use of antibiotics. Intake of current antibiotic is supposed to suppress susceptible flora and direct to an overgrowth of resistant strains (Edlund and Nord, 2000).

Escherichia coli is one of the wide ranges of very different species of bacteria found naturally in the intestinal tract of all humans and many other animal species. *E. coli* is capable of causing enteric/diarrheal and also urinary tract infection (UTI) (Horcajada et al.,

2002; Kaper et al., 2004; Sturmer et al., 2004). UTI is among the most common infections with high economic burdens.

Several pathogens are responsible for UTIs; however *E. coli* is the main culprit strain (Wullt, 2003). Recently, several studies have reported increasing resistance to antibiotic discs, so identifying efficient and suitable antibiotic is of great clinical importance (Hanberger et al., 1999, Kocazeybek, 2001). Disc diffusion test is based on the inhibited growth zones, when fixed concentrations of an antimicrobial compound diffuse from the antibiotic discs into agar plate, which has formerly been inoculated with the test organism. The inhibition zones around the discs are measured after suitable incubation and interpreted as sensitive, intermediate and resistant zones (Fritsche et al., 2007; Ge et al., 2002).

The epsilometer test (E-test) is the combination of the

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Table 1. Comparison of Iranian disc diffusion agar and epsilometer test and overall agreement percentage.

Variables	E test Sweden			Iranian disc diffusion agar			Agreement (%)
	S	I	R	S	I	R	
TMP-SMX	107(37.9)	1(0.4)	173(61.3)	97(34.4)	2(0.7)	150(53.2)	75.9
GM	203(72)	20(7.1)	58(20.6)	123(43.6)	49(17.4)	81(28.7)	39.9
FM	258(91.5)	13(4.6)	10(3.5)	173(61.3)	39(13.8)	41(14.5)	13.2
CIP	150(53.2)	2(0.7)	128(45.4)	132(46.8)	6(2.1)	115(40.8)	78.9
CAZ	182(64.5)	14(5.0)	85(30.1)	148(52.2)	12(4.3)	92(32.6)	11.1

S: Sensitive, I: Intermediate, R: Resistance, TMP-SMX: Trimetoprim sulfamethoxazole (Co-trimoxazole), GM: Gentamycin, FM: Nitrofurantoin, CIP: Ciprofloxacin, CAZ: Ceftazidim.

diffusion and the ability to establish a minimum inhibitory concentration (MIC) or breakpoint result. It is based on diffusion of a preformed antimicrobial gradient from coated plastic strips onto an agar plate inoculated with the test organism (Engberg et al., 2004; Lubber et al., 2003). Here we aimed to compare the efficacy and reliability of the Iranian disc diffusion agar (DDA) and E-test accuracy, in antibiotic susceptibility of *E. coli* isolated from patients with UTIs, attending Shariati University Hospital.

MATERIALS AND METHODS

We performed a cross sectional study on 250 patients diagnosed with urinary tract infection with *E. coli*, in microbiology research center of Tehran University of Medical Sciences. Patient recruitment was from the urology clinic at shariati hospital affiliated with Tehran University of medical science. UTI with *E. coli* was diagnosed by urine culture.

Suspensions of pure cultures were prepared in bacterial suspensions and were adjusted to give inoculums with an equivalent cell density to 0.5 McFarland turbidity standards ($\sim 1.5 \times 10^8$ cfu/ml). Cell suspensions were then swabbed evenly onto muller hinton plates and allowed to dry. The following Iranian (Padtan Teb, Iran) antibiotic discs were applied to each muller hinton agar plate: gentamycin (10 µg/disc), Trimetoprim sulfamethoxazole (25 µg/disc), ciprofloxacin (Cip 5 µg/disc), ceftazidime (30 µg/disc), Nitrofurantoin (300µg/disc), plates were incubated at 37°C for 24 h. The breakpoints were measured by calipers and interpreted according to guidelines, following incubation.

In Swedish E-test (AB.BioDisk, Solna Sweden) method, suspensions of the organism were prepared in 2.5 ml of mueller hinton broth, and the turbidity was adjusted to give a reading of 0.5 McFarland turbidity standards ($\sim 1.5 \times 10^8$ cfu/ml.). A 0.1 ml volume of the 0.5 McFarland suspension was swabbed evenly in at least three directions on the surface of a mueller hinton plate. When the surface of each plate had dried, E-test strips (one for each antimicrobial) were placed in a radial formation on the surface of the agar. The strips were placed up to the lowest concentration of the antibiotic, toward the centre of the plate. The plates were incubated lid side up at 37°C for 24 h. The MIC values were read where the edge of the inhibition ellipse intersected the strip. Readings for each culture was recorded and classified as being resistant or sensitive based on their MIC breakpoints according to the manufacturer's instructions (AB.BioDisk, Solna Sweden).

All participants gave written informed consent before participation. The research was carried out according to the principles of the declaration of Helsinki; the local ethics review

committee of Tehran University of Medical Science approved the study protocol.

RESULTS

There were 180 female and 70 male, with the mean age of 35. Nitrofurantoin had the highest sensitivity (91.5% in E-test and 61.3% in Iranian DDA), while Trimetoprim sulfamethoxazole (37.9 in Iranian DDA, and 34.4 in E test) had the lowest sensitivity in this research, Table 1).

The highest resistance were noticed for Trimetoprim sulfamethoxazole (Iranian DDA 53.2, 61.6% in E-test), while the lowest resistance in two methods were obtained for Nitrofurantoin (14.5% Iranian DDA, 3.5% in E-test). The overall concordance (based on the MIC value obtained within the two methods) between the E-test and Iranian DDA methods was 78.9% Ciprofloxacin, 75.9% for Trimetoprim sulfamethoxazole, 39.9% for gentamycin, 13.2% for Nitrofurantoin, and 11.1% for Ceftazidim, respectively (Table 1).

DISCUSSION

Here we describe the comparison of the accuracy of Iranian disc diffusion agar and E-test, in antibiotic susceptibility of *E. coli* isolated from patients with UTI. Previously several authors have studied Swedish E-test and DDA for determining bacterial sensitivity to antibiotics (Wullt, 2003, Kocazeybek, 2001; Baker et al., 1991). In current study, we tested *in vitro* activities of Trimetoprim sulfamethoxazole, gentamycin, Nitrofurantoin, ciprofloxacin and ceftazidime against *E. coli* which were identified as the causative agents of UTIs, (Kocazeybek, 2001; Wullt, 2003) by using Iranian DDA and Swedish E-test. Several studies have shown that Swedish E-test results are associated with the standard dilution techniques and are highly reproducible (Baker et al., 1991; Huang et al., 1992). So, we compared Iranian DDA with Swedish E-test to evaluating Iranian DDA accuracy.

In consistent with the previous findings we showed that the highest sensitivity was for Nitrofurantoin (91.5% in E test and 61.3% in Iranian DDA), (Noemia and Goldraich,

2002). Likewise the lowest sensitivity was for Trimetoprim sulfamethoxazole (37.9 in Iranian DDA, and 34.4 in E-test) (Table 1), similar to the results of Hanberger et al. (1999) study (Noemia and Goldraich, 2002). To evaluate Iranian DDA, we firstly compared results of two methods and then computed the overall agreement percentage in Iranian DDA and E-test techniques. We showed that the differences between two methods were strongly significant ($P < 0.001$) as follow: ceftazidim (88.9%), Nitrofurantoin (86.8%), gentamycin (60.1%) Trimetoprim sulfamethoxazole (24.1%) and ciprofloxacin (21.1%). As a result, we concluded that the differences between Iranian DDA and Swedish E-test results are prominent; therefore, the sensitivity of Iranian DDA is not enough to determine antibiotic susceptibility and results obtained from Iranian DDA method should be confirmed by other reliable method as Swedish E-test.

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