Original Article

Evaluation of Antibiotics by Disk Diffusion and Minimum Inhibitory Concentration Breakpoints in Urinary Tract Infections

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ARTICLE INFO

ABSTRACT

Antibiotic resistance (ABR) has made it more challenging to treat uropathogenic organisms. It is impossible to compromise antimicrobial susceptibility testing (AST), which is essential and has a significant impact on infection treatment strategies. Although labor-intensive and technically challenging for everyday laboratory use, the agar dilution technique is appropriate for monitoring and assessing novel antimicrobials. **Objective:** To determine the minimum inhibitory concentration (MIC), Agar dilution technique and disk diffusion as susceptibility test methodologies. **Methods:** This study was carried out at Khyber Girls Medical College (KGMC) Peshawar. Keeping in view the Clinical and Laboratory Standards Institute (CLSI) guidelines AST was executed. BIOMÉRIEUX® API® kits and gram staining were utilized for identification of bacteria. The disk diffusion was performed using Thermo Scientific™ Oxoid™ antibiotic discs of Co-trimoxazole, Levofloxacin, Nitrofurantoin and Fosfomycin. The MIC and zone of inhibitions for disk diffusion were noted according to the CLSI protocol. **Results:** 158 culture positive samples were isolated out of 680 total received. *Esherechia Coli (E. coli)* (74.1%) being the most isolated organism. In comparison of disk diffusion and agar dilution, categorical agreement for Levofloxacin, Cotrimoxazole, Nitrofurantoin and Fosfomycin were (82.28%, 72.15%, 87.97% and 82.28%) respectively. Kappa coefficients of (0.64, 0.43, 0.57 and 0.37) (p < 0.0001) were calculated for Levofloxacin, Co-trimoxazole, Nitrofurantoin, and Fosfomycin respectively, revealing considerable level of agreement for these antibiotics. **Conclusions:** It was concluded that Agar dilution is more precise than disk diffusion but being more labor intensive and technical. Disk diffusion can still produce significantly accurate results with less resource consumption.

INTRODUCTION

The increasing prevalence of antibiotic resistance (ABR) in healthcare settings and the community at large, poses a threat to the profound advantages of having availability of antibiotic therapy. We are currently combating illnesses that are practically incurable as a result of resistant bacteria [1, 2]. The inability of common infection treatments and the rise in bacterial resistance necessitate determining the root causes of the issue as well as finding ways to mitigate it and increase the efficacy of infection therapies. One potential factor contributing to treatment failure is drug selection, particularly when drugs are inadequately chosen and administered [3]. An important concern to worldwide mortality and financial burden is ABR. Developing countries are more affected by the widespread misuse of antibiotics, for purposes other than human medicine, low-quality pharmaceuticals, inadequate monitoring, and elements of individual and societal poverty. Additionally, resistance needs to be managed before we run out of strategies to combat it because there aren't any novel treatments available [4]. ABR has been

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increasing in numerous types of infections and is associated with worse outcomes, including persistent symptoms, recurrent visits to the doctor, and disease progression due to growing infection [5, 6]. The strategies that will eventually be required to control resistance include drug discovery, resistance analysis, and combinations of new techniques to diminish resistance [7, 8]. A crucial part of therapeutic medicine is carried out by antimicrobial susceptibility testing (AST). In the areas of resistance surveillance, epidemiological investigations of susceptibility, comparative assessment of novel and established drugs, in vitro efficaciousness of medication combinations, and clinical infection management, quantitative approaches for AST are very helpful. To find the minimum inhibitory concentration (MIC) of antimicrobial drugs, three procedures are now used: broth microdilution and macrodilution, gradient diffusion (Epsilometer test) and agar dilution [9]. Susceptibility determination by the Kirby-Bauer disk diffusion is achieved by placing antimicrobial disks on a Mueller-Hilton (MH) media with pathogenic bacteria grown onto it, absence of growth around the disk deems it susceptible to the antibiotic [10]. The E-test is a modified form of disk diffusion with different concentrations on a same strip gives the results of MIC breakpoints. It allows an antimicrobial gradient to diffuse from coated strips onto an agar surface and at the intersection of the zone of growth inhibition and the strip that is considered as the value and expressed in µg/ml. When examining any errors that may have occurred from using disk diffusion tests alone, determining the MIC using either E-strips or dilution tests can be significant [11]. Agar dilution or the gradient methods are now the recommended methods by the CLSI. Although the agar dilution method is quite labor-intensive and technically difficult for everyday laboratory use, it is a valuable tool for surveillance and assessment. Gradient tests are useful for single experiments and are convenient in standard laboratory settings. The gradient tests are expensive while disk diffusion is an easy and affordable process to use [12].

This study was conducted to compare and interpret antibiotic susceptibility of organism isolated from Urinary tract infections (UTI) by agar dilution and disk diffusion methods.

METHODS

The study was conducted in Khyber Girls Medical College (KGMC) Peshawar, colonies of culture positive urine samples were collected from patients who were advised urine culture in Mardan Medical complex, Mardan (MMC) for a total duration of 6 months from April 2022 to September 2022. The study was approved by the ethical committee letter no. 9039/PGMED/KGMC. Prevalence of UTI in a study conducted previously in this province was 11.6% hence by Goldberg's Equation the sample size was of 158 samples [13, 14]. The CLSI guidelines were followed for bacterial identification and AST (M100-S31) (M07-A9) [15, 16]. Urine samples were inoculated on cysteine lactose electrolyte deficient agar (CLED) deferential media and colonies from cultured organisms were subjected to gram staining and BIOMÉRIEUX® API® 10S kits were utilized for identification of bacteria. The disk diffusion was performed using Thermo ScientificTM OxoidTM antibiotic discs of Co-trimoxazole, Levofloxacin, Nitrofurantoin and Fosfomycin with zone of inhibitions in diameters recorded as ≥16mm, ≥31mm, ≥17mm and ≥16mm respectively [15]. MH agar was utilized for both disk diffusion and agar dilution. Antibiotic stock solutions of 5 serial dilutions were prepared. Raw antibiotic powders were purchased directly from manufacturer, and antibiotic solutions containing 1000µg/ml of co-trimoxazole, 10µg/ml of levofloxacin, 1020 µg/ml of nitrofurantoin, and 1020µg/ml of fosfomycin were made. For inoculation 0.5 McFarland standard solutions were prepared from stock solutions and 2µl of this inoculum ware placed on agar plates. After incubation for 24 hours at 37Co the MIC and zone of inhibitions for disk diffusion were noted. By calculating the percentages of agreement (determining the percentages of isolates being sensitive and resistant by both the methods) and Kappa coefficient was used for calculating level of agreement. Isolates sensitive by disk diffusion and resistant by agar dilution were labeled as very major error while resistant by disk diffusion and sensitive by agar dilution were labeled as major error. SPSS® version 25.0 was used for analysis.

RESULTS

The organisms that were identified and represented in table 1. *E. coli* (74.1%) being the most isolated organism (table 1).

Table 1: Spectrum of Isolated Organisms

Isolated Organisms	Frequency (%)
E.coli	117 (74.1)
Klebsiella	17 (10.8)
Pseudomonas	8 (5.1)
Enterococci	10 (6.3)
Proteus species	4 (2.5)
Citrobacter	2 (1.3)
Total	158

The number of organisms that showed the MIC values at different concentrations were, for Co-trimoxazole at 40µg/ml (122), 80µg/ml (17) and 100µg/ml (19), for Levofloxacin at 0.5µg/ml (80), 1µg/ml (3), 2µg/ml (43) and 4µg/ml (32), for Nitrofurantoin at 32 µg/ml (134), 64 µg/ml (2) and 128 µg/ml (22) and for Fosfomycin at 64 µg/ml (142), 128 µg/ml (1) and 256 µg/ml (15), The intermediate sensitivity

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was considered as sensitive, while all the resistant concentrations were combined, The susceptibility of antibiotics against isolated organisms by disk diffusion and agar dilution (table 2).

Table 2 : Study Antibiotics' Susceptibility Rates as Determined by MIC and Disk Diffusion

Antibiotic	Susceptibility	MIC (%)	Disk Diffusion (%)
Levofloxacin	S	83 (52.5)	65 (41.1)
	R	75 (47.4)	93 (58.8)
Cotrimoxazole	S	122 (77.2)	82 (51.9)
	R	36 (22.7)	76 (48.1)
Nitrofurantoin	S	137 (86.7)	126 (79.7)
	R	21 (13.2)	32 (20.2)
Fosfomycin	S	143 (90.5)	121 (76.5)
	R	15 (9.4)	37 (23.4)

MIC by agar dilution, S-Sensitive, R-Resistant

In comparison of Disk Diffusion and Agar dilution, categorical agreement for Levofloxacin, Cotrimoxazole, Nitrofurantoin and Fosfomycin were 82.28%, 72.15 %, 87.97% and 82.28% respectively. Kappa coefficients of 0.64, 0.43, 0.57 and 0.37 (p < 0.0001) were calculated for Levofloxacin, Co-trimoxazole, Nitrofurantoin, and Fosfomycin respectively, showing a high degree of agreement for selected antibiotics (table 3).

Table 3 : Disk Diffusion and Agar Dilution Analysis as Susceptibility Methods, Reporting the Correlation and Categorical Agreement Levels

Antibiotics	Percentage of categorical agreement	Kappa Co-efficient r (p-value)
Levofloxacin	82.28	0.64 (0.0001)
Cotrimoxazole	72.15	0.43 (0.0001)
Nitrofurantoin	87.97	0.57 (0.0001)
Fosfomycin	82.28	0.37 (0.0001)

Levofloxacin, Co-trimoxazole, Nitrofurantoin, and Fosfomycin revealed the very major error rates as 5, 2, 4 and 3 respectively, while a higher number of major error rate 26.5 % was observed for Co-trimoxazole (table 4).

Table 4 : Distribution of Error Rates in Susceptibility Testing

Antibiotics	Positive by DD & negative by Agar Dilution	Negative by DD & positive by Agar Dilution
	VMA (%)	MA (%)
Levofloxacin	82.28	0.64 (0.0001)
Cotrimoxazole	72.15	0.43 (0.0001)
Nitrofurantoin	87.97	0.57 (0.0001)
Fosfomycin	82.28	0.37 (0.0001)

(VMA)- Very major error, (MA)- major error, (DD)- Disk Diffusion.

DISCUSSION

It's important to understand the impact of antibiotics on not just individual health, but also on a global scale. Antibiotic resistance occurs when bacteria evolve and adapt to the antibiotics designed to kill them, making the antibiotics less effective. This can lead to longer infections, increased healthcare costs, and even higher mortality rates. To rationalize the usage of antibiotics, it's essential to consider not only the immediate benefits to an individual but also the long-term consequences for community health and the environment [17]. This study was conducted to compare and interpret antibiotic susceptibility of organism isolated from Urinary tract infections (UTI) by agar dilution and disk diffusion methods. The MIC values for the analyzed antibiotics were determined in this research using a comparison of disk diffusion and agar dilution, which showed remarkable agreement between the two techniques. We found a substantial correlation between the agar dilution method and the disk diffusion method (p < 0.0001). Our results of higher values of Kappa coefficient (0.37- 0.64) were in line with a study conducted on Neisseria gonorrhea which reported 0.89, although the organism was different the susceptibility testing had similar higher kappa index [18]. The categorical agreement between disk diffusion and agar dilution were (72.15% - 87.97%). A study of USA was reported such high concordance of disk diffusion to agar dilution with categorical results (90.4% - 93.0%) [19]. These findings as certain the reliability of either test with each other, yet higher levels of major errors were observed for Co-trimoxazole (26.5%). This may be attributed to the misreading of faint haze in zone of inhibition in disk diffusion method as CLSI guidelines recommends the reading of faint haze for co-trimoxazole [15, 16]. The very major errors were observed to be only (1.2% - 3.14%). The antibiotic susceptibility was observed to be higher with agar dilution than disk diffusion. The CLSI recommends agar dilution as standard and many studies comparing agar dilution with disk diffusion also showed similar results [18-20].

CONCLUSIONS

Agar dilution to be more precise than disk diffusion but being more labor intensive and more technical, disk diffusion can still produce significantly accurate results with less resource consumption.

Authors Contribution

Conceptualization: HQ, MAR, SLS
Methodology: HQ, MAA, MI
Formal analysis: MAR, SN, MI, SLS
Writing-review and editing: HQ, MAR, SN, MAA, MI, SLS

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Source of Funding

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