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Assessment of Minimal Inhibitory Concentration (MIC) by E Test of Ocular Antibiotics Used for Treatment of Patients with Keratitis and Postoperative Ocular Infections

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Abstract: The primary aim of this study was to validate the performance, accuracy and utility of E test and verify its reproducibility for the measurement of minimal inhibitory concentration (MIC) in comparison to reference method as broth microdilution. Methods evaluation of E test was done by testing the sensitivity of selected ocular bacteria; *Staphylococcus aureus, S. epidermidis*, *Klebsiella* and *Pseudomonas aeruginosa* to three antibiotics; gentamycin, ciprofloxacin and gatifloxacin by three independent assays; disk diffusion, broth microdilution and E-test. Results MIC of gentamycin and ciprofloxacin measured by brothmicrodilution & E- test showed significant correlation for all selected bacteria except for *S. epidermidis*. While gatifloxacin showed no significant correlation between the two methods for all staphylococci and *Pseudomonas.* When *Klebsiella* was tested against the three antibiotics their MIC values measured by the two methods showed significant correlation. Conclusion E test represents a valid and reliable method which is less laborious rapid and easy compared to the broth microdilution method. Also the data represented verify our earlier suggestion that correlations between E test and broth microdilution varies according to the type of tested bacteria as well as the antibiotic in charge.

Key words: Broth microdilution · Gentamycin · Ciprofloxacin · Gatifloxacin

INTRODUCTION

Infection of the eye results from either the acquisition of a virulent microorganism or uncontrolled growth of an existing organism because of lowered host resistance [1]. Although the fluoroquinolones were introduced for the treatment of corneal and conjunctival infections they have found yet another important role in the prophylaxis of postoperative endophthalmitis. The widespread routine use of fluoroquinolones has led to an increase in the resistance of endophthalmitis-causing microbes, particularly Gram- positive bacteria, to the commercially available second and third generation fluoroquinolones. However fluoroquinolones resistance in bacterial keratitis has been reported in *Pseudomonas*, spp. *Staphylococcus aureus, Streptococcus* and coagulase-negative *Staphylococcus species* [2].

Coagulase-negative staphylococci remain the most frequent pathogen recovered from post cataract endophthalmitis. The consensus is that the origin of pathogens recovered from post cataract infections are seeded from the patient's conjunctiva. Miller *et al*. [3] documented a high level fluoroquinolone cross resistance among coagulase negative endophthalmitis isolates. Increasing resistance to ciprofloxacin was paralleled by increasing resistance to both moxifloxacin and gatifloxacin. Isolates resistant to ciprofloxacin were in general also resistant to moxifloxacin and gatifloxacin [3, 4]. In order to detect emergence of antimicrobial resistance it is important to use a practical, consistent and standardized method that will allow comparison with national or international monitoring data. Results from antimicrobial susceptibility tests should be reported quantitatively rather than qualitatively, providing the minimal concentration of an antimicrobial required to

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inhibit the growth of the microorganism (MIC). This approach would facilitate the detection of small changes in antimicrobial susceptibility over time [5]. Broth microdilution is a technique in which standardized suspensions of bacteria are tested against varying concentrations of antimicrobial agent in a standardized liquid medium [5, 6]. Epsilometer or E- test is a gold standard test according to CDC [7] a technique for direct determination of the MIC. A gradually increasing concentration of the antibiotic is fixed along a rectangular plastic test strip which is applied to the surface of an inoculated agar plate. After overnight incubation, a tear-drop shaped inhibition zone is seen. The zone edge intersects the graded test strip at the MIC of the antibiotic [8]. The E-test method is less laborious, less expensive when testing a limited number of antimicrobials ($\leq$3) per microorganism and easier to perform than the agar dilution technique thus making it an attractive alternative for antimicrobial susceptibility testing [5].

The primary aim of this study was to validate the performance, accuracy and utility of E test and verify the reproducibility of this convenient predefined gradient methodology for MIC determination in comparison to reference method as broth microdilution. Evaluation was done by testing the sensitivity of the selected ocular bacteria; *Staphylococcus aureus*, Coagulase negative *Staphylococcus* (CNS), *Klebsiella* and *Pseudomonas aeruginosa* to three antibiotics; gentamycin, ciprofloxacin and gatifloxacin by three independent assays; disk diffusion, broth microdilution and E-test.

MATERIALS AND METHODS

Conjunctival swabs were taken from cases with ocular infections, keratitis & endophthalmitis who had been told to stop local or systemic antibiotic medication for two days prior to the swab. Culturing of the conjunctival swabs was done. Identification and isolation of bacteria was done. *Staphylococcus aureus,* Coagulase negative *Staphylococcus* (*Staphylococcus epidermidis*), *Klebsiella* and *Pseudomonas aeruginosa* were selected 10 strains for each. So finally we had four sets, each of ten bacterial stains to be studied for their sensitivity to three antibiotics; gentamycin, ciprofloxacin and gatifloxacin. By three independent assays; disk diffusion, broth microdilution and E-test. Media: Cation-adjusted Mueller-Hinton broth (MHB) was used for broth microdilution and Mueller-Hinton agar was used for disk diffusion and E test. The discs of the three antibiotics were applied from "Oxoid" and disc diffusion Modified Kirby-Bauer- method was done. Dilution procedures: Broth microdilution technique was performed by using sterile, disposable, 96–well microtiter plates (Falcon) carried out according to the Clinical Laboratory Standards Institute (CLSI) formerly the National Committee for Clinical Laboratory Standards (NCCLS) guideline [9]. MHB used for MIC determinations was prepared fresh. Microdilution plates were prepared on the day of use and the freshly prepared antibiotic stock solution was serially diluted in fresh MHB to provide a range of twofold doubling dilutions to match the E test concentration gradient range. The MIC was determined as the lowest concentration of the antibiotic that inhibited growth as judged by the unaided eye. E-test: HiComb MIC test (From HiMedia Laboratories Pvt. Limited, Mumbai, India) consisted of a strip made of inert material with 8 extensions that carry the discs of 4mm, resembling the tooth of a comb. A defined concentration of antibiotic was located on each of the disc so as to form a gradient when placed on agar plate. HiComb which was based on the principle of dilution and diffusion consists of a gradient that covers a continuous range of 16 twofold dilutions on 2 different strips A and B as per the conventional MIC method. When applied to the agar surface the antibiotic instantaneously diffuses into the surrounding medium in high to low amounts from one end of the strip to the other. The gradient remains stable after diffusion and the zone of inhibition created takes the form of ellipse. The HiComb MIC range for Gentamycin was (0.001-240 µg/ml), ciprofloxacin (0.001-240µg/ml) & gatifloxacin (0.001-64µg/ml). Inoculums preparation of each isolated ocular bacteria; a loopful of the test organism was inoculated into 5 ml of nutrient broth and incubated at 37°C for 24h. Then 0.2ml from the 24h. Culture of the organism was dispensed into 19.8ml sterile nutrient broth and incubated for 3-5h to standardize the culture to 10^6 cfu/ml according to Abalaka *et al*. [10]. Then a sterile cotton swab dipped into the suspension to evenly streak the surface of Mueller-Hinton agar plates to be used for application of the chosen antibiotic E test comb strips. Plates were then incubated for 18 to 24 hours. The MIC value was read as the point where the growth inhibition ellipse intersected the MIC on the E test gradient strip (Fig. 1).

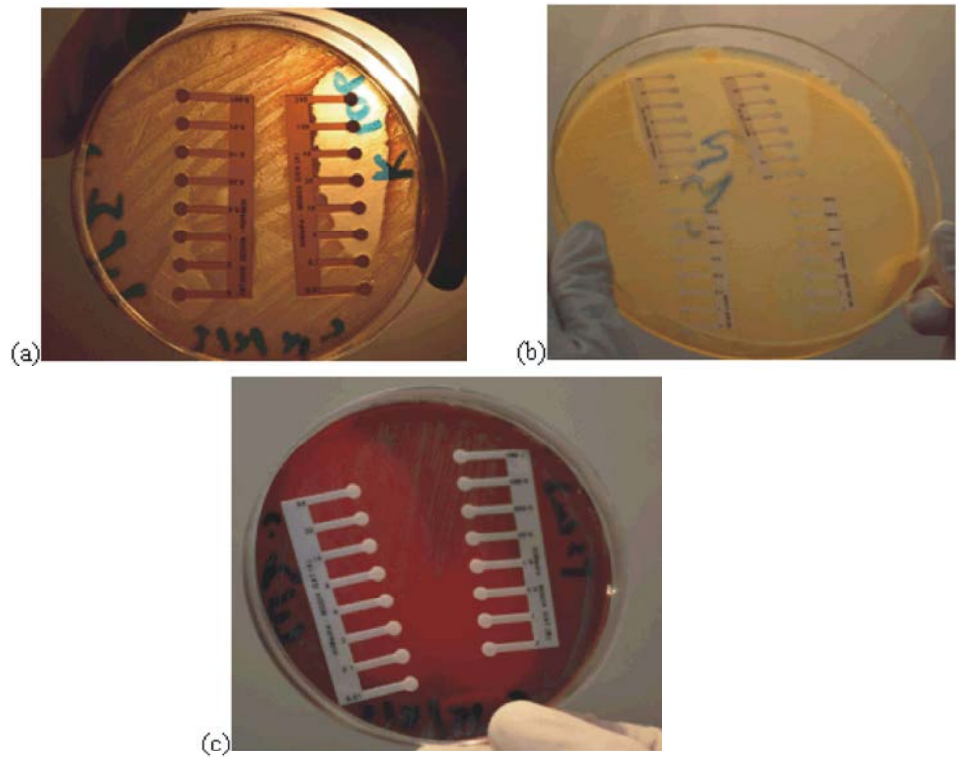

Fig. 1: a) E test comb strips of gentamycin placed on Mueller-Hinton agar plate cultured with *Klebsiella* showing MIC = 0.1µg/ml. b) 150-mm Mueller-Hinton agar plate showing MIC of ciprofloxacin and gatifloxacin by E test comb strips to *Staphylococcus aureus*. c): E test comb strips of gatifloxacin placed on blood agar plate cultured with *Pseudomonas* showing MIC=0.1µg/ml.

RESULTS

Resistant strains to any one of the three antibiotics were excluded statistically from our results. Statistical analysis of our data were subjected to statistical analysis of variance, F- test "One way ANOVA". Duncan's multiple range test is one of the multiple-comparisons procedures. It uses the "t" distribution corresponding to the number of degrees for error mean square. The significance of the measured data were considered as follows; not significant when P>0.05, significant when P <0.05 & highly significant when P < 0.01 where P is the probability "Reflect of null hypothesis" [11]. For the three antibiotics it was normal to find that an increase in the diameter of inhibition zone by the disk diffusion method was accompanied by decrease in the MIC measured by brothmicrodilution and by E-test methods for all tested bacteria (Table 1).

When testing sensitivity of *S. aureus* to gentamycin a significant correlation between MIC measured by broth microdilution as a reference method & by E- test was proved P <0.05 while no significant correlation was found for *S. epidermidis* P >0.05 (Table 2).

MIC of gentamycin sensitivity to *Klebsiella* measured by brothmicrodilution & by E-test showed significant coefficient correlation P <0.05 and highly significant for *Pseudomonas* P <0.01 (Table 2).

MIC of ciprofloxacin to *S. aureus, Klebsiella* and *Pseudomonas* measured by brothmicrodilution & by E- test showed highly significant coefficient correlation P <0.01 while no significant correlation was found for *S. epidermidis* P >0.05 (Table 3).

MIC of gatifloxacin measured by brothmicrodilution & by E-test for *S. aureus, S. epidermidis* and *Pseudomonas* showed no significant correlation P >0.05 But for *Klebsiella* significant coefficient correlation was shown P <0.05 (Table 4).

These results can be summarized in three points: MIC of gentamycin and ciprofloxacin measured by brothmicrodilution & E-test showed significant correlation for all selected bacteria except for *Staphylococcus epidermidis*. While gatifloxacin showed no significant correlation between the two methods for all staphylococci and *Pseudomonas*. MIC of the three antibiotics measured by broth microdilution & E-test for *Klebsiella* showed significant correlation.

Table 1: Ranges of antibiotic susceptibility measured by three different methods for common ocular antibiotics used for selected bacteria causing ocular infections

Ocular Antibiotic	Selected Bacteria	Disk diffusion Inhibition zone	MIC by Broth micro- dilution	MIC by E-test
Gentamycin	*S. aures*	18-20mm	0.5-4µg/ml	0.5-5µg/ml
	S. epidermidis	18-25mm	2-8µg/ml	1-5µg/ml
	Klebsiella	13-15mm	2-4µg/ml	0.01-1µg/ml
	Psuedomonas	16-21mm	0.2-4µg/ml	0.01-5µg/ml
Ciprofloxacin	*S. aures*	22-27mm	0.5-2µg/ml	1-3µg/ml
	S. epidermidis	20-30mm	0.5-2µg/ml	0.3-2µg/ml
	Klebsiella	18-22mm	1-2µg/ml	0.1-0.2µg/ml
	Psuedomonas	25-33 mm	0.7-2 µg /ml	0.5-5µg/ml
Gatifloxacin	*S. aures*	20-27mm	0.06-0.2µg/ml	0.1-0.5µg/ml
	S. epidermidis	25-30mm	0.2-0.4µg/ml	0.1µg/ml
	Klebsiella	20-25mm	0.1-0.5µg/ml	0.03-0.5µg/ml
	Psuedomonas	20-25mm	0.5-1.3µg/ml	0.01-1 µg/ml

Table 2: Correlations between three methods for gentamycin susceptibility to bacteria causing keratitis and postoperative ocular infections.

			Disk	Broth	E test
Staphylococcus aureus	Disk	Pearson Correlation	1	-0.931**	-0.654*
		Sig. (2-tailed)		0	0.04
		N	10	10	10
	Broth	Pearson Correlation	-0.931**	1	0.712*
		Sig. (2-tailed)	0		0.021
		N	10	10	10
	E test	Pearson Correlation	-0.654*	0.712*	1
		Sig. (2-tailed)	0.04	0.021	
		N	10	10	10
Staphylococcus epidermidis	Disk	Pearson Correlation	1	0	-0.134
		Sig. (2-tailed)		1	0.713
		N	10	10	10
	Broth	Pearson Correlation	0	1	0.522
		Sig. (2-tailed)	1		0.122
		N	10	10	10
	E test	Pearson Correlation	-0.134	0.522	1
		Sig. (2-tailed)	0.713	0.122	
		N	10	10	10
Klebsiella	Disk	Pearson Correlation	1	0.357	0.32
		Sig. (2-tailed)		0.312	0.367
		N	10	10	10
	Broth	Pearson Correlation	0.357	1	0.725*
		Sig. (2-tailed)	0.312		0.018
		N	10	10	10
	E test	Pearson Correlation	0.32	0.725*	1
		Sig. (2-tailed)	0.367	0.018	
		N	10	10	10
Pseudomonas aeruginosa	Disk	Pearson Correlation	1	-1.000**	-0.999**
		Sig. (2-tailed)		0	0
		N	10	10	10
	Broth	Pearson Correlation	-1.000**	1	0.999**
		Sig. (2-tailed)	0		0
		N	10	10	10
	E test	Pearson Correlation	-0.999**	0.999**	1
		Sig. (2-tailed)		0	
		N	10	10	10

*Correlation is significant at the 0.05 level **Correlation is highly significant at the 0.01 level (2-tailed).

Table 3: Correlations between three methods for ciprofloxacin susceptibility to bacteria causing keratitis and postoperative ocular infections

			Disk	Broth	E test
Staphylococcus aureus	Disk	Pearson Correlation	1	-0.898**	-0.994**
		Sig. (2-tailed)		0	0
		N	10	10	10
	Broth	Pearson Correlation	-0.898**	1	0.915**
		Sig. (2-tailed)	0		0
		N	10	10	10
	E test	Pearson Correlation	-0.994**	0.915**	1
		Sig. (2-tailed)	0	0	
		N	10	10	10
Staphylococcus epidermidis	Disk	Pearson Correlation	1	-0.235	-0.32
		Sig. (2-tailed)		0.513	0.368
		N	10	10	10
	Broth	Pearson Correlation	-0.235	1	0.592
		Sig. (2-tailed)	0.513		0.071
		N	10	10	10
	E test	Pearson Correlation	-0.32	0.592	1
		Sig. (2-tailed)	0.368	0.071	
		N	10	10	10
Klebsiella	Disk	Pearson Correlation	1	-0.889**	-0.881**
		Sig. (2-tailed)		0.001	0.001
		N	10	10	10
	Broth	Pearson Correlation	-0.889**	1	0.783**
		Sig. (2-tailed)	0.001		0.007
		N	10	10	10
	E test	Pearson Correlation	-0.881**	0.783**	1
		Sig. (2-tailed)	0.001	0.007	
		N	10	10	10
Pseudomonas aeruginosa	Disk	Pearson Correlation	1	0.764*	0.467
		Sig. (2-tailed)		0.01	0.174
		N	10	10	10
	Broth	Pearson Correlation	0.764*	1	0.802**
		Sig. (2-tailed)	0.01		0.005
		N	10	10	10
	E test	Pearson Correlation	0.467	0.802**	1
		Sig. (2-tailed)	0.174	0.005	
		N	10	10	10

*Correlation is significant at the 0.05 level **Correlation is highly significant at the 0.01 level(2- tailed).

Table 4: Correlations between three methods for gatifloxain susceptibility to bacteria causing keratitis and postoperative ocular infections

			Disk	Broth	E test
Staphylococcus aureus	Disk	Pearson Correlation	1	-0.930**	-0.608
		Sig. (2-tailed)		0	0.062
		N	10	10	10
	Broth	Pearson Correlation	-0.930**	1	0.599
		Sig. (2-tailed)	0		0.067
		N	10	10	10
	E test	Pearson Correlation	-0.608	0.599	1
		Sig. (2-tailed)	0.062	0.067	
		N	10	10	10
Staphylococcus epidermidis	Disk	Pearson Correlation	1	-0.583	-0.571
		Sig. (2-tailed)		0.077	0.085
		N	10	10	10
	Broth	Pearson Correlation	-0.583	1	0.456
		Sig. (2-tailed)	0.077		0.185
		N	10	10	10
	E test	Pearson Correlation	-0.571	0.456	1
		Sig. (2-tailed)	0.085	0.185	
		N	10	10	10

Table 4: Continued

			Disk	Broth	E test
Klebsiella	Disk	Pearson Correlation	1	-0.712*	-0.896**
		Sig. (2-tailed)		0.021	0
		N	10	10	10
	Broth	Pearson Correlation	-0.712*	1	0.637*
		Sig. (2-tailed)	0.021		0.048
		N	10	10	10
	E test	Pearson Correlation	-0.896**	0.637*	1
		Sig. (2-tailed)	0	0.048	
		N	10	10	10
Pseudomonas aeruginosa	Disk	Pearson Correlation	1	-0.557	-0.127
		Sig. (2-tailed)		0.094	0.727
		N	10	10	10
	Broth	Pearson Correlation	-0.557	1	0.221
		Sig. (2-tailed)	0.094		0.54
		N	10	10	10
	E test	Pearson Correlation	-0.127	0.221	1
		Sig. (2-tailed)	0.727	0.54	
		N	10	10	10

*correlation is significant at the 0.05 level **Correlation is highly significant at the 0.01 level (2- tailed).

Meanwhile the correlation between disk diffusion method and broth microdilution showed almost the same relationships as between E test and brothmicrodilution with two exceptions *Klebsiella* against gentamycin and *S. aureus* against gatifloxacin.

DISCUSSION

The E test predefined gradient strip can be set up as easily as a Kirby-Bauer disk diffusion test by most clinical laboratories without the need for specialized equipment. As novel antimicrobial agents become available for clinical use, the commercial availability and performance validations of E test gradient strips for these agents in comparison to reference methods becomes an essential exercise [9]. The data presented in this study suggests that correlations between E test and broth microdilution varies according to the type of tested bacteria as well as the antibiotic in charge. In a study made by Joyce *et al*. [12] comparing five methods for antimicrobial susceptibility testing of six antibiotics against *Pseudomonas aeruginosa* they reported the greatest differences in the results from the different methods were with gentamycin as it gave only 59% complete agreement between the E test and the broth microdilution using the broth microdilution as the reference method. In the present study MIC of gentamycin measured by brothmicrodilution & E test showed significant correlation for all selected bacteria except for CNS. Also Correlation between disk diffusion method and brothmicrodilution showed almost the same relationships as between E test and brothmicrodilution with an exception to *Klebsiella*. In a similar study made by Mayrhofer *et al*. [13] also tested a set of 10 strains of the *Lactobacillus acidophilus* group to four bactericidal drugs and three bacteriostatic agents in three independent assays; broth microdilution, disk diffusion and E test. They reported that in general, the broth microdilution and E test results were in good agreement. The overall agreement between these two susceptibility testing procedures, which should be higher than 90% was sufficiently achieved for the bactericidal drugs ampicillin, gentamicin, streptomycin and vancomycin. The minor satisfying agreement between the results obtained with the bacteriostatic agents clindamycin, erythromycin and especially tetracycline.

One of the strategies for improving the treatment of endophthalmitis and keratitis is to test the efficacy of newer antibiotics on the microbial spectrum. A few studies have been done using E test as a tool to assess the activity of newer fluoroquinolones against bacteria isolated from ocular infections Duggirala *et al*. [2] in their study reported gatifloxacin and moxifloxacin are equally effective and have a definite advantage over ciprofloxacin against gram-positive bacteria. Gatifloxacin and moxifloxacin are fourth generation fluoroquinoloes which are targeted against two DNA replicating enzymes, Duggirala and his coauthors hoped that Gatifloxacin and moxifloxacin will remain the drug of choice for gram-positive bacteria for a longer duration. Meanwhile they concluded that ciprofloxacin which is a second generation fluoroquinoloes acting on single DNA replicating enzyme remains the most effective

fluoroquinolone against gram-negative bacteria. In a study made by Luber *et al*. [14] where MIC values of six antimicrobial agents were determined by the broth microdilution and E test methods for *Campylobacter* isolates revealed the levels of agreement between the two methods were high for erythromycin (95.9%), tetracycline (95.9%) and gentamicin (94.6%) but slightly lower levels of agreement were shown for the quinolones ciprofloxacin (88.4%) and nalidixic acid (75.9%) due to the tendency of the E test to produce lower MICs for this class of agents. While Rennie *et al*. [15] compared the M.I.C. Evaluator strip, E test and broth microdilution by testing 14 antimicrobial agents included gentamycin and ciprofloxacin against Gram positive bacteria which included *S. aures* and *S. epidermidis* and Gram negative bacteria which included *Pseudomonas* and *Klebsiell*a, they have noted that with some microorganism-antimicrobial agent combinations, MIC by E test tend to report slightly higher MICs than broth microdilution and may well identify resistance determinants more readily (Data not shown). This phenomenon has been observed in comparisons of the E test to commercial broth dilution and they explained it is possible that the small volume used in the broth method reduces the likelihood of finding slower-growing resistant subpopulations, but this is not well understood. Recently Campana *et al*. [16] assumed that E test & Evaluator strips had to be compared with agar diffusion and not with broth microdilution as they were motivated by the results of their previous study [17] when they compared the vancomycin MICs determined by M.I.C.E. with those obtained by CLSI broth microdilution (BMD) and observed that the vancomycin MICs values determined by M.I.C.E. were higher than those obtained by BMD, a similar finding to that was reported by Rennie *et al*. [15] and they thought these results could have resulted from the different techniques, gradient agar diffusion vs. BMD, employed. However Campana *et al*. [16] according to their results where the E test showed better performance than M.I.C.E. for predicting vancomycin MICs against all staphylococci, while linezolid and teicoplanin MICs were more accurately predicted by M.I.C.E. strips, concluded that microbiologists must be aware of the different performance of commercially available gradient strips against staphylococci. All these discussed data with our present data verify our earlier suggestion that correlations between E test and broth microdilution varies according to the type of tested bacteria as well as the antibiotic in charge. The direct detection of resistance genes by polymerase chain reaction or similar techniques has limited utility, because only a few resistance genes are firmly associated with phenotypic resistance (eg, mecA, vanA and vanB) there are hundreds of â-lactamases and numerous mutations, acquisitions and expression mechanisms that result in fluoroquinolone, aminoglycoside and macrolide resistance too many to be easily detected by current molecular techniques, thus it seems likely that phenotypic measures of the level of susceptibility of bacterial isolates to antimicrobial agents will continue to be clinically relevant for years to come [18]. We agree with the conclusion made by Yah *et al*. [19] stating that E test strip method is a reliable, rapid, easy but slightly expensive susceptibility testing technique. It combines the activity of both diffusion and MIC dilution methods with a distinct intermediate sensitivity. The agar disc diffusion method also is a reliable, rapid, easy and inexpensive but does not combine the two fronts as in E test and does not have a good distinct intermediate sensitivity. We strongly recommend the use of E test sensitivity method in research in developing countries. Also we agree with the recommendation made by Liu *et al*. [20] that would likely allow for successful transition from agar dilution to the E test.

REFERENCES

1. Chung, J.L., K.Y. Seo, D.E. Yong, F.S. Mah, T. Kim, E.K. Kim and J.K. Kim, 2009. Antibiotic susceptibility of conjunctival bacterial isolates from refractive surgery patient. Ophthalmology, 116: 1067-1073.
2. Duggirala, A., J. Joseph, S. Sharma, R. Nutheti, P. Garg and T. Das, 2007. Activity of newer fluoroquinolones against gram-positive and gram-negative bacteria isolated from ocular infections: an *in vitro* comparison Indian J. Ophthalmol., 55: 15-9.
3. Miller, D., P.M. Flynn, I.U. Scott, E.C. Alfonso and H.W. Flynn Jr., 2006. In vitro fluoroquinolone resistance in staphylococcal endophthalmitis isolates. Arch. Ophthalmol., 124: 479-83.
4. Miller, D., 2008. Emerging 8-methoxy fluoroquinolone resistance among methicillin sensitive *Staphylococcus epidermidis* recovered from patients with endophthalmitis. Clin. Ophthalmol., 2: 77-91.
5. Varela, N.P., R. Friendship, C. Dewey and A. Valdivieso, 2008 Comparison of agar dilution and E-test for antimicrobial susceptibility testing of *Campylobacter coli i*solates recovered from 80 Ontario swine farms. Can. J. Vet. Res., 72: 168-174.

6. van der Heijden, I.M., A.S. Levin, E.H. De Pedri, L. Fung, F. Rossi, G. Dubo, A.A. Barone and S.F. Costa, 2007. Comparison of disc diffusion, E test and broth microdilution for testing susceptibility of carbapenem-resistant *P. aeruginosa* to polymyxins. Annals of Clinical Microbiology and Antimicrobial, 6:8 doi:10.1186/1476-0711-6-8.
7. Rao, B.N. and T. Prabhakar, 2011. Reduced susceptibility of vancomycin in methicillin resistant *Staphylococcus aureus* (MRSA) in and around Visakhapatnam andhra Pradesh. Journal of Pharmaceutical and Biomedical Sciences, 10: 1-5.
8. El- Mashad, A.M., 2002. Manual of Practical Microbiology. 7th ed. El-Ahram press, Egypt.
9. Bolmstrom, A., A. Karlsson, A. Engelhardt, P. Ho, P.J. Petersen, P.A. Bradford and C.H. Jones, 2007. Validation and reproducibility assessment of tigecycline MIC determinations by E test. J. Clin. Microbiol., 45: 2474-2479.
10. Abalaka, M.E., S.Y. Daniyan, S.B. Oyeleke and S.O. Adeyemo, 2012. The antibacterial evaluation of Moringa Oleifera leaf extracts on selected bacterial pathogens. Journal of Microbiology Research, 2: 1-4.
11. Armitage, P., 1971. Statistical Methods in Medical Research. Blackwell Scientific Publications, London.
12. Joyce, L.F., J. Downes, K. Stockman and J.H. Andrew, 1992. Comparison of five methods, including the PDM Epsilometer test (E test), for antimicrobial susceptibility testing of *Pseudomonas aeruginosa*. J. Clin. Microbiol., 30: 2709-2713.
13. Mayrhofer, S., K.J. Domig, C. Mair, U. Zitz, G. Huys and W. Kneifel, 2008. Comparison of broth microdilution, E test and agar disk diffusion methods for antimicrobial susceptibility testing of *Lactobacillus acidophilus* group. Appl. Environ. Microbiol., 74: 3745-3748.
14. Luber, P., E. Bartelt, E. Genschow, J. Wagner and H. Hahn, 2003. Comparison of broth microdilution, E test and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. J. Clin. Microbiol., 41: 1062-1068.
15. Rennie, R.P., L. Turnbull, C. Brosnikoff and J. Cloke, 2012. First comprehensive evaluation of the M.I.C. Evaluator Device compared to E test and CLSI broth microdilution for MIC testing of aerobic Gram-positive and Gram-negative bacterial species. J. Clin. Microbiol., 50: 1147-1152.
16. Campana, E.H., C.G. Carvalhaes, B. Nonato, A.M.de.O. Machado and A.C. Gales, 2014. Comparison of M.I.C.E. and E test with CLSI Agar Dilution for Antimicrobial Susceptibility Testing against Oxacillin-Resistant *Staphylococcus* spp. PLoS. ONE, 9(4): e94627. doi:10.1371/ journal.pone.0094627.
17. Carvalhaes, C.G., E.H. Campana, P.P. Barbosa, A.M. Paula and A.C. Gales, 2011. Comment on: Performance of the Oxoid M.I.C. Evaluator strips compared with the Etest and BSAC agar dilution. J. Antimicrob. Chemother. 66: 1192–1193. doi: 10.1093/ jac/ dkr065.
18. Reller, L.B., M. Weinstein, J.H. Jorgensen and M.J. Ferraro, 2012. Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clinical Infectious Disease, 49: 1749-1755.
19. Yah, S.C., F.P. Ching and E.I. Atuanya, 2008 Comparison of the E test and the routine multi-disc agar diffusion susceptibility of *Staphylococcus* species. (E test and routine agar testing of *Staphylococcus*). Sudan JMS., 3(1-4): 121-122.
20. Liu, H., T.H. Taylor Jr, K. Pettus and D. Trees, 2014. Assessment of E test as an alternative to agar dilution for antimicrobial susceptibility testing of *Neisseria gonorrhoeae.* J. Clin. Microbiol., 52: 1435-1440.