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Comparison of E-test with other conventional susceptibility testing methods for ciprofloxacin and gentamicin against gram negative enteric bacilli

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Abstract

Background: Increasing antibiotic resistance in Gram negative bacteria has led to the need for a faster and reliable method for determining antimicrobial susceptibility testing. In a resource poor setting like ours, it's also important to look for methods that will be clinically and economically beneficial to the patient.

*Aim:*This study was aimed at evaluating the Epsilometer test (E-test) and conventional methods for determining antimicrobial susceptibility of isolates of Gram-negative enteric bacteria to ciprofloxacin and gentamicin.

Methods: Disc diffusion, E-test, broth dilution and agar dilution methods were performed on 54 bacterial isolates.

Results: Using the E-test, 88.9 % of bacterial isolates were resistant to ciprofloxacin, 92.6 % were resistant using broth microdilution, 96.3 % were resistant using agar dilution and 72.2 % were resistant using disc diffusion. Minimum Inhibitory Concentration (MIC_{50}) of isolates for gentamicin showed significant difference for all the techniques ($p < 0.05$) while MIC_{90} for gentamicin and MIC_{50} and MIC_{90} for ciprofloxacin for all the techniques had no significant difference ($p > 0.05$). Both E-test and broth dilution methods showed high levels of agreement ($p > 0.05$), there were low levels of agreement between E-test and agar dilution method ($p < 0.05$), especially at MIC_{50}.

Conclusion: The E-test can therefore be considered a reliable method to determine antimicrobial susceptibility testing and it gives results which are at least as accurate as those obtained by the broth dilution method.

Keywords: *E-test, susceptibility, antibiotics, bacteria, infection*

Résumé

Contexte: L'augmentation de la résistance aux antibiotiques dans les bactéries à Gram négatif a conduit à la nécessité d'une méthode plus rapide et fiable pour déterminer les tests de sensibilité des antimicrobiens. Dans un contexte de ressources pauvre comme le nôtre, il est également important de rechercher des méthodes qui seront cliniquement et économiquement bénéfiques pour le patient.

But: Cette étude visait à évaluer le test epsilomètre (E-test) et les méthodes classiques pour la détermination de la sensibilité des antimicrobiens des isolats de bactéries à Gram négatif entériques à la ciprofloxacine et la gentamicine.

Méthodes: La méthode de la diffusion sur disque, E-test, la dilution par bouillon et de la dilution par gélose a été effectuée sur 54 isolats bactériens.

Résultats: En utilisant le E-test, 88,9% des isolats bactériens étaient résistantes à la ciprofloxacine, 92,6% étaient résistants à l'aide de micro -dilution, 96,3% étaient résistants à l'aide de dilution par gélose et 72,2% étaient résistants à l'aide de diffusion sur disque. CMI50 des isolats pour la gentamicine a montré de différence significative pour toutes les techniques ($p < 0,05$) tandis que CMI90 CMI50 pour la gentamicine et la ciprofloxacine et CMI90 pour toutes les techniques il n'y avait pas de différence significative ($P > 0,05$). Les deux méthodes de dilution E-test et le bouillon ont montré des niveaux élevés d'accord ($p > 0,05$), il y avait de faibles niveaux d'accord entre E-test et la méthode de dilution par gélose ($p < 0,05$), en particulier à la CMI50.

Conclusion: Le E-test peut donc être considéré comme une méthode fiable pour déterminer les tests de sensibilité aux antimicrobiens et donne des résultats qui sont au moins aussi précis que ceux obtenus par la méthode de dilution par bouillon.

Introduction

Antimicrobial resistance is a growing problem worldwide, requiring international approaches. Widespread use of antibiotics is thought to have spurred evolutionary changes in bacteria that allow them to survive treatment with these powerful drugs [1]. Therefore, bacterial diseases such as tuberculosis, gonorrhoea, meningitis, typhoid and childhood ear infections are now more difficult to treat

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Table 2. MIC Results using various susceptibility testing methods

Organism	Antimicrobial Agent	E-Test		Broth Dilution		Agar Dilution	
		MIC50 (μg/ml)	MIC90	MIC50 (μg/ml)	MIC90	MIC50 (μg/ml)	MIC90
E. coli (9)	Ciprofloxacin	>32	>32	>32	>32	>32	>32
	Gentamicin	32	>256	32	256	256	256
K. pneumoniae (3)	Ciprofloxacin	>32	>32	>32	>32	>32	>32
	Gentamicin	>256	>256	64	256	256	256
P. luteola (3)	Ciprofloxacin	32	>32	32	>32	>32	>32
	Gentamicin	32	64	64	64	256	256
P. oryzihabitans (12)	Ciprofloxacin	32	>32	32	>32	>32	>32
	Gentamicin	8	>256	256	>256	256	256
P. aeruginosa (9)	Ciprofloxacin	32	>32	32	>32	>32	>32
	Gentamicin	32	>256	32	256	256	256
E. cloacae (6)	Ciprofloxacin	32	>32	>32	>32	>32	>32
	Gentamicin	16	>32	32	256	256	256
P. mirabilis (9)	Ciprofloxacin	32	>32	>32	>32	>32	>32
	Gentamicin	32	>256	32	>256	256	256
C. freundii (3)	Ciprofloxacin	4	4	32	>32	>32	>32
	Gentamicin	4	4	32	32	256	256

sensitivity to ciprofloxacin and 33.3% to gentamicin. The susceptibility pattern of the rest of bacterial isolates is shown in Table 1.

Table 2 shows the MIC results of the various isolates to ciprofloxacin and gentamicin using the conventional methods and the E-test.

Figure 1 shows the summary of comparison of various antimicrobial susceptibility testing methods in form of percentage of resistant isolates. MIC_{50} of isolates for gentamicin showed significant difference for all the techniques ($P < 0.05$) while MIC_{90} for gentamicin and MIC_{50} and MIC_{90} for ciprofloxacin for all the techniques had no significant difference ($P > 0.05$). Comparing E-test with each of the techniques, E-test had high level of agreement with broth dilution (significant difference was found between E-test and agar dilution at MIC_{50}).

Discussion

Infections caused by multi-drug resistant Gram negative bacteria are increasing worldwide. The increasing resistance of many antibiotics limits a lot of therapeutic options [8] and in a resource poor setting like ours, it's important to look for methods that will be clinically and economically beneficial to the patient. With disc diffusion, *Proteus mirabilis*

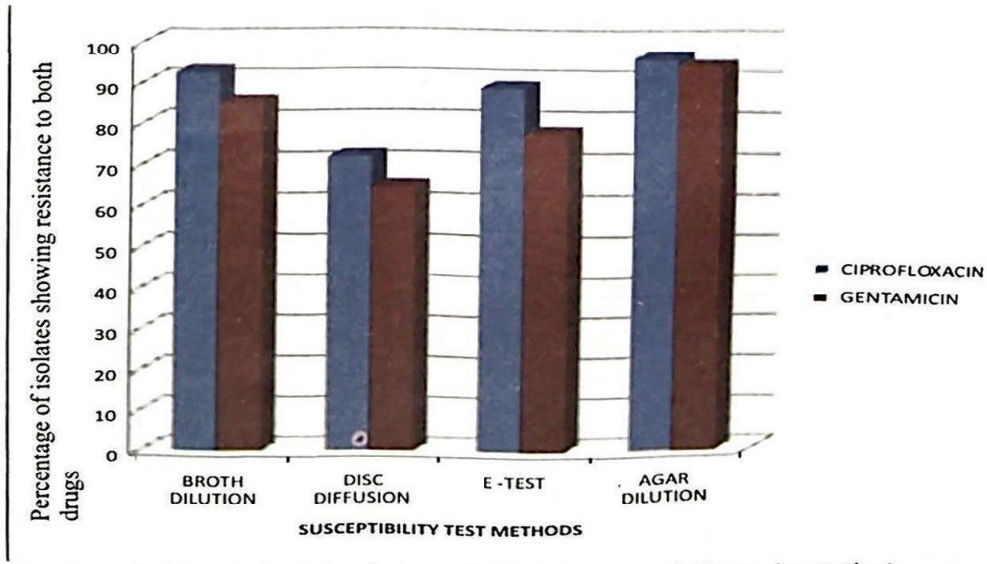

Fig 1: Percentage of resistant isolates using various susceptibility testing methods

for ciprofloxacin, ≥ 21 mm is susceptible, 16-20 mm is intermediate, and ≤ 15 mm is resistant. For gentamicin, ≥ 15 mm is susceptible, 13-14 mm is intermediate, and ≤ 12 mm is resistant [7].

Agar dilution tests

Agar plates containing serial dilutions of antibiotics were prepared. The plates were then inoculated with 1 µl of overnight broth culture of bacteria diluted to 10^6 cfu/ml using a multipoint inoculator (Denley Surrey, UK). The plates were incubated aerobically overnight at 37°C before being read the next day. The MIC was defined as the lowest concentration at which bacterial growth was inhibited. Included in all the experiments were the control organisms; *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). A start and finish plate without antibiotic was also included to ensure adequate growth from the beginning to the end of experiment.

Broth dilution

Serial doubling dilutions of the 2 antibiotics were made from the range of 0.0625 to 512 µg/ml in Mueller-Hinton broth. A drop (0.02 ml) of standard inoculum (0.5 Macfarland) of organisms was introduced into 1 ml of Mueller-Hinton broth containing varying dilutions of antibiotics and these were then incubated at 37°C for 18 hours. MIC was interpreted as the least concentration or highest dilution with no observable turbidity. Controls were set up namely; sterility control: Mueller Hinton broth only, viability control: Mueller Hinton broth and test organism, positive control: Mueller Hinton broth with antibiotics and the control organisms. They were incubated at 37°C overnight. The antibiotics were supplied in powdery formulations, namely; gentamicin (Sigma, UK) and ciprofloxacin (Fluka, UK).

E-test determination

The ciprofloxacin and gentamicin E-tests (Oxoid M.I.C.Evaluator strip) were performed and interpreted according to the manufacturer's instructions. Ciprofloxacin and gentamicin M. I. C. Evaluator strips were dispensed unto the surfaces of inoculated agar plates aseptically and incubated at 37 °C overnight, the M.I.C was read where the growth intersect with the strip (or where the growth of the organism touches the strip) [3]. It was performed using Mueller Hinton agar (Oxoid, Bakingstoke, United Kingdom). MIC breakpoints for defining ciprofloxacin susceptibility used were: susceptible, 1µg/mL; intermediate susceptible, 2 µg/mL; and resistant, 4 µg/mL. Similarly, MIC breakpoints for defining gentamicin susceptibility used were: susceptible, 4 µg/mL; intermediate susceptible, 8 µg/mL; and resistant, 16 µg/mL.

Statistical analysis

The test of significance of results obtained was determined by Chi square method, using the software, Microsoft excel 2003 edition, where the difference was considered to be statistically significant when the P value obtained was less than 0.05 ($P < 0.05$).

Results

The 54 bacterial strains included in this study were *Pseudomonas oryzihabitans* (12), *Pseudomonas aeruginosa* (9), *Pseudomona luteola* (3), *Klebsiella pneumoniae* (3), *Enterobacter cloacae* (6), *Proteus mirabilis* (9), *Escherichia coli* (9), *Citrobacter freundii* (3) from various wards and clinics with patients with varying diseases.

The antimicrobial susceptibilities of bacterial isolates using disc diffusion method showed 66.7% of *P. oryzihabitans* sensitive to ciprofloxacin and 41.7% to gentamicin. *E. cloacae* had 33.3%

Table 1. Susceptibility pattern of bacterial strains using the disc diffusion method

Organism	Ciprofloxacin		Gentamicin	
	S	R	S	R
P.oryzihabitans, 12	8 (66.7)	4 (33.3)	5 (41.7)	7 (58.3)
P. aeruginosa, 9	0 (0)	9 (100)	0 (0)	9 (100)
P. luteola, 3	0 (0)	3 (100)	0 (0)	3 (100)
K. pneumoniae, 3	0 (0)	3 (100)	0 (0)	3 (100)
E. cloacae, 6	2 (33.3)	4 (66.7)	2 (33.3)	4 (66.7)
P. mirabilis	0 (0)	9 (100)	3 (33.3)	6 (66.7)
Escherichia coli, 9	5 (55.6)	4 (44.4)	6 (66.7)	3 (33.3)
C. freundii, 3	3 (100)	0 (0)	3 (100)	0 (0)

() – Numbers in parentheses are percentages

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and *Escherichia coli* seemed to be more susceptible to gentamicin than to ciprofloxacin; this could be as a result of oral administration of ciprofloxacin which makes it to be easily abused compared to gentamicin which is given parenterally.

In this study, E-test and broth dilution method showed high levels of agreement for both ciprofloxacin and gentamicin. Breakpoints for ciprofloxacin resistance are Minimum Inhibitory Concentration (MIC) ≤ 1, susceptible and MIC ≤ 4 μg/ml, resistant and for gentamicin MIC ≤ 4, susceptible and MIC ≤ 8 μg/ml, resistant. This agrees with the work of Jerome *et al.* who showed a high level of agreement between the E-test and broth dilution [8]. Also, findings from study done by Arroyo *et al.* [9] showed that E-test has an excellent agreement with broth dilution methods. E-test and agar dilution showed low levels of agreement in our work.

Similarly, evaluation of the E-test for antimicrobial sensitivity testing of *P. aeruginosa* isolates to ciprofloxacin showed high level of agreement in our study. This is similar to report of Nicodemo *et al.* who observed a high level of agreement between the E-test and agar dilution [10]. E-test has been validated for many organisms against the broth / agar dilution method and shown to have excellent correlation; for example, *Pseudomonas aeruginosa:* amikacin, ceftazidime, gentamicin, piperacillin, ticarcillin, tobramycin [4]. Broth dilution method was considered to be the reference method as was done previously [9]. However, this is different from other reports that stated that E-test has been shown to be more reliable compared to the agar dilution method [11, 12]. In Evangel Hospital, Jos, Nigeria E-test has also been found to be especially useful for susceptibility testing of *Helicobacter pylori* to amoxicillin [13].

E-test was not compared with disc diffusion, it has been reported that results of zone diameter do not always correlate with MIC results [8, 14]. The disc diffusion is more suitable for routine testing in a clinical laboratory for large number of isolates, although, it cannot give a quantitative result and therefore cannot be used for effective monitor of emergence of resistant organisms effectively. Agar dilution is usually employed when many organisms are to be tested e. g. when new agents are to be screened for the spectrum of activity. Although broth dilution test is fairly precise, the test is laborious because serial dilutions of the antibiotic must be made and only one isolate can be tested in each series of dilutions. Both agar dilution and broth dilution take a long time to set up before incubation. E-test is easy to perform, take short time to set up before incubation and it also gives a quantitative result.

In conclusion, evaluation of the E-test and other conventional methods shows that E-test had a high level of agreement with broth dilution but a relatively low level of agreement with agar dilution. E-test will be recommended for susceptibility testing since it combines the ease of disc diffusion and quantitative susceptibility testing. However, the higher cost of the E-test may likely discourage most laboratories, therefore, if quantitative susceptibility testing is required, broth dilution will be recommended if E-test cannot be obtained either due to cost or availability.

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increase occurring in developing countries [1]. In Nigeria, diabetes mellitus is the second most common Non-Communicable Disease (NCD) with a prevalence of 2.2% [2], accounts for 7.6% of medical admission in Sagamu, Nigeria with case fatality of 30% [3]. The major challenges in diabetes care include achieving and maintaining ideal body weight, optimal glycaemic, lipids and blood pressure control as well as increased level of physical activity with good healthy dietary habits all aimed at preventing or suppressing development of diabetes-related complications. Poor control of diabetes mellitus is consequently associated with increased burden of complications, increased health expenditure, poor quality of life (QOL) and attendant mood disorders [4,5]

The significant increase in disease burden of diabetes associated negative effect on quality of life (QOL) and attendant psychosocial effects on the patients necessitated the need to evaluate the psychosocial status in them. Previous studies reported incidence of emotional distress of between 15 to 20% for depression in patients suffering from diabetes mellitus [6]. The coexistence of diabetes and psychological disorders is associated with varying degrees of increased adverse diabetes outcome [7] that include increased risk of morbidity, functional impairment, diabetes related complications and poor quality of life [8,9].

In developing countries where health care expenditure is mainly out of pocket, there are limited reports on psychological profiles of the diabetic population. In Africans and where such studies exist, focus has been mainly on the socioeconomic impact of the disease [10,11]. Akinlade and Ohaeri (1996), in Ibadan reported depression in 25%, anxiety in 4.8% of the studied diabetic population, similar to reports from developed countries [12,13]. The negative impact of psychological disorders in diabetes results in poor adherence to diabetes treatment regimens and health behaviours needed for good management. These include increased physical activity, smoking cessation, healthy dietary habits and good drug compliance [8,9].

For optimum diabetes care, assessment of disease related emotional problems are important for improved clinical outcome. Factors associated with the high co-morbidity of psychological disorders in diabetes have not been fully evaluated in Africans [10]. Some of these factors are related to age, gender, disease state and progression, peripheral neuropathy, neurogenic bladder and erectile dysfunction [5,6]. Others are disease related stressors of poor glycaemic control, hypertension, obesity, dyslipidaemia and some treatment options, particularly insulin regimen. Improved quality of life and diabetes control have been reported when mood disorders are recognized early and followed with prompt and effective treatment [8,15,16].

The study sought to determine the pattern and prevalence of anxiety and depressive illnesses among type 2 diabetic patients attending the medical out-patient clinic of Olabisi Onabanjo University Teaching Hospital, (OOUTH), Sagamu Nigeria, and define the psychological burden with the aim of improving their quality of life, reduce health burden of diabetes as well as identify diabetes-related factors relationship with mood disorders. It also aimed to determine relationship, if any, between mood disorders and diabetes specific complications.

Materials and methods

The study population involved adult T2DM patients attending the medical out-patient clinic of Olabisi Onabanjo University Teaching Hospital Sagamu, Nigeria over a 3 month period. Diagnosis of diabetes mellitus was based on the International Diabetes Federation (IDF) criteria [1]. After obtaining informed consent, each participant was interviewed over a 15-20 minutes period using both the General Health Questionnaire (GHQ-12) and the Hospital Anxiety and Depression Scale (HADS) questionnaire [17,18]. A score of three and above on the GHQ-12 is taken as indicative of psycho-pathology while a score of 8 and above on either part of HADS is diagnostic for anxiety and depression respectively [17]. HADS comprises two 7-item scales, one each for anxiety and depression and each item is rated on a 4-point scale giving a maximum of 21 each for anxiety and depression. The questionnaire was translated by health educators to the native Yoruba language and back translated to ensure reliability. They were administered by trained interns and health educators. Information on demographic data, disease duration, co-morbid factors and diabetes-related complications including degree of glycaemic control were extracted from patients' case records.

Excluded from the study were people below age 18 years, those who declined to participate in the study, the acutely ill and inability to communicate freely in either of Yoruba or English language.

Results

One hundred and two questionnaires were administered of which 98 (96.1%) were returned fully filled. There were 56 (55.1%) females and 46 (44.9%) males with age range between 40-78years (mean 60.4±9.2years) and no significant sex