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Full Length Research Paper

E-test versus disk diffusion method in determining multidrug resistant strains of *Escherichia coli* in urinary tract infection

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Multidrug resistant strains of *Escherichia coli* are becoming a major challenge in treatment of patients with urinary tract infection. Developing effective screening methods for selection of antimicrobial resistant strains is necessary. In this study, we have compared descriptively results of E-test with Disk diffusion agar method in selection of multidrug resistant strains of *E. coli* among patients with urinary tract infection. In disk diffusion test, 19 of 256 urine collections were resistant to 5 antibiotics used in the study. Later E-test was performed on these 19 collections with the same 5 antibiotics. In E-test, 10.5% of the specimens were sensitive to Bacterim, 21.1% to gentamicin, 47.7% to nitrofurantoin, 10.5% to ciprofloxacin and 10.5% to Ceftazidime. Results indicate that performing E-test on strains that were resistant in disk diffusion test may increase specificity in determination of multidrug resistant strains of *E. coli* in patients with urinary tract infection. This antibiotic susceptibility study showed difference between E-test and disk diffusion agar in assessing the antibiotic susceptibility and found E-test accuracy and its superiority to disk diffusion in detecting multidrug resistance.

Key words: *Escherichia coli*, resistance, E-test, disk diffusion.

INTRODUCTION

Urinary tract infections (UTI) are the most common nosocomial infections which accounts for 40% of hospital acquired infections (Gales et al., 2000; Talebi and Golestanpour, 2009). *Escherichia coli* is the most frequently found bacteria in both community and hospital acquired UTIs (Daza et al., 2001; Farrell et al., 2003). In recent years antimicrobial resistance has emerged explosively in many diverse bacterial types largely as a consequence of unrestrained antimicrobial use in medicine (Johson et al., 1999). This affects the management of UTI by increasing prevalence of multidrug resistant strains of *E. coli* (Rafay and Nsanze, 2003). Therefore developing methods for accurate identification of multidrug resistant strains of *E .coli* is mandatory (Giamarellou and Poulakou, 2009; Katz et al., 2004). In recent years several methods have been developed for antimicrobial susceptibility testing. Disk diffusion agar is a traditional and routine method of antimicrobial sensitivity testing. E-test provides a rapid and convenient means for determining minimal inhibitory concentration (MIC) for a variety of antimicrobial agents. Studies have shown that E-test shows good agreement with reference “agar dilution” susceptibility testing methods (Rosser et al., 1999).

MIC determining methods like E-test, although provide quantitative measurement of antimicrobial sensitivity (Erfani et al., 2008) because of their cost and limited availability in developing countries, their appli-cation is not as frequent as disk diffusion method (Khan and Zaman, 2006; Rahbar et al., 2006). Although, previous reports have compared E-test with disk diffusion in determining antimicrobial susceptibility, differences in their capabilities for selection of multidrug resistant strains of *E. coli* in UTI has not been fully encountered. In this study we have compared E-test and disk diffusion results in finding out multidrug resistant strains of *E. coli* in urinary tract infections. The

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Table 1. Distribution of resistant specimens in different hospital wards.

Hospital ward	Frequency	percent
OP and G	1	5.3
Urology	3	15.8
Nephrology	2	10.5
CCU	1	5.3
Hospital clinic	1	5.3
ICU	1	5.3
Surgery 2	1	5.3
Endocrinology	2	10.5
Renal transplantation	2	10.5
Social support	2	10.5
Medical treatment 1	2	10.5
Unidentified	1	5.3
Total	19	100

aim of this study was to investigate effectiveness of performing E-test in multi- antibacterial resistant strain of *E. coli*, determined by disk diffusion method in urinary tract infections.

MATERIALS AND METHODS

256 urinary specimens from patients with hospital acquired *E. coli* induced urinary tract infections were collected. Patients were from 10 different wards of Shariati Hospital affiliated by Tehran University of Medical Sciences. The study protocol was approved in ethics committee of Tehran University of Medical Sciences. An antibiogram was carried out using five antibiotics:

Trimetoprim sulfometoxazole, nitrofurantoin, ciprofloxacin, gentamicin, and ceftazidime.

First antimicrobial susceptibility was carried out with disk diffusion agar as a routine method. In this method bacterial suspensions were prepared using 0.5 McFarland methods. Then the bacteria transferred on Muller Hinton agar plates. Antibiotic disks were placed on the surface of the plate using sterile forceps. The degree of resistance or susceptibility was determined by measuring the inhibited growth areas around the disk after 24 h of incubating the plates in 37 according to NCCLS guidelines. Specimens showing resistance to all five antibiotics were selected for determining susceptibility with E-test as second method. For E-test also a bacterial suspension using the 0.5 McFarland methods was prepared and bacteria transferred to Muller Hinton agar plates. The E-test strip for each antibiotic was placed on the surface of the plate (Erfani et al., 2008). The triangle inhibited growth areas were studied after 24 h incubation in 37°C.

The susceptibility of *E. coli* was determined as MIC using the reference table provided by producer of the E -test strips (AB. Biodisk, solna, Sweden) and was converted to three sensitivity classes (sensitive, intermediate, resistant) according to breakpoints of NCCLS guidelines. Thereafter results of E-test antimicrobial sensitivity compared with multidrug resistant reported by Disk diffusion method descriptively.

RESULTS

In present study, 19 specimens from of 256 urine collections were resistant to all five antibiotics in Disk Diffusion agar test. The distribution of these resistant collections in different hospital wards is presented in Table 1. E-test was carried out on these 19 specimens. Results of E-test for different antibiotics are summarized in Table 2. E- test showed the following differences with disk Diffusion method:

i) In sensitivity testing for TMP-SMX, 2 of 19 strains (10.5%) were sensitive to this antibiotic in comparism to disk diffusion which all 19 specimens were resistant to TMP-SMX.
ii) In sensitivity testing for “gentamicin” 4 of 19 strains (21.1%) were sensitive, 3 of 19 (15.3%) were in intermediate group of susceptibility, in comparism to disk diffusion which all 19 specimens were resistant to gentamycin.
iii) In sensitivity testing for “nitrofurantoin” 9 of 19 strains (47.7%) were sensitive, 6 of 19 (31.6 %) were in intermediate group, in comparism to disk diffusion which all 19 specimens were resistant to “nitrofurantoin”.
iv) In sensitivity testing with “ciprofloxacin”, 2 of 19 strains (10.5%) were sensitive, in compare to disk diffusion which all 19 specimens were resistant to “ciprofloxacin”.
v) In sensitivity testing with “ceftazidime” 2 of 19 strains (10.5 %) were sensitive, 1 of 19 (5.1%) in intermediate group, in compare to disk diffusion which all 19 specimens were resistant to “ceftazidime”.

DISCUSSION

Increasing emergence of multidrug resistant bacteria impose a challenge for their selection and appropriate treatment (Rossolini and Mantengoli, 2008). Over prescription of different classes of antibiotics in hospital and community acquired infections, is proposed as a possible mechanism for their development (Johson, 1999). Therefore optimizing laboratory methods for finding resistant strains is crucial.

Disk diffusion agar is a common qualitative method for determining antibiotic sensitivity. Because of its possible limitations, MIC based methods like E-test has

Table 2. Results of antibiotic susceptibility testing with disk diffusion and E-test methods.

Method	Disk diffusion	E-test			
Antibiotic	**S**	**R (%)**	**R (%)**	**I (%)**	**S (%)**
Trimetoprim sulfometoxazole	0	19(100)	2 (10.5)	0	17 (89.5)
Gentamycin	0	19(100)	4 (21.1)	3 (15.8)	12(63.2)
Nitroforantoin	0	19(100)	9 (47.7)	6 (31.6)	4 (21.1)
Ciprofloxacin	0	19(100)	2 (10.5)	0	17 (89.5)
Ceftazidime	0	19(100)	2 (10.5)	1 (5.3)	16 (84.2)

R: Resistance, S: sensitive, I: intermediate.

been developed (Mnoharan et al., 2003). Previous studies have shown that E-test is a good alternative to reference methods like agar dilution and Broth microdilution methods (Katz et al., 2004, Kelly etal.,1999). In present study E-test shows sensitive strains of *E. coli* that were resistant to all five antibiotics in disk diffusion test. This difference was higher in sensitivity testing for "nitroflorantoin". 47.5% of strains that was reported as resistant in disk diffusion were sensitive to "nitroflorantoin". This result is consistent with previous reports where most of bacteria were sensitive to "nitroflorantoin" (Erfani et al., 2008; Barry et al., 2001).

Sahm et al. (2001) have found that 7.7% of multidrug resistant *E. coli* strains were resistant to "nitroflorantoin", in comparism to TMP-SMX where 86.6% of strains were resistant (Sahm et al., 2001). Disk diffusion and E-test had minimal differences in sensitivity test for "trimetoprim" sulfometoxazole and ciprofloxacin. While all specimens were resistant to these antibiotics in Disk diffusion test, 10.5% were sensitive to "trimetoprim" sulfometoxazole and ciprofloxacin in E- test. Findings are in concordance with other studies, showing resistance to "bacterim" in most of the strains (Yilmaz et al., 2009). In previous studies different levels of agreement between E-test and disk diffusion in determining antimicrobial sensitivity have been reported, depending on types of specific organisms and antibiotics used in the studies (Katz et al., 2004; Rahbar et al., 2006; Hsueh et al., 1997; Lo-Ten-Foe et al., 2007). Erfani et al. (2008) found good agreement between two methods in sensitivity testing of *E. coli* for TMP- SMX and Nitrofurantoin in UTI. The agreement reported weak for gentamicin, ciprofloxacin and ceftazidime.

Manoharam et al. (2003) study indicates that for TMP-SMX in susceptibility testing of "haemophilus influenza", Disk diffusion shows a very major (2%) and minor(4%) interpretative errors when compared with reference method, while E-test produced only minor interpretative errors (Mnoharan et al., 2003). These results undermine more accuracy in respect to reference methods for E-test in compare to Disk diffusion testing. While E-test has a more sensitivity and specificity in susceptibility testing, because of its cost and limited availability, disk diffusion has been suggested as a preliminary screening test before conduction of E-test (Katz et al., 2004). This two level antibacterial sensitivity testing in which all strains that are multidrug resistant in disk diffusion method are retested by E-test method is supported by findings of present study. This strategy would provide effective antibacterial treatment for UTI patients while reducing incidence of drug resistant strains of *E. coli*.

Conclusion

These antibiotic susceptibility data revealed that there is an important difference between E-test and disk diffusion agar in assessing the antibiotic susceptibility. It seems that E-test is superior to disk diffusion in deter-mining multidrug resistance more accurately. Therefore the E-test method is recommended for multidrug resistant *E. coli* in order to prevent spreading of antibiotic resistant bacteria.

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REFERENCES

Barry AL, Fuchs PC, Brown SD (2001). Identification of beta-lactamase-negative, ampicillin-resistant strains of Haemophilus influenzae with four methods and eight media. Antimicrob. Agents. Chemother., 45: 1585-1588.

Daza R, Gutie´rrez J, Pie´drola G (2001). Antibiotic susceptibility of bacterial strains isolated from patients with community-acquired urinary tract infections. Int. J. Antimicrob. Agents., 18: 211-215.

Erfani Y, Choobineh H, Safdari R, Rasti A, Alizadeh S(2008). Comparison of E-test and Disk Diffusion Agar in amtibiotic Suseceptibility of *E. coli* Isolated from patients with urinary tract infections in Shariati Hospital(Iran). Res. J. Biol. Sci., 3: 24-27

Farrell DJ, Morrissey I, Robbins M, Felmingham D (2003). A UK mulicenre sud of The Antimicrobial Susceptibility of Bacterial Pathogens Causing Urinary tract Infections. J. Infect., 46: 94-100.

Gales AC, Jones RN, Gordon KA, Sader HS, Wilke WW, Beach ML, Pfaller MA, Doern GV (2000). The SENTRY Study Group Latin America. A GV and spectrum of 22 antimicrobial agents tested against urinary tract infection pathogens in hospitalized patients in Latin America: report from the second year of the SENTRY antimicrobial surveillance program (1998). J. Antimicrob. Chemother., 45: 295-303.

Giamarellou H, Poulakou G (2009). Multidrug-resistant Gram-negative infections: what are the treatment options? Drugs, 69: 1879-1901.

Hsueh PR, Chang GC, Teng LJ, Yang PC, Ho SW (1997). Comparison of Etest and Agar Dilution Method for Antimicrobial

Susceptibility Testing of Flavobacterium Isolates. J. Clin. Microb., 35: 1021-1023.
Johson JR, Delavari P, Azar A (1999). Activities of a Nitrofurazone-Containing Urinary Catheter and a Silver Hydrogel Catheter against Multidrug-Resistant Bacteria Characteristic of Catheter-Associated Urinary Tract Infection. Antimicrob. Agents. Chemother., 43: 2990-2995
Katz OT, Peled N, Yagupsky P (2004). Evaluation of the current NCCLS guidelines for screening and confirming extended – spectrum beta-lactamase production in isolates of *E. coli* and *Klebsiella* species from bacteremic patients. Eur. J. Clin. Microbiol. Infect. Dis., 23: 813-817.
Kelly LM, Jacobs MR, Appelbaum PC (1999). Comparison of agar dilution, microdilution, E-test, and disk diffusion methods for testing activity of cefditoren against Streptococcus pneumoniae. J. Clin. Microbiol., 37: 3296-3299.
Khan AU, Zaman MS (2006). Multiple drug resistance in Urinary Tract Infection patients in Aligarh. Biomed. Res., 17: 179-181.
Lo-Ten-Foe JR, De Smet AM, Diederen BM, Kluytmans JA, Van Keulen PH (2007). Comparative evaluation of the VITEK 2, disk diffusion, E-test, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant Enterobacter cloacae and Acinetobacter baumannii strains. Antimicrob. Agents. Chemother., 51: 3726-3730.
Mnoharan A, Pai R, Shankar V, Thomas K, Lalitha MK (2003). Comparison of disk diffusion and E-test methods with agar dilution for antimicrobial susceptibility testing of Haemophilus influenza. Indian J. Med. Res., 117: 81-87.
Rafay AM, Nsanze HN (2003). Multi-drug resistance of *E. coli* from the urinary tract.Saudi. Med. J., 24: 261-264.
Rahbar M, Yaghoobi M, Fattahi A (2006). Comparison of different laboratory methods for detection of Methicillin Resistant *Staphylococcus Aureus*. Pak. J. Med. Sci., 22: 442-445.
Rosser SJ, Alfa MJ, Hoban S, Kennedy J, Harding GK (1999). E-test versus agar dilution for antimicrobial susceptibility testing of viridans group streptococci. J. Clin. Microbiol., 37: 26-30.
Rossolini GM, Mantengoli E (2008). Antimicrobial resistance in Europe and its potential impact on empirical therapy. Clin. Microbiol. Infect., 14(6): 2-8.
Sahm DF, Thornsberry C, Mayfield DC, Jones ME, Karlowsky JA (2001). Multidrug Resistant Urinary Tract Isolates of Escherichia coli: Prevalence and patient Demographics in the United States in 2000. Antimicrob. Agents Chemother., 45: 1402-1406.
Talebi M, Golestanpour A (2009). Symptomatic nosocomial urinary tract infection in ICU patients. Iranian J. Clin. Infect. Dis., 4: 25-29.
Yilmaz N, Agus N, Yurtsever SG, Pullukcu H, Gulay Z, Coskuner A, Kose S, Aydemir S, Gulenc N, Ozgenc O (2009). Prevalence and antimicrobial susceptibility of *E. coli* in outpatient urinary isolates in Izmir, Turkey. Med. Sci. Monit., 15: 161-165.