Comparison of Epsilometer Test and Disc Diffusion Methods for Antibiotic Susceptibility Testing of Pseudomonas Aeruginosa

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Background: Good methods for detecting antimicrobial resistance are of fundamental importance in the curing of infections caused by Pseudomonas aeruginosa.
Objective: The target of this study was to compare and evaluate the performance of the E-test (Epsilometer test) with disc diffusion for Pseudomonas aeruginosa.
Methods: A Total of 300 isolates of Pseudomonas aeruginosa from wound were included as test strains. Antibacterial susceptibility testing was performed by Kirby-Bauer disc Diffusion method. Minimum inhibitory concentration (MIC) determination was performed by E test. MIC values were read where the inhibition ellipses intersect the E. test strip. Breakpoint values were used for interpretation as Sensitive, Intermediate or Resistant. Interpretation of result according to the (CLSI).
Results: Susceptibility testing of 300 Pseudomonas aeruginosa isolates against 11 antimicrobials revealed that cefotaxime showed the highest resistance rate (86.3%) when tested using the disc diffusion method and the E test. Tobramycin exhibited the highest susceptibility rates, with 46.3% and 44% susceptibility observed for the disc diffusion and E test method, respectively. Intermediate susceptibility was most commonly observed with tobramycin, with rates of 7.0% and 7.7% using the disc diffusion and E test methods, respectively. E test showed good agreement with disc diffusion for each of the antimicrobial tested.
Conclusion: Disc diffusion test may be used as a preliminary screen for susceptibility testing of Pseudomonas aeruginosa. E test is simple, easy to perform and a reliable method for determination of resistance in Pseudomonas aeruginosa. However its cost and limited availability in Sudan may limit its use. Disc diffusion method can be used reliably in routine susceptibility testing of Pseudomonas aeruginosa.

Keywords: A Pseudomonas aeruginosa, Antimicrobial, minimum inhibitory concentration, disk diffusion, Epsilometer test, Clinical laboratory standard institute.

1. Introduction

Pseudomonas aeruginosa is aerobic gram-negative rod [1]. It is oxidase and catalase positive, motile with polar flagella and do not ferment carbohydrates. Pseudomonas aeruginosa is a major infection problem in nosocomial settings. Most intense infection occurs in patients with serious underlying condition (eg, malignancy burns or as a result of therapeutic procedures (eg, urinary catheterization and mechanical ventilation support). Previous antibiotic therapy may also favor infection with Pseudomonas aeruginosa [2]. Pseudomonas aeruginosa has high resistance to many antibiotics at levels that reach the body's tissues. Antibiotics likely to be most effective are the amino gentamicin, glycosides and tobramicin . Newer agents which have a good activity include the imipenem, carbapenem and monobactam [3]. Quinolones, especially ciprofloxacin, provided a major advance as the first highly effective oral anti pseudomonas agent. [3]. Although the anti-Pseudomonas activity of these antibiotics is greatly improved, higher than usual doses are necessary in severe infections. [3]. Another problem with Pseudomona aeruginosa is that many strains do not respond clinically even though they are sensitive to antibiotics in vitro with amino glycosides. This phenomenon is partly explained by tissue antagonism, ionizing diluent effect, and poor tissue penetration. The exopolysaccharide produce by mucoid Pseudomonas aeruginosa in the presence of physiological level of ca^2 and other diluent cations protect the bacteria within a gelled ionized biofilm or micro colony. Gaining additional resistance superimposed on the normal resistance is also a problem. Plasmid resistance involving modification of enzymes is also particularly associated with topical antibiotic use and with sites. An additional form of acquired resistance results from a reduction in permeability associated with a change in the outer membrane protein [3]

2. Methodology:

Prospective randomized cross-sectional study of 300 Pseudomonas aeruginosa isolates obtained from wounds of the patients in Khartoum State. Study was conducted in the period from 2013 to 2016. Personal data were collected via questionnaire. Questionnaire containing demographic data (Age, Sex, coming from…etc), Types of wounds. Identification of bacteria was carried out according to [4]. Pseudomonas isolates were tested for antibiotic susceptibility test using standard microbiological technique [3].

Antimicrobial susceptibility test: -

Antimicrobial susceptibility testing of Pseudomonas aeruginosa isolates were performed by Kirby-Bauer disk diffusion Method. Isolated colonies were tested on Muller-Hinton agar medium against 11 commonly prescribed antibiotic by using Standard antibiotic disc which include: gentamicin (10µg), ofloxacin (5µg), tobramycin (10µg), amikacin (30µg), ceftazidime (30µ), cefotaxime (30µg), cefepime (30), ciprofloxacin (30µg), imipenem (10µ), meropenem (10µg) and piperacillin (100µg). Isolated colonies were suspended in sterile normal saline, and suspension was adjusted to 0.5 McFarland reactions, and then inoculated

on Muller-Hinton agar plate, then antibiotic discs were added and incubated at 37 ℃ for 24 hours. Sensitive or resistance to each antibiotic was determined on the basis of the size of zone of the growth inhibition, according to the chart of interpretive standards for disc susceptibility testing (CLSI).

Detection of minimum inhibitory concentrations (MICs):-

Minimum inhibitory concentrations of Pseudomonas aeruginosa isolates were achieved by using E.test strips. This procedure was as follows; Fresh colonies from overnight culture of bacterial isolates were used to prepare a 0.5McFarland turbidity standard. The bacterial suspension was then inoculated onto Mueller-Hinton agar plates using a cotton swab and spread it throughout the plate. Test strips were placed on the surface of a Mueller-Hinton agar plate in a position such that the entire length of the strip was in full contact with the agar surface. After incubation at 37°C for 16–18 h, MIC values were read where the ellipse marks intersected the E. test strip. Breakpoint values were used for interpretation as Sensitive, Intermediate or Resistant. Interpretation of result according to the (CLSI).

3. Results:

Total of 300 Pseudomonas aeruginosa isolated from biological specimens were included in the study. Pseudomonas aeruginosa included were isolated and identified by using morphological and biochemical test. Most isolates (72%) were from males. The majority of the isolates were from patients more than 18 years old, with equal percentages in the age groups (18-49years) and 50 and more years (45.3%and 45.5%) respectively. Regarding the type of wounds from which Pseudomonas aeruginosa were isolated, 52.7% were from acute wounds and the remaining 47.3% were from chronic wounds.

Evaluation of the antibiotic sensitivity of the isolates: -

Disc diffusion method: -

Susceptibility testing of the 300 Pseudomonas aeruginosa isolates showed that the most resistance was found against cefotaxime (84.7%), imipenem (78.7), amikacin (64.7%), cefepime (63.7%) ceftazidime (60.0%), ofloxacin (58.0%), piperacillin (55.3%), gentamicin (54.3%), meropenem and ciprofloxacin (53.3%), and lastly tobramycin (46.7%). Regarding susceptibility the most was found against tobramycin (46.3%) and meropenem (44.0%) In case of intermediate the most were intermediate resistant to tobramycin (7.0%) and imipenem (4.7%). as in figure (1) and table (1).

Table(1):-Antibiotics Susceptibility of P. aeruginosa by Disk Diffusion

P. aeruginosa isolates (300)						
Antibiotics	Sensitive[1]		Intermediate[2]		Resistant[3]	
	No of isolates	% of isolates	No of isolates	% of isolates	No of isolates	% of isolates
Imipenem	50	16.6	14	4.7	236	78.7
Cefepime	103	34.3	6	2.0	191	63.7
Amikacin	103	34.3	3	1.0	194	64.7
Ofloxacin	117	39.0	9	3.0	174	58.0

Meropenem	132	44.0	8	2.7	160	53.3
Gentamicin	127	42.3	10	3.3	163	54.3
Ciprofloxacin	128	42.7	12	4.0	160	53.3
Tobramycin	139	46.3	21	7.0	140	46.7
Ceftazidime	111	37.0	3	1.0	186	62.0
Cefotaxime	42	14.0	4	1.3	254	84.7
Piperacillin	127	42.3	7	2.3	166	55.3

1, 2 and 3 according to the Clinical laboratory standard institute.

MIC.E TEST:-

Susceptibility testing of the 300 Pseudomonas aeruginosa isolates showed that the most resistance found against cefotaxime (86.3%), followed by imipenem (77.3%), ceftazidime (63.3%) cefepime (63.0%), Amikacin (62.7%), ofloxacin (58.2%), Gentamicin (55.0%), ciprofloxacin and piperacillin (54.0%), meropenem (52.7%), Tobramycin (48.0%). Pseudomonas aeruginosa isolate reacted better susceptibility to meropenem and tobramycin (44 %) to each. The isolates showed intermediate resistance to tobramycin (7.7) followed by imipenem (5.3%) (Table 2) (Figure 2)

Table (2):-Antibiotics Susceptibility of Pseudomonas aeruginosa by (MIC.E) test strips.

Pseudomona aeruginosa isolates (300)						
Antibiotics	Sensitive[1]		Intermediate[2]		Resistant[3]	
	No of isolates	% of isolates	No of isolates	% of isolates	No of isolates	% of isolates
Imipenem	52	17.3	16	5.3	232	77.3
Cefepime	103	34.3	8	2.7	189	63.0
Amikacin	102	34	10	3.3	188	62.7
Ofloxacin	116	38.7	10	3.3	174	58
Meropenem	132	44	10	3.3	158	52.7
Gentamicin	127	42.3	8	2.7	165	55.0
Ciprofloxacin	131	43.7	7	2.3	162	54.0
Tobramycin	133	44.3	23	7.7	144	48.0
Ceftazidime	107	35.7	3	1.0	190	63.3
Cefotaxime	35	11.7	6	2.0	259	86.3
Piperacillin	123	41	15	5.0	162	54.0

1, 2 and 3 according to the Clinical laboratory standard institute.

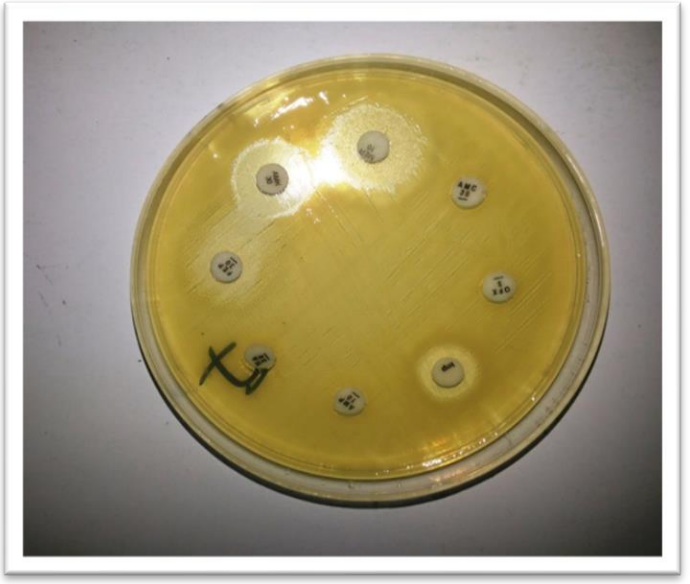

Figure (1):- Antibiotic susceptibility of P.aeruginosa on Mueller Hinton agar

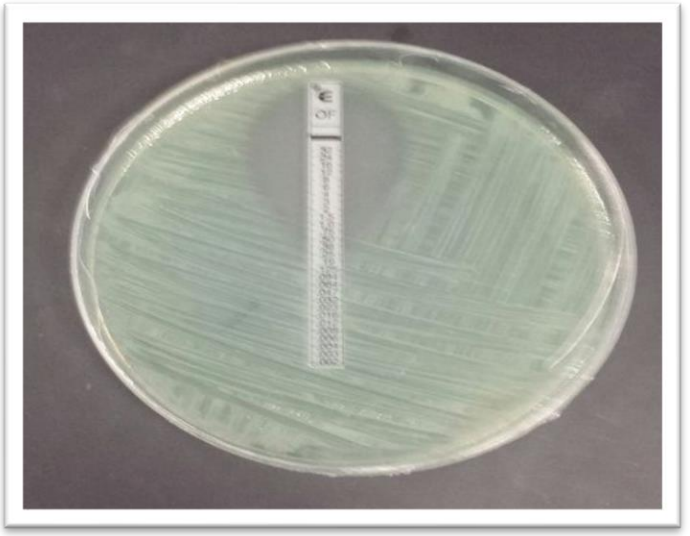

Figure (2):- Mic of P.aeruginosa showing highly sensitive reaction.

4. Discussion:

In this study sample from males patients were (72.7) and from females patients were (27.3) this is in agreement with research done by Thomas etal .Who found the majority of Pseudomonas isolates to be from males (61%) also he found that the mean age of the patient to be 60.5 ± 18.9 yrs and this similar to what was observed in this study. [5]. Most of

P.aeruginosa strains were isolated from chronic wounds finding which is concordant with Power et al [6].

In this study only 16.7% and 17.3% of Pseudomonas isolates were sensitivity to imipenem by the conventional method and E test respectively and the resistance rate was 78.7% and 77.3% by conventional method a and E test respectively. This is in concordant with what was reported by Souli et al, [7], who found the resistance rate of Pseudomonas aeruginosa to imipenem in Europe to range from less than 1% up to 85%. But in disagreement with what was found by Fatemeh et al, who found the sensitivity average of Pseudomonas aeruginosa to imipenem to be 44.4% and the resistance to imipenem to be 53.7%.[8].The sensitivity of Pseudomonas aeruginosa to cefepime in the present study by disc diffusion was 34.3% and by E test was 34. % and the resistance by disc diffusion was 63.7% and by E test 63.0%. This result is to some extend near to what was found by Zulfiqar et al [9], who found the resistance to cefepime to be 50% and sensitivity (50%). This is different by what was found by Mirsalehian et al, who found the resistance to be 100%. [10].

The sensitivity of Pseudomonas aeruginosa to amikacin by disc diffusion was 34.3% and by E test was 34. % and the resistance by disc diffusion was 64.7% and by E test was 62.7% .This result was similar to what was found by Estahbanati, [11], who found the resistance against amikacin to be (67.4%) and this slightly different by what was found by Fatemeh et al [8], who found resistance to amikacin to be 46.2% and sensitive to be 51.9%.The sensitivity of Pseudomonas aeruginosa to ofloxacin by disc diffusion was 39.0% and by E test was 38. % and the resistance by disc diffusion was 58.0% and by E test was 58.2%.This is similar by what was found by Fatemeh et al [8], who was found the resistance to ofloxacin to be 44.4%and sensitive to ofloxacin to be 50%.

The sensitivity of Pseudomonas aeruginosa to the meropenem by disc diffusion was 44.0% and by E test was 44. % And the resistance by disc diffusion was 53.3% and by E test was 52.7%. And this result is similar to what was found by Gad et al [12], Who was found the resistance to meropenem to be 68%. The sensitivity of Pseudomonas aeruginosa to the gentamicin by disc diffusion was 42.3% and by E test was 42. % and the resistance by disc diffusion was 54.3% and by E test was 55.0% this result in agreement by what was found by Fatemeh [8], who was found the resistance to gentamicin to be 55.5% and the sensitive to gentamicin to be 38.9% and this was disagree by what was found by Lari, who was found the sensitive to be 95% [13]. The sensitivity of Pseudomonas aeruginosa to ciprofloxacin by disc diffusion was 42.7% and by E test was 43. % and the resistance by disc diffusion was 53.3%and bye E test was 54.0% this result is similar to what was found by Zulfiqar et al [9], who found the sensitivity to ciprofloxacin to be 54.5% and the resistance to be 45.5%. And this result was different from Shahnaz and Mohammadali Zia., who was found the resistance to be 75%. [14]. The sensitivity of Pseudomonas aeruginosa to tobramycin by disc diffusion was 46.3% and by E test was 44. % and the resistance by disc diffusion was 46.7%. And by E test was 48.0%. This result is different by what was found by Zulfiqar et al. who was found that the sensitivity to be 4.5 % and the resistance to be 95.5%. [9]. The sensitivity of Pseudomonas aeruginosa to the ceftazidime by disc diffusion was 37.0% and by E test was 35. % and the resistance by disc diffusion was 70.7% and by E test was 63.3%. This result is similar to what was found by Osundiya et al [15], who was found that resistance to ceftazidime to be 79.4%, and different by what was found by Fatemeh et al, who was found the resistance

to ceftazidime to be 92.6% and the sensitive ceftazidime to be 3.7%. [8]. The sensitivity of Pseudomonas aeruginosa to cefotaxime by disc diffusion was 14.0% and by E test was 11.7% and the resistance by disc diffusion was 84.7% and by E test was 86.3%. And this result is not far by what was found by Singh et al., who was found the resistance to be 95%.[16]. The sensitivity of Pseudomonas aeruginosa to piperacillin by disc diffusion was 42.3% and by E test was 41% and the resistance by disc diffusion was 55.3%. And by E test was 54.0%. This result is in agreement by what was found by Zulfiqar et al who was found the resistance to piperacillin to be 44.1% and the sensitive to piperacillin to be 55.9% [9].

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