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Original Research Article

Ticking the clock: Modified Kirby-Bauer's technique for accelerated antibiotic susceptibility reporting

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Abstract

Background: Antibiotic resistance poses a global health threat, potentially leading to a post-antibiotic era with severe consequences. Timely and accurate antimicrobial susceptibility testing (AST) is critical for effective treatment, reducing mortality, and combating antimicrobial resistance (AMR). This study evaluates the efficacy of early disc diffusion (EDD) testing by using 6-hour and 10-hour old growth compared to the standard 18-24 hours growth in performing the Kirby-Bauer disc diffusion method.

Materials and Methods: A cross-sectional study was conducted at a tertiary care hospital on five quality control (QC) strains and 100 clinical isolates. Additionally, growth from primary isolation plates of 25 turbid urine and 25 positive flagged blood culture samples were also analyzed.

Results: EDD 6-hour and 10-hour testing of QC strains showed 100% CA with the 24-hour growth to reference method. Clinical isolates demonstrated 97.48% CA for EDD 6-hour testing and 97.1% CA for EDD 10-hour testing, with minimal discrepancies (VME ≤ 0.63%, ME ≤ 0.58%, mE ≤ 1.94%). Early growth (EDD 10-hour) from primary isolation plates of urine and positive flagged blood cultures showed >99% CA, with no major or very major discrepancies.

Conclusion: EDD 10-hour testing demonstrated high accuracy and concordance with the standard 18-24 hour method, offering a cost-effective alternative method for faster AST reporting. Early reporting using EDD can enhance timely antibiotic administration, reduce AMR, and improve patient outcomes without additional costs. This method aligns with FDA-recommended thresholds for AST systems and provide significant advantage in clinical settings.

Keywords: Antimicrobial susceptibility testing, Early disc diffusion, Kirby Bauer's disc diffusion, Turnaround time.

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1. Introduction

Antibiotic resistance refers to a bacterium's capacity to genetically encode resistance genes that overturn the inhibitory effects of antibiotics in order to thrive. It may be acquired through horizontal gene mutation processes like conjugation, transformation, and transduction or it may arise intrinsically through integration and spontaneous recombination into the bacterial genome.[1] Antimicrobial resistance (AMR) is now a global health emergency. We are moving towards a post-antibiotic age where our current arsenal antibiotics won't work because of the overuse and injudicious prescription of antibiotics. Globally, AMR causes 700,000 deaths annually, but if the above-mentioned urgent steps to address drug-resistant infections are not taken, this number is expected to rise to 10 million deaths annually by 2050.[2,3]

Antimicrobial susceptibility testing (AST) of microorganisms can be performed using various methods, one of which is the disc diffusion technique, first standardized by Bauer *et al.* in 1966.[4] Kirby-Bauer disc diffusion is the most common routine traditional method used for AST reporting. Kirby-Bauer disk diffusion method is easier, reproducible dependable and materials required are

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cost effective and allows visibility of growth and can be used for isolates from mixed cultures. This method provides the laboratory with a great deal of flexibility and antibiotic panels can be created based on antibiogram and the presence of antibiotic-resistant strains in that particular area.[5,6]

Prompt administration of the appropriate antibiotic treatment is essential for saving lives in severe infections and can also help lower healthcare costs.[7-10] Studies have demonstrated that timely and effective antibiotic therapy significantly improves clinical outcomes and reduces mortality.[11] Fast access to accurate results from antimicrobial susceptibility tests (AST) is seen as a major unaddressed need in treating infectious diseases. Immediate and accurate diagnostic approaches for AST are essential for lowering dependence on broad-spectrum antibiotics and addressing the increasing challenge of resistance.[12]

Delayed release of Microbiological culture reports leads to starting of empirical treatment with broad spectrum antibiotics leading to emergence of multidrug resistance. There are published data on shortened incubation period for disc diffusion testing, but these protocols are yet to be standardized for recommendations by CLSI or EUCAST.

In this study, an approach to decrease the turnaround time for disc diffusion testing was made by reducing the incubation period required for the growth of the bacteria from clinical sample that precedes disk diffusion testing. This study was performed to evaluate the disc diffusion susceptibility test results on early growth of isolates i.e., at 6 and 10 hours of incubation by comparing with the susceptibility test results performed on 18-24 hours growth.

Early disc diffusion (EDD) testing will decrease the turnaround time (TAT) of AST reports and this method would be the most cost-effective method to accelerate AST results.[13]

2. Materials and Methods

A laboratory based cross sectional study was conducted in the tertiary care hospital, *Staphylococcus aureus* [ATCC 29213], *Staphylococcus aureus* [ATCC 25923], *Enterococcus faecalis* [ATCC 29212], *Escherichia coli* [ATCC 25922] and *Pseudomonas aeruginosa* [ATCC 27853] were used as quality control (QC) strains.

2.1. Clinical isolates

One hundred clinical isolates, both sensitive and resistant strains, isolated from various clinical samples were included in the study. They included 25 isolates each of *Staphylococcus aureus*, *Enterococcus* species, *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae)* and *Pseudomonas aeruginosa* identified with standard biochemical reactions.[14]

Both Quality control (QC) strains and clinical isolates were inoculated onto peptone water and turbidity adjusted to 0.5 McFarland standard. A loopful of this inoculum was then inoculated by quadrant streaking onto Blood Agar (BA) and MacConkey Agar (MA) plates. These plates were incubated at 37°C in ambient air for 6, 10, and 18-24 hours.

Bacterial inoculum for Kirby Bauer's disc diffusion was prepared by taking similar type of colonies to be tested using sterile inoculation straight wire. 3-5 individual colonies from the culture growth of clinical isolates and QC strains were selected from plates incubated for 6, 10, and 18-24 hours of incubation (**Figure 1**). The colonies were suspended in peptone broth, the turbidity of this suspension was adjusted to 0.5 McFarland standard. A sterile swab was soaked into the inoculum tube and rotated against the sides of the tube to remove excess fluid. The dried surface of a cation adjusted Muller Hinton Agar (MHA) plate was inoculated by streaking the swab three times over the entire agar surface rotating the plates approximately 60 degree each time and lastly along the rim of the plate with the swab to pick up any excess inoculum. The plates were allowed to dry for 3-5mins to allow evaporation of excess moisture and appropriate antibiotic discs were applied onto the agar surface depending on the organism (**Table 1**) using sterile forceps.

The MHA plates were incubated aerobically at 37°C for 18-24 hours as per CLSI guidelines. The size of the zone of inhibition from all the plates inoculated with growth at 6 hours, 10 hours and 18-24 hours was measured manually and interpreted as Sensitive (S), Intermediate (I) and Resistant (R) based on CLSI (16) guidelines 2024 (**Figure 2** and **Figure 3**).

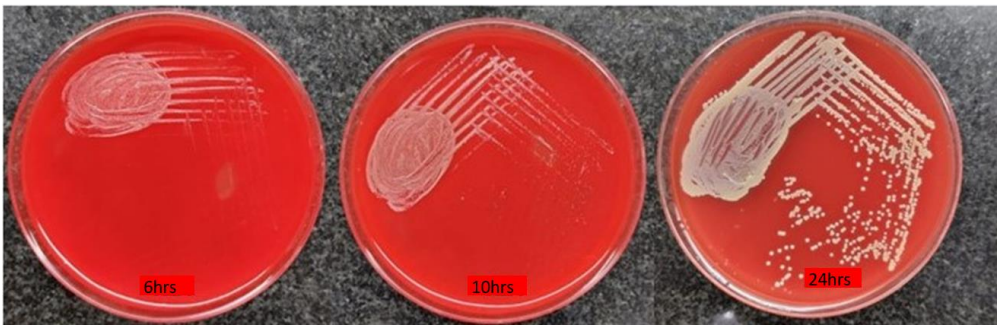


Figure 1: Growth of *S. aureus* on blood agar at 6, 10 and 18-24 hours of incubation

Table 1: Displays the number of organisms, quality control strains with ATCC numbers, clinical isolates distribution of resistance patters and clinical samples

Staphylococcus	***Enterococcus***	***Enterobacterales***	***Pseudomonas***
Clinical Isolates (Count)			
S. aureus (25)	*Enterococcus species* (25)	*E.coli* (12)	*P. aeruginosa* (25)
		K. pneumoniae (13)	
QC Strains (Triplets)			
S. aureus (ATCC 25923)	*E. faecalis* (ATCC 29212)	*E.coli* (ATCC 25922)	*P. aeruginosa* (ATCC 27853)
S. aureus(ATCC 29213)			
100 Clinical Isolates (Distribution of resistance pattern)			
Methicillin - resistance *Staphylococcus aureus* (MRSA) - 20	Vancomycin Resistant *Enterococcus* (VRE) - 02	*E.coli* (Carbapenem-resistant) - 03 *E.coli* (Sensitive-) - 04 *E.coli* (Variable -) - 05	*P. aeruginosa*(Multi-drug resistant) – 05 *P. aeruginosa* (Sensitive) - 14 *P. aeruginosa* (Variable) - 06
Methicillin - sensitive *Staphylococcus aureus* (MSSA) – 05	Vancomycin sensitive *Enterococcus* - 23	*K. pneumoniae* (Multi-drug resistant) - 05 *K. pneumoniae* (Sensitive) - 03 *K. pneumoniae* (Variable) - 05	
25 Urine samples (Count)			
S. aureus - 0	*Enterococcus* species - 7	*E.coli* -11	*P. aeruginosa* -3
		K. pneumoniae -04	
25 Positive flagged blood samples(Count)			
S. aureus -10	*Enterococcus* species -0	*E. coli* -08	*P. aeruginosa* -0
		K. pneumoniae -07	

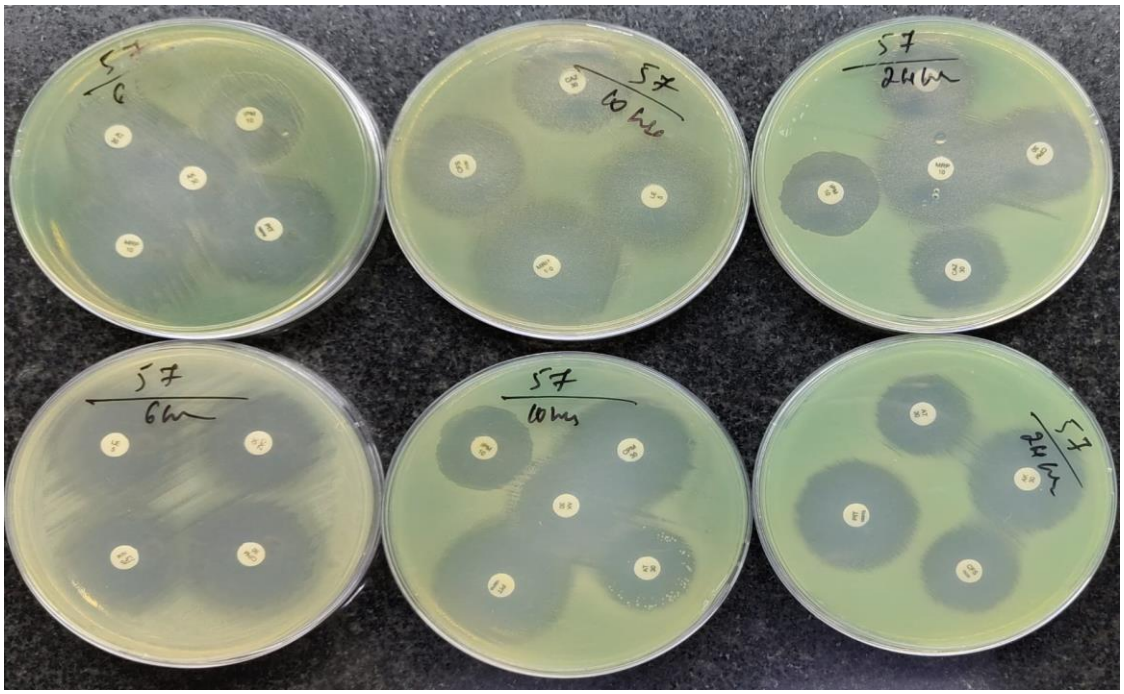
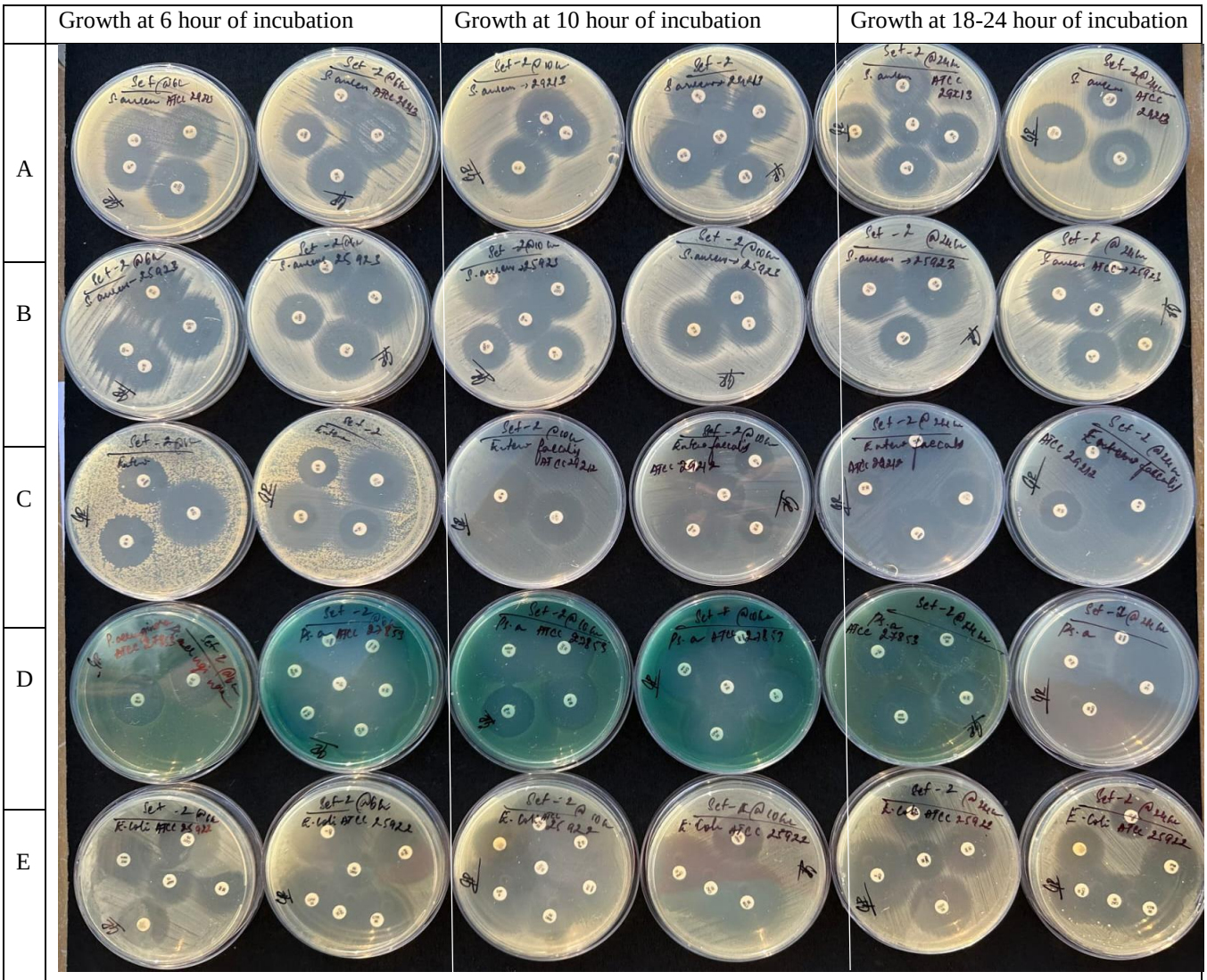


Figure 2: EDD Susceptibility testing of *P. aeruginosa* at 6, 10 and 18-24 hours growth

2.2. Clinical sample

Fifty clinical samples (25 turbid Urine samples and 25 Positive flagged blood culture samples) were also inoculated. Urine samples were inoculated onto chromogenic Urichrome agar (UCA) (**Figure 4**A) and Positive flagged blood cultures were sub cultured onto Blood agar (BA) and MacConkey agar (MA) (**Figure 4** B&C). The BA, MA and UCA plates were incubated aerobically at 37℃. Those clinical samples which yielded single type of growth were included in the study. The growth at 10 hour and 18-24 hours of incubation were used to perform Kirby Bauer's disc diffusion as per CLSI guidelines.[15] The size of the zone of inhibition of 10-hour old growth was compared with the standard 18-24 hour old growth and interpreted as Sensitive (S), Intermediate (I) and Resistant (R) based on Clinical and Laboratory Standards Institute (CLSI) guideline 2024.[15]



Row A: Susceptibility testing of QC strain of *Staphylococcus aureus* [ATCC 29213] at 6, 10 and 18-24 hours growth.

Row B: Susceptibility testing of QC strain of *Staphylococcus aureus* [ATCC 25923] at 6, 10 and 18-24 hours growth.

Row C: Susceptibility testing of QC strain of *Enterococcus faecalis* [ATCC 29212] at 6, 10 and 18-24 hours growth.

Row D: Susceptibility testing of QC strain of *Pseudomonas aeruginosa* [ATCC 27853] at 6, 10 and 18-24 hours growth.

Row E: Susceptibility testing of QC strain of *Escherichia coli* [ATCC 25922] at 6, 10 and 18-24 hours growth.

Figure 3: EDD Susceptibility testing of QC strains at 6h, 10h and 18-24h growth

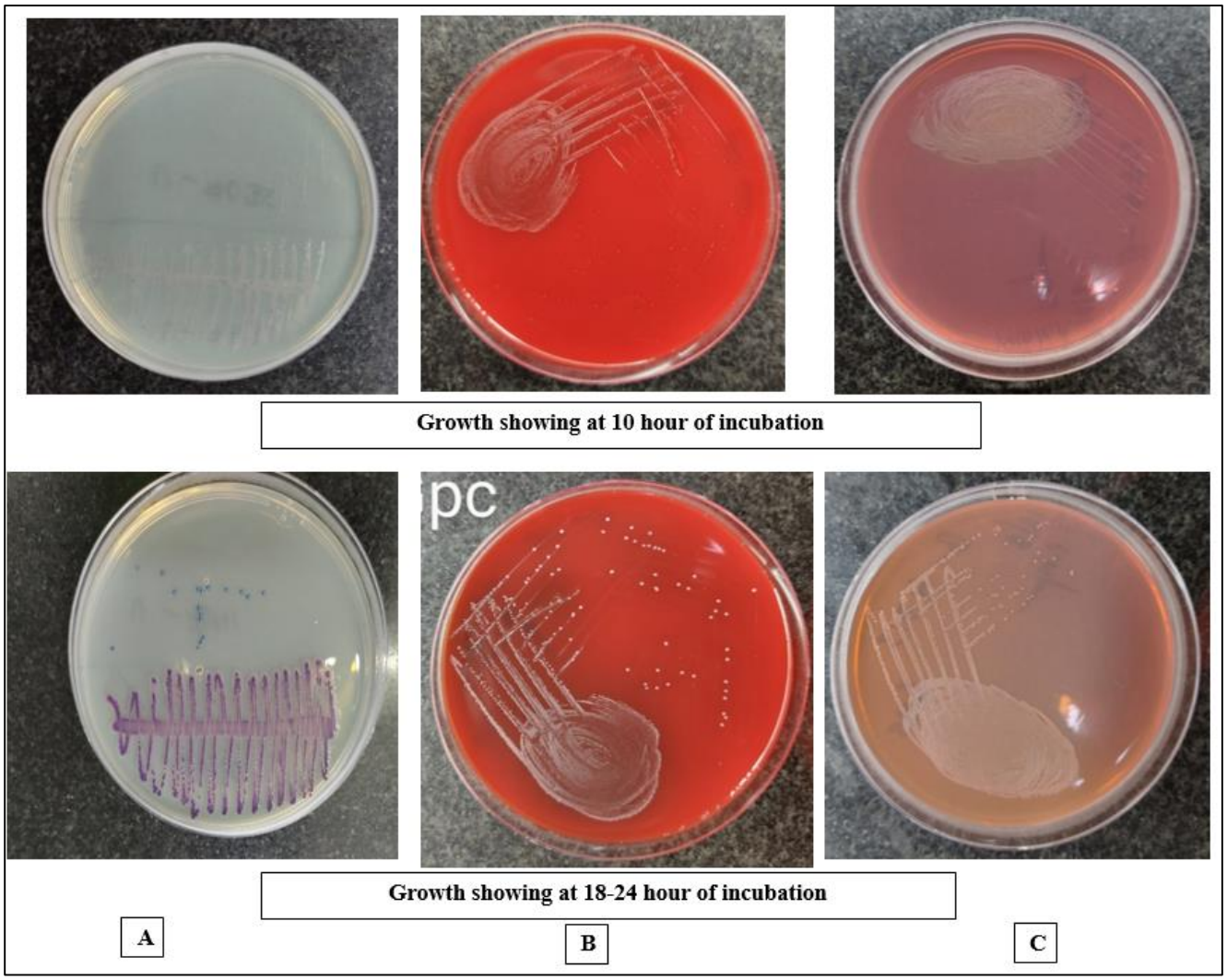


Figure 4: A): Culture growth of urine sample inoculation on Urichrome agar after 10 hour and 18- 24 hours of incubation; **B)**: Culture growth of Gram Positive Cocci on blood agar from Positive flagged blood culture sample after 10 hour and 18-24 hours of incubation; **C)**: Culture growth of Gram Negative Bacilli on Mac Conkey agar from Positive flagged blood culture sample after 10 hour and 18-24 hours of incubation

The following antibiotic disks panels were used for AST testing as per CLSI guidelines.

1. *Staphylococcus aureus*: Cefoxitin (30µg), Ciprofloxacin (5µg), Gentamicin (10µg), Tetracycline (30µg), Linezolid (30µg), Cotrimoxazole (23.5/1.25µg), Erythromycin (15µg), Clindamycin (2µg)
2. *Enterocccus spp*: Ampicillin (10µg), Penicillin (10µg), Tetracycline (30µg), Erythromycin (15µg), high level Gentamicin (120µg), Linezolid (30µg), Vancomycin (30µg), Teicoplanin (30µg).
3. *Escherichia coli* and *Klebsiella pneumoniae*: Ampicillin (30µg), Ceftriaxone (30µg), Cefepime (30µg), Amoxycillin clavulanicacid (20/10µg), Pipericillin tazobactam (100/10µg), Imipenem (10µg), Amikacin (30µg), Ciprofloxacin (5µg), Cotrimoxazole (10µg).
4. *Pseudomonas aeruginosa*: Ceftazidime (30µg), Cefepime (30µg), Cefoperazone sulbactam (75/30µg), Piperacillin tazobactum (100/10µg), Aztreonam (30µg), Meropenem (10µg), Imipenem (10µg), Amikacin (30µg), Levofloxacin (5µg).

Categorical agreement (CA), minor error (mE), major error (ME), and very major error (VMEs) of the Early disc diffusion (EDD) test methods were calculated in reference to the approved 18-24 hours growth and quality control isolates antibiotic susceptibility testing as per CLSI guidelines 2024 (16). Categorical agreement (CA) means similar interpretive criteria (susceptible/intermediate/resistant) was agreed upon between the two methods. Minor error (mE) means a susceptible or resistant result was shown as intermediate and vice versa. Major error (ME) denotes a susceptible isolate shown as resistant and calculated only for susceptible isolates. Very major error (VME) suggests a resistant isolate shown as susceptible and calculated only for resistant isolates.

3. Results

In this study, the new methodology, early disc diffusion (EDD) method from 6- and 10-hour growth of the clinical isolates and quality control isolates were studied in comparison to the established 18-24 hours growth Kirby-Bauer antimicrobial susceptibility test method. But for the isolates directly from clinical samples, only 10-hour early growth culture were included in the study and 6-hour early growth were not included due to heterogeneous culture yield and extremely small colonies that made it difficult to distinguish the mixed type of growth.

Quality control strains *Staphylococcus aureus* [ATCC 29213], *Staphylococcus aureus* [ATCC 25923], *Enterococcus faecalis* [ATCC 29212], *Escherichia coli* [ATCC 25922] and *Pseudomonas aeruginosa* [ATCC 27853] were tested in triplicates for early disc diffusion (EDD) method at 6hour and 10hour of growth. The susceptibility results showed 100% agreement with 18-24h growth standard method, with no major or minor discrepancies.

The susceptibility testing results of clinical isolates from 6-hour growth (EDD6) compared with the Standard 18-24 hour growth (St24) reference method showed 97.48% categorical agreement (CA) with 0.63% very major error (VME), 0.58% major error (ME) and 1.94% minor error (mE) discrepancies. A linear regression analysis of inhibitory zones from EDD6 and St24 revealed an r^2 value of 0.87 and a slope value of 0.94 which suggests a high level of correlation between the two methods (**Figure 5**).

EDD for 6h growth of *S. aureus* showed 96% categorical agreement with 1.29% VME, 0.87% ME, 3% mE, *Enterococcus* showed 99% with 1% mE discrepancy, *P. aeruginosa* showed 96.4% CA with 2.94% VME, 0.59% ME, 2.66% mE, *E. coli* showed 97.5% CA with 2.55% mE and *K. pneumoniae* showed the highest rate of 99.2% CA with 2.43% ME compared to the 24h standard reference method respectively (**Table 2**).

EDD for 10h growth of *S. aureus* showed 94.5% CA with 0.87% ME, 5% mE, *Enterococcus* showed 98.5% CA with 1.5% mE, *P. aeruginosa* showed 96% CA with 5.88% VME, 0.59% ME, 2.66% mE, *E. coli* showed 100% CA with no errors and *K. pneumoniae* showed 98.46% CA with 2.43% ME and 0.76% mE compared to the 18-24h standard reference method respectively (**Table 3**).

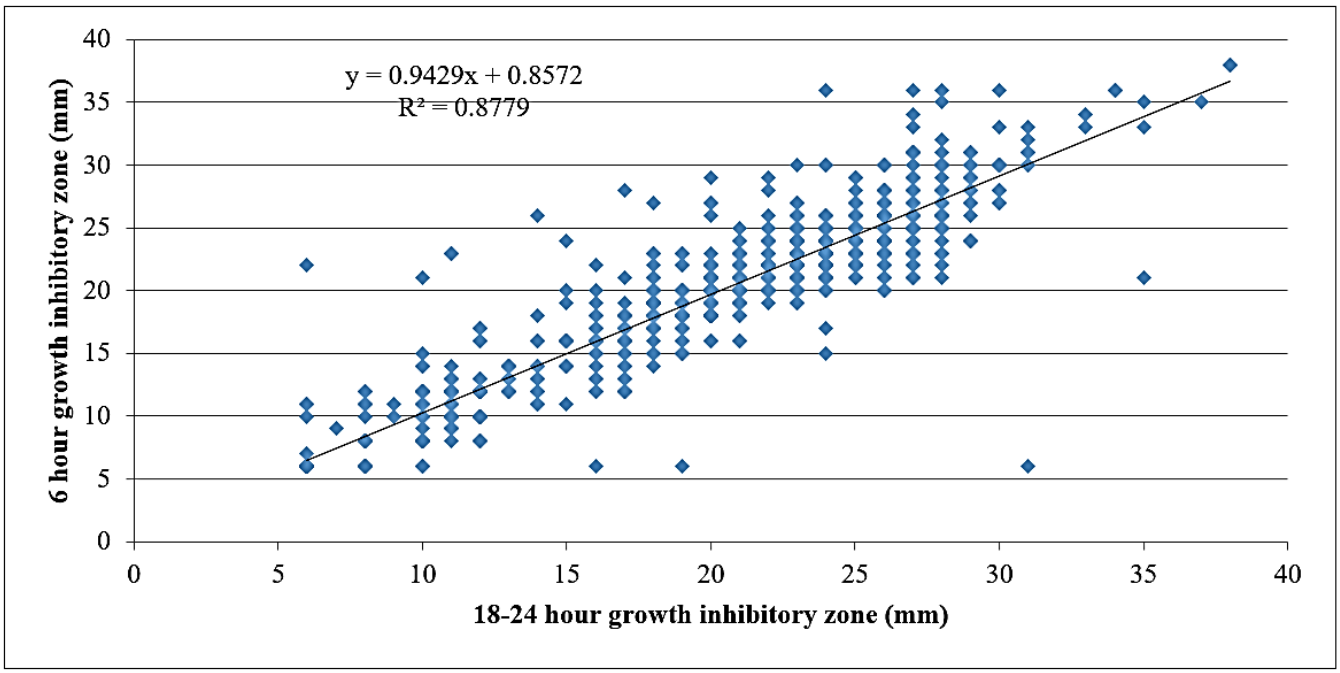


Figure 5: Linear regression analysis of inhibitory zones from EDD 6-hour growth compared to standard 18-24 hours growth of clinical isolates

Table 2: Comparison of AST testing by disk diffusion from 6-hours old growth method versus 18-24 hours standard growth with clinical isolates and QC strains.

Clinical isolates 6-hour incubation												
6-hour incubation AST report					**Reference method (18-24 hour) AST report**				**Discrepancies (n/n %)**			
Organism	**S**	**I**	**R**	**Total**	**S**	**I**	**R**	**Total**	**Agreement (CA)**	**Very major (VME)**	**Major (ME)**	**Minor (mE)**
S.aureus	113	11	76	200	114	9	77	200	192/200 (96%)	1/77 (1.29%)	1/114 (0.87%)	6/200 (3%)
Enterococcus	129	2	69	200	127	4	69	200	198/200 (99%)	0/69 (0%)	0/127 (0%)	2/200 (1%)
P.aeruginosa	171	20	34	225	167	24	34	225	217/225 (96.4%)	1/34 (2.94%)	1/167 (0.59%)	6/225 (2.66%)
E.coli	62	6	52	120	64	5	51	120	117/120 (97.5%)	0/51 (0%)	0/64 (0%)	3/120 (2.5%)
K.pneumoniae	40	5	85	130	41	5	84	130	129/130 (99.2%)	0/84 (0%)	1/41 (2.43%)	0/130 (0%)
Total	515	44	316	875	513	47	315	875	853/875 (97.48%)	2/315 (0.63%)	3/513 (0.58%)	17/875 (1.94%)
QC Strains 6-hours incubation												
S.aureus	48	0	0	48	48	0	0	48	48/48 (100%)	0 (0%)	0/48 (0%)	0/48 (0%)
Enterococcus	24	0	0	24	24	0	0	24	24/24 (100%)	0 (0%)	0/24 (0%)	0/24 (0%)
P.aeruginosa	27	0	0	27	27	0	0	27	27/27 (100%)	0 (0%)	0/27 (0%)	0/27 (0%)
E.coli	30	0	0	30	30	0	0	30	30/30 (100%)	0 (0%)	0/30 (0%)	0/30 (0%)
Total	129	0	0	129	129	0	0	129	129/129 (100%)	0 (0%)	0/129 (0%)	0/129 (0%)
Total clinical isolates and QC isolates												
All organisms	644	44	316	1004	642	47	315	1004	982/1004 (97.8%)	2/315 (0.63%)	3/642 (0.46%)	17/1004 (1.6%)

Table 3: Comparison of AST testing by disk diffusion from 10-hours old growth method versus 18-24 hour standard growth with clinical isolates and QC strains

Clinical Isolates 10-hour incubation												
10hour incubation AST report					**Reference method (18-24 hour) AST report**				**Discrepancies (n/n %)**			
Organism	**S**	**I**	**R**	**Total**	**S**	**I**	**R**	**Total**	**Agreement (CA)**	**Very major (VME)**	**Major (ME)**	**Minor (mE)**
S.aureus	110	13	77	200	114	9	77	200	189/200 (94.5%)	0/77 (0%)	1/114 (0.87%)	10/200 (5%)
Entercoccus	127	3	70	200	127	4	69	200	197/200 (98.5%)	0/69 (0%)	0/127 (0%)	3/200 (1.5%)
P.aeruginosa	170	20	35	225	167	24	34	225	216/225 (96%)	2/34 (5.88%)	1/167 (0.59%)	6/225 (2.66%)
E.coli	65	5	51	120	64	5	51	120	120/120 (100%)	0/51 (0%)	0/64 (0%)	0/12 (0%)
K.pneumoniae	39	6	85	130	41	5	84	130	128/130 (98.46%)	0/84 (0%)	1/41 (2.43%)	1/130 (0.76%)
Total	511	47	318	875	513	47	315	875	850/875 (97.1%)	2/315 (0.63%)	3/513 (0.58%)	20/875 (2.28%)

Table 3 Continued…												
QC Strains 10-hours incubation												
S.aureus	48	0	0	48	48	0	0	48	48/48 (100%)	0 (0%)	0/48 (0%)	0/48 (0%)
Enterococcus	24	0	0	24	24	0	0	24	24/24 (100%)	0(0%)	0/24 (0%)	0/24 (0%)
P.aeruginosa	27	0	0	27	27	0	0	27	27/27 (100%)	0 (0%)	0/2 (0%)	0/2 (0%)
E.coli	30	0	0	30	30	0	0	30	30/30 (100%)	0 (0%)	0/30 (0%)	0/30 (0%)
Total	129	0	0	129	129	0	0	129	129/129 (100%)	0 (0%)	0/1 (0%)	0/129 (0%)
Clinical Samples												
Urine Samples												
Enterococcus	41	1	14	56	41	1	14	56	56/56 (100%)	0/24 (0%)	0/41 (0%)	0/56 (0%)
P.aeruginosa	9	2	16	27	9	2	16	27	27/27 (100%)	0/16 (0%)	0/9 (0%)	0/27 (0%)
E.coli	51	4	55	110	52	3	55	110	109/110 (99.09%)	0/55 (0%)	0/52 (0%)	1/110 (0.90%)
K.pneumoniae	10	1	29	40	11	2	27	40	39/40 (99.09%)	0/27 (0%)	0/11 (0%)	1/40 (2.5%)
Total	113	8	112	233	113	8	112	233	231/233 (99.14%)	0/122 (0%)	0/113 (0%)	2/233 (0.85%)
Positive Flagged Blood (culture) samples												
S.aureus	49	2	29	80	49	3	28	80	79/80 (98.75%)	0/28(0%)	0/49 (0%)	1/80 (1.25%)
E.coli	39	2	54	96	40	3	53	96	95/96 (98.95%)	0/53 (0%)	0/40(0%)	1/96 (1.04%)
K.pneumoniae	47	4	33	84	47	4	33	84	84/84 (100%)	0/33 (0%)	0/47 (0%)	0/84 (0%)
Total	136	8	116	260	136	10	114	260	258/260 (99.23%)	0/114 (0%)	0/136 (0%)	2/260 (0.76%)
Total clinical isolates, QC isolates and clinical samples												
All organisms	884	67	546	1497	891	65	541	1497	1468/1497 (98.06%)	2/551(0.36%)	3/891(0.33%)	24/1497 (1.60%)

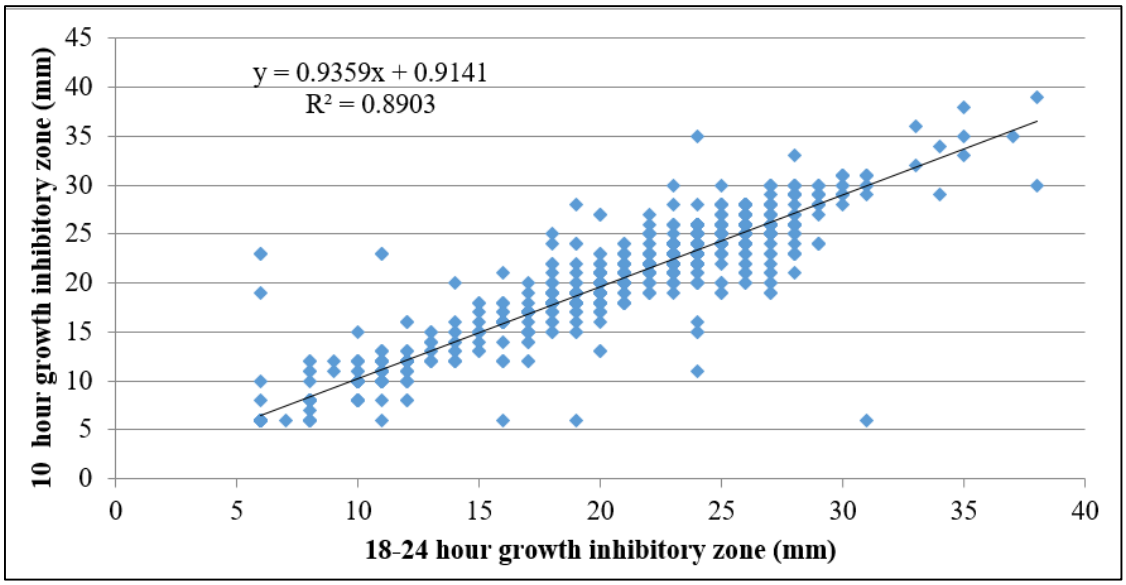


Figure 6: Linear regression analysis of inhibitory zones from EDD10 hour growth compared to standard 18-24 hours growth of clinical isolates

97.1% categorical agreement (CA) with 0.63% very major error (VME), 0.58% major error (ME) and 2.28% minor error (mE) discrepancies were observed for the susceptibility results of 10-hour growth (EDD10) compared with the standard 18-24 hours (St24) hour growth. A linear regression analysis of inhibitory zones from EDD10 and St24 revealed an r^2 value of 0.89 and a slope value of 0.93 which suggests a high level of correlation between the two methods (**Figure 6**).

3.1. Clinical samples

Twenty-five clinical isolates isolated turbid urine samples showed greater rate of categorical agreement of 99.14% with only 0.85% minor discrepancy for 10-hour growth (EDD10). *Enterococcus* and *P. aeruginosa* showed complete agreement to the 18-24h standard reference method. *E. coli* and *K. pneumoniae* both showed 99.09% CA with only 0.90% and 2.5% mE respectively.

The EDD 10-hour growth of 25 isolates isolated from positive flagged blood cultures showed much higher rate of 99.23% CA with only 0.76% minor discrepancy when compared to 18-24 hours growth reference method. *S. aureus* showed 98.75% CA with 1.25% mE, *E. coli* showed 98.95% CA with 1.04% mE and *K. pneumoniae* showed absolute CA to the 18-24h standard reference method.

Overall clinical samples isolate linear regression analysis of inhibitory zones from EDD10 and St24 revealed an r^2 value of 0.91 and a slope value of 0.95 and this indicates a significantly higher correlation between the two methodologies (**Figure 7**).

In the study, it was also observed that D zone test in *S. aureus* for detection of inducible to clindamycin also showed 100% agreement in 10h EDD to standard 24-hour growth reference method. Detection of EBSL production also accurately matched with 10hour growth susceptibility testing. All resistance mechanisms detection could also be done accurately with 10h EDD method.

4. Discussion

This study highlights the importance of using the early growth of the culture isolates in testing antibiotic sensitivity pattern and hence the reducing the turnaround time for culture and sensitivity report.

The AST results of the QC isolates were accurate and showed 100% agreement with both EDD 6h and 10h compared to the standard reference 18-24 hours AST results. In the study conducted by Weber *et al*., the QC strains showed only 98.3% CA. QC strains of *S. aureus* 6-hour EDD AST reports showed 96.7% agreement with 3.3% major discrepancy. QC strains of *E. faecalis* 6-hour AST reports showed 95.8% CA and 4.7% mE. QC strain for EDD 6h of *E. coli*, *K. pneumoniae* and *P. aeruginosa* showed 100% agreement to the standard reference 24-hour AST results. QC strain for EDD 10h showed 96.6% CA with 0.5% ME and 3.4% mE and *S. aureus* showed 98.3% CA with 1.7% ME. *E. faecalis* showed 87.5% CA with 12.5% mE. *E. coli* has 98.6% CA with 1.4 mE. *K. pneumoniae* and *P. aeruginosa* showed 100% agreement to the standard reference 18-24 hours AST results which was comparable with the present study.[5]

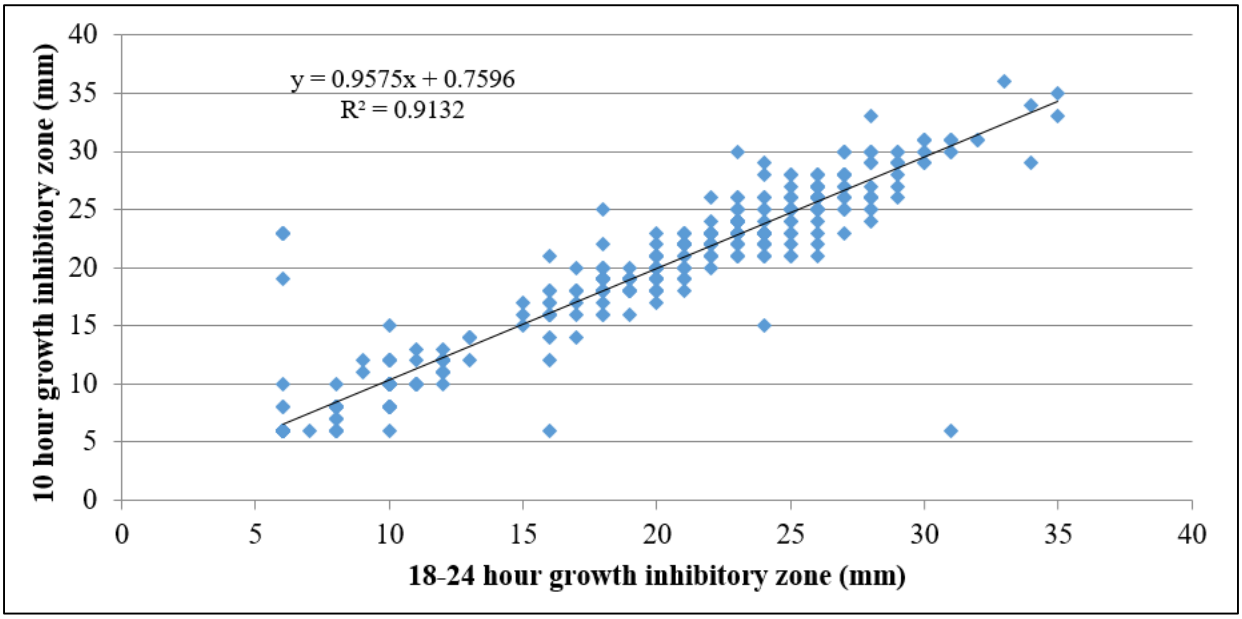


Figure 7: Linear regression analysis of inhibitory zones from EDD10 hour primary growth compared to standard 18-24 hours growth from clinical samples

A study conducted by Biswas *et al*., evaluating this EDD testing for QC strains, they observed a similarly high level of correlation between EDD6 and standard 24h as well as between EDD10 and St24. For the 20 quality control strains, they observed the categorical agreement of EDD6 with standard incubation was 94.38% with 5.10% minor error (mE) and 0.69% major errors (MEs). Likewise, 10-h growth comparisons yielded a same pattern of 5.10% mE and 0.69% MEs and no VMEs with 94.38% categorical agreement with standard incubation.[13]

In the study conducted by Weber *et al*., EDD 6h testing of clinical isolates showed 95.6% categorical agreement with 2.5% very major, 1.6% major and 2.5% minor errors. EDD 10h testing of clinical isolates showed 96.7% categorical agreement with 0.8% major and 2.7% minor errors.[5]

In the study conducted by Biswas *et al*., evaluating this EDD testing for the 48 clinical isolates they observed the categorical agreement of EDD6 with standard incubation was 98.15% with 1.22% major error and 1.29% minor errors and no very major errors.[5]

In our study for clinical samples for EDD 6h with the 18-24 hours reference method showed 97.48% agreement with 0.63% very major, 0.58% major and 1.94% minor discrepancies and for clinical isolates of EDD 10h with the 24h-reference method showed 97.1% agreement with 0.63% very major, 0.58% minor and 2.28% minor discrepancies.

The novelty of this study is that growth from clinical samples urine and positive flagged blood culture were also included in the study. Even though there are studies using early growth for testing disc diffusion, there are no published data on EDD studies performed directly on isolates isolated from clinical samples. Six-hour old growth was not tested as it was difficult to identify mixed growth as the colonies were extremely small. In this study, EDD for 10h growth isolated from Urine samples showed 99.14% agreement and 0.85% minor error with no very major or major discrepancies. In urine samples *E.coli* and *K. pneumoniae* showed more than 99% agreement whereas *Enterococcus* and *P. aeruginosa* showed complete agreement to the 18-24h standard reference method.

The 10-hour growth of clinical isolates isolated from Positive flagged blood culture samples showed 99.23% agreement with 0.76% minor discrepancy. *S. aureus* and *E. coli* showed more than 98% categorical agreement whereas *K. pneumoniae* showed complete agreement with the 18-24h standard reference method.

In this study, EDD for 10hour growth showed more accurate results than 6 hour growth of AST reports. Also, the drug resistance mechanisms such as inducible clindamycin (D-zone) test and ESBL production were also detected in the EDD performed form 10-hour incubated growth and the results correlated well with that performed with 18-24 hours growth standard method. In 10h growth colonies are moderate in size and it's easy to pick up isolated colonies whereas it was very difficult to pick isolated colonies 6hour growth and also to identify mixed or heterogeneous growth. By performing the AST testing using 10-hour old growth, the reports can be dispatched 10 to 12 hours early decreasing the turnaround time which will have a significant impact on early initiation of patient treatment and antimicrobial stewardship.

These values of categorical agreement were well above the threshold (90% or more) provided by the FDA Class II Special Controls Guidance for AST systems. Likewise, AST results from EDD6 and EDD10 met the FDA-recommended threshold of ME of 3% or less and VME upper and lower 95% CIs less than or equal to 7.5 and 1.5%, respectively (Food and Drug Administration).[16] All the statistical analysis indicates that early disc diffusion method using 6- and 10-hour growth is highly reliable and can be considered for routine reporting of antibiotic susceptibility test results.

5. Conclusion

Kirby-Bauer disc diffusion is the most common method of performing sensitivity testing in the most of the laboratories. This study shows that the culture isolates as old as 6 and 10 hours have yielded comparable results with that of CLSI approved standard 18-24 hours growth. Hence 6 hours and 10 hours old culture can be used in the routine sensitivity testing and reporting of bacterial isolates such as *Staphylococcus aureus*, *Enterococcus*, *P. aeruginosa*, *E. coli* and *K. pneumoniae*. 10hour early disc diffusion is ideal method for replacement for standard method, as it is highly concordant with reports of 18-24 hours standard reference method. Just by reducing growth period without adding extra cost is possible to release early report of AST which in turn helps to reduce AMR and improve antimicrobial stewardship.

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7. Conflicts of Interest

All the authors declare no conflict of interest.

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