Al Ameen J Med Sci 2022;15(4):285-294 ● US National Library of Medicine enlisted journal ● ISSN 0974-1143

ORIGINAL ARTICLE CODEN: AAJMBG

Determination of minimum inhibitory concentration is more informative than disc diffusion for decision making purpose: A study on the cases of urinary tract infection from a tertiary care hospital of eastern India

Hossain Najma Banu[1], Kumkum Bhattacharyya[2], Raveendra D. Totad[3] and Hirak Jyoti Raj[2*]

[1]Department of Microbiology, Barasat Government Medical College and Hospital, Barasat, North 24 Parganas, Kolkata-700124, West Bengal, India, [2]Department of Microbiology, IPGME & R, SSKM Hospital, 244 AJC Bose Road, Kolkata-700020, West Bengal, India and [3]Department of Microbiology, Al Ameen Medical College and Hospital, Athani Road, Vijayapur-586108, Karnataka, India

Received: 16th March 2022; ***Accepted:*** 13th September 2022; ***Published:*** 01st October 2022

Abstract: *Background:* Urinary Tract Infection ranks second among the hospital acquired infections after respiratory tract infections in adults, a major section of which is treatable by oral antimicrobials. *Objectives:* Detection of prevalent uropathogens in adults and comparison of minimum inhibitory concentration of apt oral antimicrobials by disc diffusion and micro-broth dilution methods, to assess the effect on reporting and detection of antimicrobial resistance. *Methods:* Samples from suspected urinary tract infection cases from outdoor and indoor departments of a medical college hospital were included in this prospective observational study following exclusion and inclusion criteria. Isolates were processed as per standard conventional techniques. Antimicrobial susceptibility test (AST) and detection of minimum inhibitory concentration were done by both dilution and diffusion methods following the international and national guidelines. Data had been summarized by simple descriptive statistics with 95% Confidence Interval (CI) for selected results. The extent of agreement between different methods of susceptibility technique was assessed using Chi-square test and p-value calculation using www.vssarstat.net *Result:* Out of 506 culture-confirmed adult urine samples only 318 were included in the study. *Escherichia coli* was the commonest isolate followed by *Klebsiella spp*. The asynchrony of results between the two methods of AST was compared by statistical methods. For *E. coli* and *Klebsiella spp.,* AST patterns were similar for nitrofurantoin, levofloxacin, cefuroxime, fosfomycin (for *E. coli*), where methods could not be used interchangeably. The pairs of levofloxacin-*Pseudomonas aeruginosa*, cefuroxime –*Proteus mirabilis* cannot be tested by alternative methods; whereas; those of *Enterococcus faecalis* and nitrofurantoin, levofloxacin and fosfomycin; as well as *Staphylococcus spp.* including *CoNS* and levofloxacin can be. However, nitrofurantoin needs to be tested separately for *Staphylococci. Conclusion:* Oral antibiotics for treatment of UTI needed testing by different methodology in order to obtain exact results. Validity of extrapolation of results did not hold true for all cases.

Keywords: Uropathogens, MIC, Oral Antimicrobials, Broth Dilution

Introduction

Urinary Tract Infection (UTI) ranks second among the hospital acquired infections after respiratory tract infections in adults [1]. It is also the third most common infection in all age groups [2]. A sample of urine from a patient with suspected UTI is the most common type of specimen received by any clinical microbiological laboratory. Although greater part of the urinary tract is devoid of commensal flora, bladder urine is free from bacteria in an uninfected person. Spontaneously voided urine is apt to be contaminated with some commensal bacteria from urethral orifice and perineum, particularly in females even after proper precautions [1].

In order to make an accurate diagnosis, it is essential for physicians to understand the

value and limitations of urine culture and sensitivity. Use of these tests in conjunction with an assessment of urinary symptoms will yield a diagnosis of either asymptomatic bacteriuria or symptomatic UTI. During the last decade we have seen significant changes in the field of urinary tract infections regarding pathogens and antibiotic treatment calling for an update of current trends. A paradigm shift concerning asymptomatic bacteriuria has had a great impact on the definition and management of UTIs today [3].

Unfortunately, there are only few new antimicrobial drugs available with prospects to overcome the problem of multi and extended drug resistant uropathogens [4]. Antimicrobial resistance is a major public health problem worldwide, caused in part by the misuse of antimicrobials in clinical situations where they are not necessary or overuse when shorter durations are as effective [5]. It is the responsibility of all healthcare providers to practice antimicrobial stewardship and to avoid the unnecessary use of antimicrobials [6].

Minimum inhibitory concentration (MIC) is the lowest concentration of antibiotics that inhibits visible growth of organism after due period of incubation [1]. So, calculation of MICs of the commonly prescribed oral antibiotics for culture confirmed cases of adult UTI would certainly help in these existing problems. There are several studies in India as well as abroad which have dealt with individual oral antibiotics for UTI, but unfortunately there are very few studies from our state. MIC is the lowest concentration of antibiotics that will inhibit visible growth of an organism after due period of incubation [1].

Antibiotics with low MICs are more effective than those with high MICs, as only a low dosage is necessary to eradicate the microbes [1]. MIC can be calculated conventionally by Agar Dilution, Micro-broth dilution, Macro-broth dilution and E-test (Epsilon strip test) [7]. It can also be performed by the automated system (VITEK -2 compact). The pros of using MIC are being easy to perform, widely used in reference laboratories, usually automated, highly reproducible due to simplicity and rapid turnaround time (TAT); whereas cons of using it remains variability in conventional methodology like increase in apparent MIC with prolong incubation, decrease in apparent MIC with smaller inoculums concentration. Even MIC may change with freezer storage of samples [8]. Many organisms can infect urinary tract, but by far the most common agents are Gram-negative bacilli. *Escherichia coli* cause 80% of acute infections. Other Gram-negative bacilli, *Proteu*s spp., *Klebsiella* spp. and occasionally *Enterobacter* spp. accounts for uncomplicated UTI [9]. *Escherichia coli* are the most frequently isolated species in community acquired as well as nosocomial UTI [9].

Material and Methods

The study was carried out in the Department of Microbiology of a tertiary-care hospital after acquiring written approval from the Institutional Ethics Committee. It was a cross-sectional study (analytical observational), conducted from January, 2019 to June, 2020.The study population were all culture-confirmed adult UTI isolates that fitted into the inclusion-exclusion criteria of this study, obtained from the patient's urine of different OPDs.318 samples were included in this study following the inclusion criteria that included suspected cases of UTI in adult age group, attending OPDs, self-voided samples.

Geriatric (above 60 years) and pediatric (below 18 years) age group patients [10], samples from non-consenting and post-instrumentation or post-surgical patients, those with apparent gastrointestinal disorder and renal tuberculosis were excluded from this study. Urine samples were collected and processed as per the methodology described in standard text book of microbiology, with the logistic support, available in our laboratory [1,7,11]. Bacterial identification was done according to standard microbiological guidelines [7]. Antibiotic susceptibility test (AST) was done by modified Kirby-Bauer disc diffusion method [1].

We have used following control strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Proteus mirabilis* ATCC 29906 and *Klebsiella pneumoniae* ATCC 700603.

Microbroth-dilution method was chosen for the purpose of additional testing of the antimicrobial susceptibility for the commonly prescribed oral antibiotics *viz.* nitrofurantoin, levofloxacin, cefuroxime, fosfomycin, trimethoprim and sulfamethoxazole [7]. Antimicrobials were selected following the CLSI guideline 2019 [12].

MIC determination of antimicrobial agents [13-15]: MIC values of the antibiotics were determined by broth microdilution technique in microtiter plate for each isolate with cation adjusted Muller Hinton broth being used as medium. The crude antibiotics powder procured were nitrofurantoin, levofloxacin, cefuroxime, fosfomycin, trimethoprim and sulfamethoxazole from MERCK (Sigma-Aldrich), Bangalore, India. The highest dilution showing no visible growth in naked eye was considered as MIC of the respective drug.

Steps of MIC determination:

Preparation of antibiotic stock solution

↓

Inoculum preparation (Direct colony suspension method – applied *Staphylococcus aureus* and Growth method –applied for other isolates)

↓

Microbroth dilution and incubation

↓

Interpretation of Result

a) Preparation of antibiotic stock solution:

For the preparation of stock solution following formula is used –

Weight of antibiotic powder =
[Volume of solution (ml) × Concentration (µg/ml)] ÷ [Potency of powder (µg/mg)]
While, Potency = Assay purity ×Active Fraction × (1- water content)

The agent was dissolved in solvents as per the instructions of the manufacturer and then kept in aliquots at -20˚ C. The mouth of the aliquot was sealed with parafilm. When it was brought out of the refrigerator for use, it was allowed to defrost fully and then used promptly. The remaining solution in the aliquot was discarded.

b) Inoculum preparation [16]:

I) Direct colony suspension method–1). This method was applied *Staphylococcus aureus* inoculums preparation as recommended in CLSI 2019. Here, the inoculum was prepared by making a direct broth suspension of isolated colonies selected from an 18-hour blood agar plate. 2). Adjustment of the suspension to achieve a turbidity equivalent to a 0.5 McFarland standard was done by using spectrophotometer. This resulted in a suspension containing approximately 1 to 2×10^8 colony-forming units (CFU)/ml for *Escherichia coli* ATCC 25922.

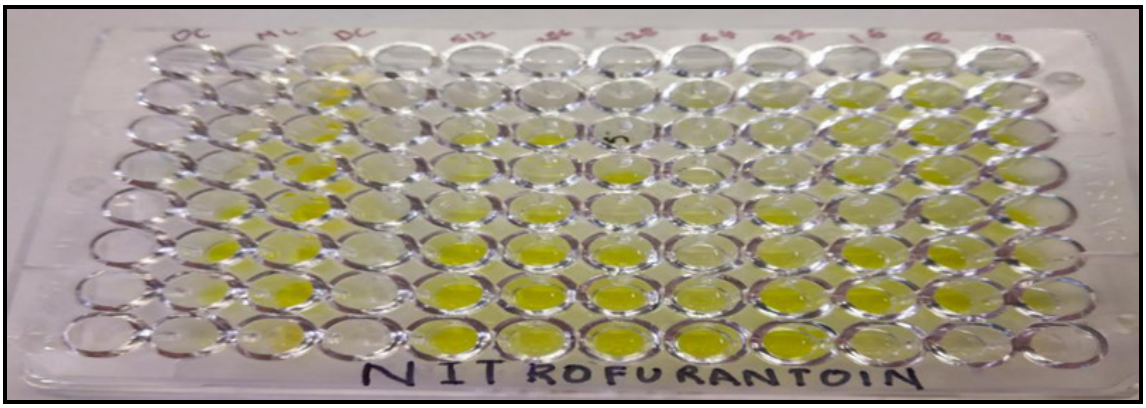
II) Growth method –The growth method was used alternatively and sometimes preferable when colony growth was difficult to suspend directly and a smooth suspension cannot be made. It can also be used for non-fastidious organisms (except staphylococci) when fresh (24-hour) colonies, as required for the direct colony suspension method, are not available. At least three to five well-isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a sterile loop and transferred the growth into a tube containing 5 ml of a CAMH broth medium. The broth is incubated for culture at 35 ± 2°C until it achieves or exceeds the turbidity of the 0.5 McFarland standards (usually two to six hours).

Adjustment of the suspension to achieve a turbidity equivalent to a 0.5 McFarland standard was done by using spectrophotometer. This resulted in a suspension containing approximately 1 to 2×10^8 colony-forming units (CFU)/ml for *Escherichia coli* ATCC 25922. The Log phase or stationary phase growth inoculum, colonies grown for 1 day on non-selective (Blood agar) medium was used. In case of stock culture of ATCC strains, the colonies were sub-cultured three times before inoculation. It was kept in the incubator for 3-4 hours at 35^0 C until the suspension is visibly turbid (Exponential phase of growth).

The suspension is adjusted to obtain a turbidity comparable to that of a McFarland 0.5 turbidity. That means each ml of the broth was then having approximately 1 to 2 × 10^8 colony-forming unit (CFU)/ml for *Escherichia coli* ATCC 25922. McFarland 0.5 suspension =1.5 ×10^8 CFU/ml for *Escherichia coli* ATCC 25922. Plates were to be inoculated within 30 minutes, otherwise the colony count will go beyond 1.5×10^8.

c) Broth Micro dilution procedure for MIC determination: A sterile 96 well microtitre plate (U bottom) was taken. The plate (Figure 1) was labeled according to the antibiotic and bacterial suspension to be taken. The first 3 wells on extreme left (well 1, 2, 3) were labelled as MC (media control/ negative control), OC (organism control/ positive control) and DC (drug control having antimicrobial concentration twice the concentration required) respectively. One well after the DC was kept blank (well 4) and then the remaining 8 wells (well 5 to 12) were marked accordingly.

Fig-1: Round bottom 96- welled micro-titre plate with Nitrofurantoin for MIC calculation



By using a micropipette, 100µl of the CAMHB is dispensed into the MC (well-1) and each of the remaining eight wells (well 5 to 12). Similarly, 100 µl of antibiotic solution was dispensed in the DC well (well 3) and the first well after the blank (well 5). The antibiotic solution was obtained from the stock solution that was already been prepared. It was to be thawed before use. The concentration of the antibiotic should be 2X, where X was the desired concentration to be added in the well 5. Upon dissolving in the medium which was already been taken in the microtiter plate, the concentration of the antibiotic will become half.

Using the micropipette, the antibiotic in the well 5 was mixed by sucking up and down several times (7-8 times). Care was taken not to splash the well and avoid formation of air bubble. This was followed by withdrawal of 100 µl of the mixture from the well no. 5 and transfer to well no. 6. This would make well no. 6, a two-fold dilution of well 5. Mixing is done 7-8 times. Then 100 µl transferred to well 7. The procedure was repeated till the well 12 (last). The same tip could be used for the entire dilution series. 100 µl was now discarded from well no. 12. The bacterial suspension was taken and its turbidity was adjusted (as described above) to match 0.5 McFarland turbidity standard by using spectrophotometer at OD625. So, the suspension will then contain bacterial count approximately 1.5×10^8 colony-forming unit (CFU)/ ml.

The dilution took place as follows-

1ml ~ 1.5×10^8 CFU

So, 0.8ml of the suspension was added to 25ml of water diluents. That made 1:31 dilution. That will achieve 4-5 ×10^6 CFU/ ml suspension. So, 10 µl (0.01ml) of this suspension would contain 4-5 ×10^4 CFU that was the appropriate inoculums size for standard MIC. 10 µl of the suspension was then dispensed in each of the eight wells (5 to 12) in that order. The microtiter plate was then incubated at 35˚C (± 2˚C) for the next 20 hours. From the reserved bacterial suspension, a stock solution was prepared in 30% Brain

Heart infusion broth glycerol solution. After 20 hours of incubation, positive (OC) and negative control (MC) wells were examined first:

- Positive control well should demonstrate sediments/turbidity (button size ≥ 2mm in size) indicating adequate growth. The growth pattern should be the same on all positive control wells throughout the microtiter plate.
- Negative control wells should show no growth.
- If either type of growth control well is unacceptable, repeat the test.

d) Interpretation of Result:

MIC was read only if the positive control well was adequately turbid (button size ≥ 2mm in size). The MIC was the lowest concentration of antimicrobial agent showing complete inhibition of growth, or, for sulfonamides and trimethoprim, the MIC was the lowest concentration that inhibits 80% of the growth compared to the growth control well. However, for trimethoprim and sulfamethoxazole, very slight hazes and/or a pin point button (2 mm) that persists through several dilutions are ignored. MIC was reported along with its categorical interpretation: susceptible (S), intermediate (I), or resistant after comparing it with corresponding breakpoints.

Agar dilution for Fosfomycin [13-15]:

Organisms to be tested are brought together on an agar-based medium rather than in liquid broth. For each doubling dilution requires a single agar plate. After inoculum preparation it was seeded onto antibiotic screen agar of different concentration to be tested. Concentration of fosfomycin in plates with no growth of the organism was the MIC.MIC was reported along with its categorical interpretation viz. susceptible (S), intermediate (I), or resistant(R) after comparing it with corresponding breakpoints.

Statistical analysis plan: Data was summarized by simple descriptive statistics, mainly counts, tables and percentages with Chi-square test for selected results. *P*-value calculation was done using online calculator, available at www.vssarstat.net. Statistical tools used are counts, tables, percentages and *p*-value. No financial support was received from any extramural or commercial organization. No conflict of interest was associated with this study.

Results

Out of 2046 culture positive samples, 506 were from adults. Amongst these 506, only 318 samples were included. Susceptibility pattern of the Gram-negative and Gram-positive isolates obtained from both the methods of AST are shown in Table 1. Proportion of resistance against the antimicrobials (CI=95%) by microbroth dilution and disc diffusion methods have been depicted in Table 2. In Table 3, the result of Chi square test has been shown.

Table-1: Susceptibility pattern of the Gram-negative bacilli and Gram-positive cocci among the isolates obtained

AMA	*Escherichia coli (n=176)*		*Klebsiella spp. (n=85)*		*Pseudomonas Aeruginosa (n=31)*		*Proteus mirabilis (n=13)*	
	Disc Diffusion (mm)	**MIC[a] (ug/ml)**	**Disc Diffusion (mm)**	**MIC (ug/ml)**	**Disc Diffusion (mm)**	**MIC (ug/ml)**	**Disc Diffusion (mm)**	**MIC (ug/ml)**
NIT[b]	S=69.88% I=4.54%	S=62% I=4.54%	S=24.7% I=0%	S=24.7% I=0%	NA	NA	NA	NA
LEV[c]	S=34.65% I=0%	S=39.77% I=0.56%	S=35.29% I=0%	S=41.17% I=2.35%	S=41.93% I=0%	S=29.03% I=0%	S=61.53% I=0%	S=38.46% I=0%
CXM[d]	S=26.13% I=0%	S=22.72% I=0%	S=24.7% I=0%	S=17.67% I=2.35%	NA	NA	S=38.46% I=0%	S=30.76% I=0%
FOS[e]	S=78.40% I=0%	S=74.43% I=0%	NA	NA	NA	NA	NA	NA
COT[f]	S=47.72% I=0%	**SMX[g]** S=39.20% I=0% **TMP[h]** S=39.20% I=0%	S= 24.7% I= 0%	**SMX** S=16.47% I=0% **TMP** S=24.7% I=0%.	NA	**SMX** NA **TMP** NA	S=60% I=0%	**SMX** S=46.15% I=0% **TMP** S=30.76% I=0%

AMA	*Enterococcus faecalis (6)*		*Staphylococcus aureus (5)*		*Coagulase Negative Staphylococcus (2)*	
	Disc diffusion (mm)	**MIC[a] (ug/ml)**	**Disc diffusion (mm)**	**MIC (ug/ml)**	**Disc diffusion (mm)**	**MIC (ug/ml)**
NIT[b]	S=66.66% I=0%	S=50% I=0%	S=20% I=0%	S=20% I=0%	S=0% I= 0%	S=0% I= 0%
LEV[c]	S=66.66% I=0%	S=66.66% I=0%	S=40% I=0%	S=40% I=20%	S=50% I=0%	S=50% I=0%
CXM[d]	NA	NA	NA	NA	NA	NA
FOS[e]	S=83.33% I=0%	S=83.33% I=0%	NA	NA	NA	NA
COT[f]	NA	**SMX[g]** NA **TMP[h]** NA	S= 60% I= 0%	**SMX** S=66.67% I=0% **TMP** S=33.33% I=0%.	S= 100% I= 0%	**SMX** S=50% I=0% **TMP** S=50% I=0%.

MINIMUM INHIBITORY CONCENTRATION, b-NITROFURANTOIN, c-LEVOFLOXACIN, d-CEFUROXIME, e-FOSFOMYCIN, f-COTRIMOXAZOLE, g-SULFAMETHOXAZOLE, h-TRIMETHOPRIM, NA- THIS DRUG IS NOT APPLICABLE FOR THAT PARTICULAR ORGANISM, S-SUSCEPTIBLE, I-INTERMEDIATE. AMA-Antimicrobial agent

Table-2: Proportion of resistance against the antimicrobials (CI=95%) by conventional MIC calculation and by disc diffusion method

	Total Samples	**Sensitive (S)**	**Resistant (R)**	**95% CI (R/Total)**
Conventional MIC calculation by MBD method				
Nitrofurantoin	274	135 (49.27%)	139 (50.73%)	45.81-57.29%
Levofloxacin	318	126 (39.62%)	192 (60.38%)	54.91-65.60%
Cefuroxime	274	59 (21.54%)	215 (78.46%)	73.23-82.93%
Fosfomycin	182	136 (74.73%)	46 (25.27%)	19.51-32.05%
Sulfamethoxazole	281	94 (33.45%)	187 (66.55%)	60.84-71.81%
Trimethoprim	281	97 (34.52%)	184 (65.48%)	59.75-70.80%
Zone diameter measurement by disc diffusion method				
Nitrofurantoin	274	149 (54.38%)	125 (45.62%)	39.64-51.08%
Levofloxacin	318	119 (37.42%)	199 (62.58%)	57.14-67.72%
Cefuroxime	274	72 (26.28%)	202 (73.72%)	68.21-78.58%
Fosfomycin	182	143 (78.57%)	39 (21.43%)	16.09-27.95%
Cotrimoxazole	281	118 (41.99%)	163 (58.01%)	52.17-63.63%

Table-3: CHI square test results for diffusion and dilution methods			
Organism	**Antibiotic***	***p*-Value**	**Remark (Significance)**
1. *Escherichia coli*	NIT	0.0276	Significant
2. *Escherichia coli*	LEV	0.0185	Significant
3. *Escherichia coli*	CEF	0.0071	Significant
4. *Escherichia coli*	FOS	0.0016	Significant
5.*Klebsiella spp.*	NIT	0.0469	Significant
6.*Klebsiella spp.*	LEV	0.0316	Significant
7.*Klebsiella spp.*	CEF	0.0498	Significant
8. *Pseudomonas aeruginosa*	LEV	0.0332	Significant
9. *Proteus mirabilis*	LEV	0.2384	Non-significant
10.*Proteus mirabilis*	CEF	0.0439	Significant
11. *Enterococcus faecalis*	NIT	0.1821	Non-significant
12. *Enterococcus faecalis*	LEV	0.2418	Non-significant
13. *Enterococcus faecalis*	FOS	0.5657	Non-significant
14. *Staphylococcus aureus*	NIT	0.4274	Non-significant
15. *Staphylococcus aureus*	LEV	0.4274	Non-significant
16. *Coagulase Negative Staphylococcus*	NIT	<0.0001	Significant
17. *Coagulase Negative Staphylococcus*	LEV	0.3173	Non-significant
NIT= Nitrofurantoin, LEV= Levofloxacin, CEF= Cefuroxime, FOS= Fosfomycin			

Discussion

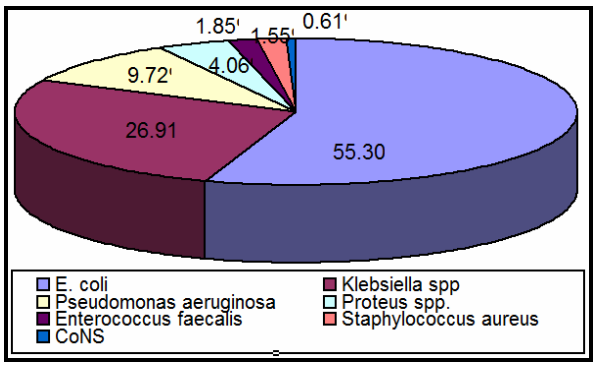
Urinary Tract Infection (UTI) has been a common presentation in community and hospital set up since long time. Isolation and identification of etiological agents and determination of antimicrobial susceptibility play a central role in diagnosis and management of UTI. Since MIC helps in effective dosage formulation for therapy, determination of MIC for the prescribed drugs enables earlier initiation of therapy with appropriate dosage. So, there is a need for rapid, efficient and accurate system for determination of appropriate dosage.

The rationale of this study is to avoid the empirical treatment so that the misuse, overuse and/or abuse of antibiotics can be minimized. This can be done by identifying the uropathogens and suggesting the appropriate drug along with its MIC. The male and female percentage of culture-confirmed cases of adult UTI in the current study were 39.31% and 60.69%, respectively. An incidence of 12.6% in women as compared with 3% among men has been described by Moue et-al., Ghadage et al [15, 17].

Another study by Yang et-al observed the similar incidence of UTI among females [18]. Higher incidence of UTI is in females can be explained by the facts that females have shorter urethra, proximity to anus and genital opening. So, it can be easily colonized by colonic bacilli [15, 17].

Sexual intercourse, pregnancy and postmenopausal state also favor occurrence of UTI in females [19]. In the present study, *E. coli* (55.30%) was the commonest organism isolated followed by *Klebsiella spp.* (26.91%), *Pseudomonas aeruginosa* (9.72%), *Proteus spp.* (4.06 %), *Enterococcus faecalis* (1.85%), *Staphylococcus aureus* (1.55%) and *CoNS* (0.61%) as shown in Figure 2.

Fig-2: 3-D pie chart showing percentage distribution of the isolates obtained



According to the studies conducted in India by Akram et-al. and in Latin America by Gales et-al. *Escherichia coli* was the commonest organism isolated with isolation rate of 61% and 64%, respectively; followed by *Klebsiella spp* [20-21]. In our study, out of 318 adult UTI cases, 305 (95.91%) were Gram negative and 13 (4.09%) were Gram positive organisms. These findings are similar to that of Prakash et-al. from Uttar Pradesh, where out of 155 isolates, 140 (90.32%) were Gram negative and 15 (9.68%) were Gram-positive [22]. Even Girma et-al also found *E. coli* to be the most common pathogen causing UTI [23].

Among the uropathogens isolated and antibiotics studied, maximum resistance is observed against Cefuroxime (78.56% with 95% CI of 73.23-82.93%) and least in case of Fosfomycin (25.27% with 95% CI of 19.51-32.05%). Similar findings were observed by Singhal et-al from Rajasthan [24].

Two methods of antimicrobial susceptibility testing (Modified Kirby-Bauer's disc diffusion and microbroth dilution/agar dilution method) were compared in this study. For*Escherichia coli*, microbroth dilution (or agar dilution for fosfomycin) and disc diffusion methods cannot be used as a replacement of each other for AST of the following antibiotics: Nitrofurantoin (*p*-value=0.0276), Levofloxacin (*p*-value=0.0185), Cefuroxime (*p*-value=0.0071) and Fosfomycin (*p*-value=0.0016). For *Klebsiella spp.,* microbroth dilution (or agar dilution) and disc diffusion methods cannot be used as a replacement of each other for AST of the following antibiotics: Nitrofurantoin (*p*-value=0.0469), Levofloxacin(*p*-value=0.0316) and Cefuroxime (*p*-value=0.0498). Among the drugs studied, only Levofloxacin is used against *Pseudomonas aeruginosa*. For this combination, microbroth dilution and disc diffusion methods of AST cannot be used interchangeably (*p*-value=0.0332). For *Proteus mirabilis*, only in case of Cefuroxime, microbroth dilution and disc diffusion methods cannot be used as a replacement of each other, asp-value=0.0439. For *Enterococcus faecalis*, the AST results in two methods disclosed that significant difference did not exist and the methods could be used interchangeably (*p*-value>0.05). For *Staphylococcus aureus*, both nitrofurantoin and levofloxacin, microbroth dilution and disc diffusion methods can be used interchangeably as per the findings of chi square test.

Coagulase Negative Staphylococcus, Nitrofurantoin (*p*-value=0.3173) cannot be tested interchangeably by two mentioned methodologies, whereas, levofloxacin can be (*p*-value<0.0001). The results of the broth-dilution tests and diffusion tests are expected to be similar [25].

There is an inverse linear relationship between the size of the zone and the MIC value-the larger the zone of inhibited growth (more susceptible the organism to the antibiotic), the smaller the MIC value. Thus, it is possible to extrapolate from the measured size of the inhibitory zone to the corresponding MIC value. In addition, the interpretive criteria that are applied to MIC tests apply to the diffusion tests. Thus, for most organism-antibiotic tests, the diffusion tests and dilution tests are equally accurate in predicting antimicrobial susceptibility. Similar relationships were observed in a study by Matthew Luc from University of Washington, where he did a comparison of disc diffusion and micro-broth dilution methods for Gram negative bacilli [26].

Conclusion

The present study has its share of limitations. Cotrimoxazole, an easily available and cheap antimicrobial option could not be tested because lack of availability of different compatible solvents for its ingredients namely sulpha-methoxazole and trimethoprim separately. Hence, the outcome or the result of

AST by disc diffusion and micro-broth dilution was not comparable. The parenteral drugs could not be tested for limitation of time and further resources. Geriatric and pediatric age group were excluded in this study in the ground of compliance and follow up.

Antimicrobial dosing requires consideration of the interactions between the body's metabolomics, the susceptibility or MIC of the pathogen, the microbiological spectrum of activity and chemical properties of the antimicrobial agents. MIC calculation of the oral antibiotics can re-establish the role of oral antimicrobial agents in the treatment of UTI. Use of oral antibiotics can restore higher generation newer antimicrobial and prevent their abuse/misuse/overuse thereby decreasing the development of drug resistance.

The MIC value allows the clinician to select the most appropriate antimicrobial, customize antimicrobial dosing taking into account the susceptibility of the pathogen (MIC) combined with patient profile and the PK (pharmacokinetic) parameters of the drug through use of TDM (therapeutic drug monitoring). The MIC helps to define the target exposure that an optimized antimicrobial dosing regimen should reach. Moreover, the Turn Around Time (TAT) for micro-broth dilution and modified Kirby Bauer's Disc Diffusion methods for AST are almost similar and in majority of the isolates in this study both the conventional methods can be used as a replacement of each other. So, MIC calculation can be used in antibiotic stewardship policy.

Acknowledgement

All faculty and non-teaching staff of Department of Microbiology, IPGME&R and SSKM Hospital.

Financial Support and sponsorship: Nil

Conflicts of interest: There are no conflicts of interest.

References

1. Mackie TJ, Collee JG, McCartney JE. Mackie and McCartney practical medical microbiology. 14th edi. *Elsevier, New Delhi (India),* 2007.
2. Najan M, Saldanha C, Banday K. Approach to urinary tract infections. *Indian Journal of Nephrology,* 2009; 19(4); 129.
3. Wagenlehner FME, Naber KG. Asymptomatic bacteriuria–Shift of paradigm. *Clin. infect. Dis.* 2012; 55:778-780.
4. Magiorakos AP. Srinivasan A, Carey B, Carmeli YY, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, olson Liljequist B et-al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin.Microbiol.Infect.* 2012; 18:268-281.
5. Tremolieres F. Short or long course antibiotics. Is there a debate on the duration of treatment? *Presse Med.* 2002; 31:1495-1501.
6. Herr HW. Should antibiotics be given prior to outpatient cystoscopy? A plea to urologists to practice antibiotic stewardship. *Eur Urol.* 2014; 65(4):839-842.
7. Koneman E. Color atlas and textbook of diagnostic microbiology. 7th ed. *Philadelphia: Lippincott;* 1997.
8. Andrews J. Determination of Minimum Inhibitory Concentration. *Journal of Antimicrobial Chemotherapy,* 2001; 48(suppl-1);5-16.
9. Stamm W. Urinary Tract Infections Infectiology. *Karger [Internet]*. 1997; 1:1-7.
10. [Internet]. 2022 [cited 7 October 2022]. Available from: h*ttp://www.un.org/en/sections/issues-depth/ageing/*
11. Tille P. Bailey & Scott's Diagnostic Microbiology. 14th edition. *Mosby,* 2018;842-55
12. Turner GC. Bacilluriaa in pregnancy. *Lancet*, 1961; II: 1062-1064.
13. Chua AT, Ariceo Editha and Pena A. Comparison of initial verses midstream voided urine culture among men. *Phil. J. Microbiol Infect. Dis.* 1988; 17(1):22-24.
14. Morris RW et al. Perineal cleansing before midstream urine, a necessary ritual?. *Lancet.* 1979; II:158-159.
15. Moue A, Aktaruzzaman AQM, Ferdous N, Karim, Md R, Khalil MMR, Das AK. Prevalence of urinary tract infection in both outpatient department and in patient department at a medical college setting of Bangladesh. *International Journal of Biosciences* 2015; 7(5):146-152.
16. Sanchez GV, Master RN, Karlowsky JA, Bordon JM. In vitro antimicrobial resistance of urinary *Escherichia coli* isolates among U.S. outpatients from 2000 to 2010. *Antimicrobial Agents Chemother,* 2012; 56:2181-2183.
17. Ghadage DP, Muley VA, Sharma J, Bhore, AV. Bacteriological profile and antibiogram of Urinary Tract Infections at a tertiary care hospital. *National Journal of Laboratory Medicines,* 2016; 5(4):20-24.
18. Yang X, Chen H, Zheng Y, Qu S, Wang H, Yi F. Disease burden and long-term trends of urinary tract infections: A worldwide report. *Frontiers in Public Health,* 2022; 10.
19. Sobel JD, Kaye D. Urinary tract infections. In Gerald L. Mandell, John E. Bennett, Raphael Dolin.

Editors. Mandell, Douglas, and Bennett's principles and practice of infectious diseases.7th ed. *Philadelphia: Churchill livingstone,* 2010; 964.
20. Akram M, Shahid M, Khan A. Etiology and antibiotic resistance patterns of community-acquired urinary tract infections in JNMC Hospital Aligarh, India. *Annals of Clinical Microbiology and Antimicrobials* 2007; 6(1):4.
21. Sader H, Jones R, Gales A, Silva J. SENTRY antimicrobial surveillance program report: Latin American and Brazilian results for 1997 through 2001. *The Brazilian journal of Infectious disease,* 2004; 8(1):25-79.
22. Prakash D, Saxena RS. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of meerut city, India. *ISRN Microbiol.* 2013; 749629.
23. Girma A, Aemiro A. The Bacterial Profile and Antimicrobial Susceptibility Patterns of Urinary Tract Infection Patients at Pawe General Hospital, Northwest Ethiopia. *Scientifica (Cairo).* 2022; 2022:3085950.
24. Singhal A, Sharma R, Jain M, Vyas L. Hospital and Community Isolates of Uropathogens and their Antibiotic Sensitivity Pattern from a Tertiary Care Hospital in North West India. *Ann Med Health Sci Res.* 2014; 4(1):51-6.
25. Bennet J. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases + Clinics Review Articles. 8th ed. *Elsevier Science Health Science,* 2015; 312-326.
26. Luc M. A Comparison of Disc Diffusion Methods for the detection of Antibiotic Resistant Subpopulations in Gram Negative Bacilli [Internet]. *Digital.lib.washington.edu.* 2015[cited 7 September 2019].

Cite this article as: Banu HN, Bhattacharyya K, Totad RD and Raj HJ. Determination of minimum inhibitory concentration is more informative than disc diffusion for decision making purpose: A study on the cases of urinary tract infection from a tertiary care hospital of eastern India. *Al Ameen J Med Sci* 2022; 15(4): 285-294.

*All correspondences to: Dr. Hirak Jyoti Raj, Associate Professor, Department of Microbiology, UCM Building-5th Floor, IPGME&R-SSKM Hospital, 244 AJC BOSE Road, Kolkata-700020, West Bengal, India. E-mail: drhjrmicro@gmail.com