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Original Article

Comparison of Direct Gram Stain directed Antibiotic Susceptibility testing with Standard Disk Diffusion Method in Urine

Asima Niazi[1], Umar Khurshid[2], Irfan Mirza[3] Nayab Ali[4], Wajid Hussain[5] and Adeel Gardezi[6]

[1] Department of Pathology, Quetta Institute of Medical Sciences, [2, 3,4,5,6] Department of Pathology, Armed forces Institute of Pathology

ABSTRACT

Background: Majority of Urinary tract infections are treated empirically. This not only results in antibiotic resistance in current patient but also there is spread of the resistance in community.

Objective: To compare direct antibiotic susceptibility testing method in urine with standard disk diffusion method.

Methods: The cross-sectional descriptive study was conducted at Armed Forces Institute of Rawalpindi Pakistan. Direct antimicrobial susceptibility was done after performing Gram stain on urine samples containing > 5 pus cells. The Gram stain of sample containing >2 Gram negative rods (GNR) was further processed. Results of Direct susceptibility were compared with Standard disk diffusion testing and minor, major and very major errors were calculated. Agreement between two methods was calculated by Pearson coefficient and significance was calculated by using T test.

Result: Out of the 70 samples with Gram negative rods on Gram stain,54.2% were minor, 12.85% were major and 4.29% were vey major errors. The Pearson Coefficient was 0.866 which was high positive. The P value was (<0.01), it was statistically significant.

Conclusion: Direct susceptibility of urine culture produced reliable results for majority of the antibiotics. Though not recommended, Direct Susceptibility Testing can be used in patients giving the clinician a preliminary report before the results of AST are available.

Key Words: Direct Disk Diffusion, Standard Disk Diffusion, antibiotic resistance, Urine Culture.

Introduction

Urinary tract infections constitute the majority of infections that are treated empirically. The timely specific antibiotics are required to decrease the morbidity and mortality and to help with antibiotic stewardship. Routine cultures take 72 hours till the results of antibiotic susceptibility are available. Patients are already started on broad spectrum antibiotics which are only de-escalated after the availability of the results. This not only results in antibiotic resistance in current patient but also there is spread of the resistance in community.

Gram stain helps in the early recognition of type of organism and direct susceptibility reduces the time to 16 hours and thus helping the clinician to make a better clinical decision.

CORRESPONDENCE AUTHOR

Dr. Asima Niazi

Department of Pathology

Quetta Institute of Medical Sciences

Email: asimaahmed1983@hotmail.com

Many assays like Matrix Assisted Laser Desorption Time of Flight assay and Fast Antibiotic susceptibility test (fASTest) are developed but in resource limited settings they are not available. [1] So, Direct susceptibility can be used as an alternative for preliminary antibiotic susceptibility reporting. The limited guidelines are provided by EUCAST for the direct susceptibility testing to be used with discreetness in urine samples.[2] One of the studies from Iran showed research that included identification and anti-microbial sensitivity testing, The results of the study showed a high concordance between Standard Disk Diffusion and Direct Disk Diffusion method in urine sample.[3]

There are currently no studies on urine samples from Pakistan. Our study was planned to evaluate simple, cost-effective antibiotic testing done on urine directly.

Materials and Methods

The cross-sectional descriptive study was conducted in Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan after

approval from the institute's ethic board. Sample size was calculated by WHO calculator. Due to large number of samples containing more than one type of organisms on microscopy, the sample size of 70 was considered cost effective, could be conveniently collected and had a strong statistical power. The media used was Mueller Hinton Agar that was prepared in-house.

Urine samples that were clean caught, mid-stream taken in sterile containers were collected from Combined Military Hospital Rawalpindi and Pak Emirates Military Hospital Rawalpindi by simple random sampling technique. Repeat and duplicate samples were not included and so were the samples containing Gram positive cocci, yeast cells or mixed organisms on Gram stain. The samples were processed within 2 hours of urine collection. Pus cells were seen and calculated by Urine analyzer FUS 2000.The uncentrifuged well mixed urine was then Gram stained. Only those samples that contained >2 single type of GNR /field were further processed.

Direct susceptibility was performed on undiluted urine by swabbing the surface of Mueller Hinton agar plates after excess urine was expressed by pressing against the walls of the container. The plates were air dried for 10 minutes and antimicrobial disks were placed by multidisc dispenser. The discs were pressed firmly on the agar with flamed forceps. The plates were incubated at 37 ^{o}C for 18 to 24 hours. The zone diameters were measured using zone scale according to the criteria of Clinical and Laboratory standard Institute (CLSI). [4]

The standard Disk diffusion method was performed by Kirby-Bauer method on pure cultures and zone diameters measured by CLSI. [(5)] Escherichia coli ATCC (25922) was used as quality control. Zone diameters with each method were measured. The discrepancies were described as very major if resistant by standard method and sensitive by direct method; major discrepancy was if susceptibility by standard method and resistant by direct method; minor discrepancy represented intermediate results by any of the methods. Meropenem(10g), Cotrimoxazole (1.25/23.75g), Amoxicillin-clavulanate (20/10g), Ceftriaxone (30g), Fosfomycin (200g) and Nitrofurantoin(300g) were used for comparison. The antibiotic disks were chosen keeping in view the organism, antibiotics in common use, broad spectrum antibiotics, time duration and cost effectiveness of the study. The discs were frozen at -18^{o}C with a desiccant intact. The discs were thawed and used as per requirement on day to day basis. The Mueller Hinton agar plates were prepared in the laboratory by commercially available powders. They were placed at 8^{o}C and allowed to reach room temperature before use.

Data was analyzed using SPSS 26 software. Descriptive statistics and percentages of the errors were calculated. Simple mathematical and Pearson correlation coefficient were developed for each antibiotic. The following values of Pearson coefficient were applied as follows; 0.9-1 =very high positive, 0.70-0.90 (high positive), 0.50-0.70 =moderate positive, 0.30-0.50=low positive and 0.00-0.30=negligible correlation.

Results

The 70 urine samples selected for direct antibiotic susceptibility were compared with corresponding Standard disc diffusion method. The zone diameters were measured for each antibiotic (table 1)

Table 1: Difference in zone diameters observed between direct and standard disk diffusion method (n=70)

Antibiotics	0-1 mm	1.1-2 mm	2.1-3 mm	3.1-4 mm	>4mm
Meropenem	27(38.57%)	34 (48.5%)	03 (4.29%)	03 (4.29%)	3 (4.29%)
Cotrimoxazole	37(52.85%)	07 (10%)	12(17.41%)	04 (5.71%)	10 (14.28%)
Amoxicillin-clavulanate	35 (50%)	10(14.28%)	00 (0.0%)	06 (8.57%)	19 (27.14%)
Ceftriaxone	42(60%)	03 (4.29%)	03 (4.29%)	06 (8.57%)	16 (22.85%)
Fosfomycin	19(27.14%)	19(27.14%)	06 (8.57%)	00 (0.0%)	26 (37.14%)
Nitrofurantoin	38(54.28%)	03 (4.29%)	03 (4.29%)	03 (4.29%)	23 (32.85%)
Percentage	**47.14%**	**18.08%**	**6.43%**	**5.23%**	**23.09%**

Most of the differences were minor (54.2%), 12.85% discrepancies were in major range and 4.28% were in very major range (Table 2). The overall agreement was

86.65%. The highest discordant results were seen with Amoxicillin-clavulanate. The very major discrepancy was only found in Fosfomycin with most of the major discrepancies were found in zone > 4mm.Majority of the discrepancies were minor and in zone 0-1mm which reflected measurement error. The Pearson coefficient was 0.86 that was very high positive with a P value <0.01 that was significant.

Table 2: % of discrepant results and correlation between direct and standard disk diffusion method

Antibiotics	Minor	Major	Very Major	Correlation
Meropenem	00	00	00	100%
Trimethoprim-sulfamethoxazole	10	00	00	85.7%
Amoxicillin-clavulanate	19	03	00	68.57%
Ceftriaxone	06	00	00	91.4%
Fosfomycin	03	03	03	87.14%
Nitrofurantoin	06	03	00	87.14%
Total	**54.2%**	**12.85%**	**4.29%**	**86.65%**

Discussion

Multiple studies performed on different samples show significant correlation between Standard disk diffusion and Direct susceptibility testing. EUCAST does not recommend primary susceptibility testing and any laboratory using this approach must take responsibility for ensuring that results are reliable. [2] In our study with unstandardized inoculum there were 54.2% minor errors, 12.85% major errors and 4.28% very major errors. The Pearson correlation coefficient was 0.86 that was strong positive. A study on urine samples from Iran showed 0.6% very major errors, 0.5% major errors, 1.8%minor errors, and 97.1% were in agreement.[3] Another study from Spain had the error rate of 2.4 % overall with 0.2 % very major, 0.4 % major and 1.8 % minor errors.[5] One of the studies from Rochester, Minnesota had percentage of major discrepancies of 1.4%.[6] There have been studies on different samples including blood, respiratory samples had 1.6 % minor discordances, 4.6 % major discordances and 0.4 % very major discordances.[7]Some studies on blood samples showed overall error rate of 2.1% for Gram-negative rods, with 0.1% very major, 0.4% major, and 1.6% minor discrepancies validating the use of DST [8] ·A study on blood samples from Pakistan showed that the number of Very Major Errors as 3(0.4%), Major as 7 (0.9%) and Minor errors were 12(1.5%).[9] Further studies are required on urine samples , so as to establish the ascendancy of Direct method of susceptibility. Whether or not to perform the direct susceptibility testing depends upon the cost and labor and thus has to be evaluated. [10]

The ASM does not approve of unstandardized inoculum to be used for sensitivity. [7] This is because the quantitative analysis of urine sample requires standardized inoculum in routine sensitivity of pure cultures. Nevertheless, ASM and EUCAST allow DST under some conditions including certain ICU settings and where immediate results are required by the clinicians for treatment.[11, 12] EUCAST emphasizes that there are no validated methods for precise inoculums, but recommends a minimal incubation period of 16 hours and looking out for light inoculum, as strains could be reported falsely susceptible. They assert that a reliable interpretation requires an exact identification of the species and repeat testing on pure cultures. We did not standardize the inoculum. We tipped the swab after dipping in urine and removing excess by pressing against the wall of the container.

Direct antibiotic susceptibility may be non-interpretable with low bacterial concentrations and in mixed cultures that lead to higher error rates. Some isolates with similar properties can be easily considered as a single isolate, especially when present in small numbers. Another problem is that urine specimens have the high number of negative tests if direct susceptibility is performed on all urine specimens, most of which are culture-negative or contain fewer than 10^5 CFU/ml. Direct susceptibility of these types of specimens are both the waste of time and of antimicrobial drug disks, so that the cost for this method is higher than AST after primary cultivation.[6,9] Nonetheless under certain circumstances such as ICU settings and patients with asymptomatic UTI where timely antibiotic therapy is important DST with gram stain can give the clinicians a better foresight and help in antibiotic stewardship.[12,13,14]

Conclusion

Though not recommended, Direct Susceptibility Testing can be used in patients giving the clinician a preliminary report before the results of AST are available.

Conflict of Interest: Authors declare no conflict of interest.

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References

1. Baltekin Ö, Boucharin A, Tano E, Andersson DI, Elf J. Antibiotic susceptibility testing in less than 30 min using direct single-cell imaging. Proceedings of the National Academy of Sciences. 2017;114(34):9170.https://doi.org/10.1073/pnas.1708558114
2. Sundqvist M, Olafsson J, Matuschek E. EUCAST breakpoints can be used to interpret direct susceptibility testing of Enterobacteriaceae from urine samples. Apmis. 2015 Feb; 123(2):152-5.
3. Mohammad RN, Omer SA. Direct disk testing versus isolation and antimicrobial susceptibility testing of urine from urinary tract infection. Iranian journal of Microbiology. 2018 Feb; 10(1):37.
4. Weinstein MP, Lewis JS. The clinical and laboratory standards institute subcommittee on antimicrobial susceptibility testing: background, organization, functions, and processes. Journal of clinical microbiology. 2020 Feb 24; 58(3):10-128.
5. Munoz-Dávila MJ, Roig M, Yagüe G, Blázquez A, Salvador C, Segovia M. Comparative evaluation of Vitek 2 identification and susceptibility testing of urinary tract pathogens directly and isolated from chromogenic media. European journal of clinical microbiology & infectious diseases. 2013 Jun;32:773-80.
6. Hollick GE, Washington JA. Comaprison of Direct and Standardized Disk Diffusio'n Susceptibility Testing of Urine Cult res. Antimicroagen and Chemo 1976:804-809.
7. Coorevits L, Boelens J, Claeys G. Direct susceptibility testing by disk diffusion on clinical samples: a rapid and accurate tool for antibiotic v stewardship. European Journal of clinical microbiology & infect Dis. 2015; 34(6):1207-12.
8. Kong H, Zhapg S, Chen X, Zhang W, Yang Q, Fu Y, et al. Rapid identification and antimicrobial susceptibility testing directly from blood cultures of Gram positive and Gram negative isolates. Clinica lab .2013;59:1305-10.
9. Imtiaz A, Ikram A, Zaman G, Satti L, Sana F. Evaluation of direct drug susceptibility testing of blood culture isolates comparing it with conventional disk diffusion testing. JPMA. 2020 Sep 4;70:105.https://doi.org/10.5455/JPMA.10801
10. Oakes AR, Badger R, Grove DI. Comparison of direct and standardized testing of infected urine for antimicrobial susceptibilities by disk diffusion. J ClinMicrobiol 1994; 32:40–45
11. Barry AL, Joyce LJ, Adams AP, Benner EJ. Rapid determination of antimicrobial susceptibility for urgent clinical situations. Am. J. Clin. Pathol. 1973; 59:693-699.
12. McCarter YS, Burd EM, Hall GS, Zervos M (2009) Cumitech 2C, laboratory diagnosis of urinary tract infections. In: Sharp SE, co-ordinating editor. ASM Press, Washington, DC
13. Little P, Merriman R, Turner S, Rumsby K, Warner G, Lowes JA, Smith H, Hawke C, Leydon G, Mullee M, Moore MV. Presentation, pattern, and natural course of severe symptoms, and role of antibiotics and antibiotic resistance among patients presenting with suspected uncomplicated urinary tract infection in primary care: observational study. Bmj. 2010 Feb 5; 340.
14. Klein Breteler KB, Rentenaar RJ, Verkaart G, Sturm PD. Performance and clinical significance of direct antimicrobial susceptibility testing on urine from hospitalized patients. Scandinavian journal of infectious diseases. 2011 Oct 1;43(10):771-6.doi: 10.3109/ 00365548.2011. 588609.

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CONTRIBUTION OF AUTHORS	
AUTHOR	**CONTRIBUTION**
Asima Niazi	A,B,C
Umar Khurshid	A,C
Irfan Mirza	C
Nayab Ali	B
Wajid Hussain	C
Adeel Gardezi	C