



### RESEARCH ARTICLE

#### A COMPARATIVE STUDY TO DETERMINE THE ACCURACY OF DIRECT SUSCEPTIBILITY TESTING WITH CONVENTIONAL ANTIMICROBIAL SUSCEPTIBILITY TESTING

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#### Abstract

**Introduction:** Clinically significant bacteria are identified in the laboratory and the accurate information is provided with antimicrobial susceptibility testing (AST) which is essential for an accurate management of patients suffering with a bacterial infection or bacterial disease<sup>[1]</sup>. As the process of AST is time consuming and Results of AST are provided within a timespan of 48–72 h after sampling. Disk diffusion has many benefits, which includes low expenditure, time duration reduction of results and few more basic benefits. One of the benefits is the probability or chances of executing direct susceptibility testing (DST). When is potentially very useful in the management of critically used selectively and interpreted carefully, DST on clinical samples of ill patients, as the time to results is shortened by approximately 24 h.

**Aim:** To do a comparative study for determining the accuracy of direct susceptibility testing with conventional antimicrobial susceptibility testing.

**Material And Method:** This cross-sectional study was conducted in the Department of Microbiology, People's College of Medical Sciences and Research Centre, over a period of 1 year—from February 2021 to February 2022.

**Result:** A total of 311 samples of urine, pus and body fluid were collected and positive samples processed from 124 males and 187 females from age groups 1 to 90 years. From total of 311 samples out of all the samples 132 were of pus, 175 were of urine specimen 4 were of fluid samples. Comparison of the bacterial response to 14 antimicrobial agents using direct antimicrobial sensitivity testing (DST) versus standard antimicrobial sensitivity testing (AST) for Gram positive bacteria. Out of 91 gram positive bacteria we found that on performing AST and DST on norfloxacin probability value was 1, which shows that there is no significant difference in results by these

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two methods. Similarly, Ciprofloxacin, Cefoxitin, Doxycycline, Amoxy-clavulanic acid, Erythromycin, Clindamycin, Linezolid, Chloramphenicol, Cefuroxime all have a p-value as 1 on performing both the different types of antimicrobials susceptibility testing method which indicates a similarity in their results.

**Conclusion:** After 1 year of systematic study even though we found that AST and DST both gives almost similar result, but keeping in mind major and minor differences in antibiotic sensitivity test. We should consider it only for emergency purposes. In emergency it is observed that DST proved to be more successful because DST provides results within 24 to 32 hours, this providing results faster but it should always be followed by AST for confirmation of results. With DST, we have to apply more no. of antibiotics disk to cover all bacteria increasing cost of ABST.

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### Introduction:-

Clinically significant bacteria are identified in the laboratory and the accurate information is provided with antimicrobial susceptibility testing (AST) which is essential for an accurate management of patients suffering with a bacterial infection or bacterial disease<sup>[1]</sup>. As the process of AST is time consuming and Results of AST are provided within a timespan of 48–72 h after sampling. Bacteria is needed to be cultured before AST can be executed because culturing the bacteria is an important task before performing (AST). The unpredictable and diminishing receptive to antibiotic agents may result in inadequate therapy and urges the empiric use of broad-spectrum antibiotics. Downscale of treatment must be practiced only when results from AST are available, with immediate and long-term outcomes such as the exposure of multidrug-resistant microorganisms and an increased risk of severe superinfections, morbidity, mortality and costs<sup>[2]</sup>. This method of detection of microbes and Antimicrobial susceptibility testing (AST) was one of the earliest forms of personalized medicine which was adopted for detecting the information regarding specific antibiotics needed in treatment of a patient's infection<sup>[3]</sup>. Various methods are used for performing susceptibility testing of microbes, but

Conventional phenotypic methods is one of the most commonly used method for performing of antimicrobials susceptibility testing which is based on culturing on agar (example- disk diffusion tests) or on microtitration plates (example- broth dilution tests).<sup>[4]</sup> Disk diffusion has many benefits, which includes low expenditure, time duration reduction of results and few more basic benefits. One of the benefits is the probability or chances of executing direct susceptibility testing (DST). DST has been practiced in some

Laboratories and reported in multiple papers, it is better when results are aimed to be in short span of time [5]. Providing clinician with early microbiological information has a better impact on the patient which is beneficial, permitting tailored antibiotic use and a decrease in antimicrobial-related adverse events<sup>[6]</sup>. The administration of appropriate antimicrobial therapy at the earliest time is essential in reducing the high incidence of mortality associated with bacteremia. In an attempt to shorten reporting time for susceptibility results, several authors have suggested that in urgent situations susceptibility plates may be inoculated directly with clinical material. The results of these direct or preliminary tests are then confirmed the following day by using one of the accepted standardized methods [7-8] when is potentially very useful in the management of critically used selectively and interpreted carefully, DST on clinical samples ill patients, as the time to results is shortened by approximately 24 h. However, are recommended to communicate results with reservations and confirm by conventional AST. Direct disk diffusion susceptibility testing of the organisms in clinical samples has been shown to be reliable for most microorganisms and antimicrobial agents [10,11,12,13]. This technique can save 18 to 24 h compared to the times required for the standardized protocols. Additional time savings can be obtained by early reading (6 to 10 h) of the plates after direct incubation [14,15,16]. Direct susceptibility testing has an additional advantage for the testing of a broader representation of the bacterial population present in clinical samples and is more likely to detect the heterogeneous resistant bacteria which represent only a minor subpopulation in positive clinical samples. This might explain the observation that on most occasions in which discrepant results occurred the direct method detected the more resistant organism of the mixed cultures and very major errors were not found [17,18].

**Aim:-**

To do a comparative study for determining the accuracy of direct susceptibility testing with conventional antimicrobial susceptibility testing.

**Objectives:-**

To determine antibiogram through direct susceptibility testing, To determine antibiogram of conventional AST, Comparison between the accuracy of direct susceptibility testing & conventional AST.

**Material And Method:-**

This cross-sectional study was conducted in the Department of Microbiology, people's College of Medical Sciences and Research Centre, over a period of 1 year-from February 2021 to February 2022. A total of 311 samples of urine, pus and body fluid were collected and positive sample processed from 12 males and 187 females from age groups 1 to 90 years.

**Sample Collection:**

A total of 311 clinical sample were selected on various indications, such as request by the clinician for DST urine, pus and body fluid were collected and positive sample processed, Gram stain showing predominantly GPC or GNB. The studied specimens 132 pus, 175 urine and 4 body fluids.

**Culture:**

Urine sample was spread onto cysteine-Lactose-Electrolyte Deficient Agar (CLED) solid media. Pus & body fluids inoculated on Blood agar and MacConkey agar. The inoculated plates were incubated aerobically at 37°C for overnight and then examined for growth. Preliminary tests - Gram stain, Catalase test and Motility testing, were done and further processing and biochemical reactions were done using standard techniques to identify the organisms.

**Antimicrobial Susceptibility Testing:**

Disk diffusion AST & DST was performed using paper disks on Mueller hinton agar (Hi-media laboratories Pvt. Ltd. Mumbai) for GPC 14 antibiotics were tested Nitrofurantion (NIT), Norfloxacin (NX), Co-trimoxazole (COT), Ciprofloxacin (CIP), Cefoxitin (CX), Doxycycline (DO), Amoxy-clavulanic acid (AMC), Gentamicin (GEN), Erythromycin (E), Clindamycin (CD), Linezolid (LZ), Penicillin (P), Chloramphenicol (C), Cefuroxime (CFX). For GNB 14 antibiotics were tested Nitrofurantion (NIT), Norfloxacin NX, Co-trimoxazole (COT), Ciprofloxacin (CIP), Cefoxitin (CX), Doxycycline (DO), Amoxy-clavulanic acid (AMC), Gentamicin (GEN), Ampicillin/Sulbactam (A/S), Piperacillin/Tazobactam (PIT), Meropenem (MRP), Cefepime (CPM), Ceftriaxone (CTR), Amikacin (AK). Zone of inhibition were interpreted as susceptible (S) or resistant (R) according to the CLSI guidelines. For DST, a sterile cotton swab was dipped into a vortexed sample and inoculated onto a Mueller-Hinton agar plate, following a massive three direction pattern. AST with disk diffusion was executed according to the CLSI guidelines. Both AST and DST Plates were read simultaneously after overnight incubation at 37°C for 18-24 hours aerobically and observed for zone of inhibition. The results of DST were compared with the cumulative susceptibility of the different isolates found with the regular technique.

**Result:-**

This cross-sectional study was conducted in the Department of Microbiology, people's College of Medical Sciences and Research Centre, over a period of 1 year-from February 2021 to February 2022. A total of 311 samples of urine, pus and body fluid were collected and positive sample processed from 124 males and 187 females from age groups 1 to 90 years. From total of 311 samples Out of all the samples 132 were of pus, 175 were of urine specimen 4 were of fluid sample. Direct microscopic examination of all the samples by Gram staining revealed 91 Gram positive cocci mostly in clusters and 220 Gram negative bacilli.

Sample	Sample Size
Urine	175(65.2)
Pus	132(42.4)
Fluid	4(1.3)
<b>Total</b>	<b>311</b>

Out of 91 Gram positive bacteria isolates, Staphylococcus spp. (29.3%) out of 221 Gram negative bacterial the most commonly isolated organism was Escherichia coli 132 (42.44%), Klebsiella spp. 72 (23.15%), Citrobacter Freundii 11 (3.53%), Proteus mirabilis 6 (1.92%).

32 Comparison of the bacterial response to 14 antimicrobial agents using direct antimicrobial sensitivity testing (DST) versus standard antimicrobial sensitivity testing (AST) for Gram positive bacteria

Out of 91 gram positive bacteria we found that on performing AST and DST on norfloxacin probability value was 1, which shows that there is no significant difference in results by these two methods. Similarly, Ciprofloxacin, Cefoxitin, Doxycycline, Amoxy-clavulanic acid, Erythromycin, Clindamycin, Linezolid, Chloramphenicol, Cefuroxime all have a p-value as 1 on performing both the different types of antimicrobial susceptibility testing method which indicates a similarity in their results. Other antimicrobial agent have recorded probability (p) value between 0.85-0.87, which is not statistically significant. Out of 220 gram negative bacteria we found that on performing AST and DST on Norfloxacin probability value was 1, which shows that there is no significant difference in results by these two methods. Similarly, all Doxycycline, Gentamicin, Piperacillin/Tazobactam, Meropenem, Ceftriaxone have a p-value as 1 on performing both the different types of antimicrobial susceptibility testing method which indicates a similarity in their results. Other antimicrobial agent have recorded probability (p) value between 0.85-0.87, which is not statistically significant. When 311 sample were tested against the 20 antimicrobial agents (a total of 2,542 microorganism-antibiotic combinations) by the Kirby-Bauer disc diffusion method, the overall agreement between the two methods in term of the interpretive categories were 12 (0.6%) major errors caused by the direct method. The major discrepancies were observed for strain of E.coli, Klebsiella spp., and Staphylococcus aureus when testing Ceftriaxone, Penicillin, Nitrofurantion, Ciprofloxacin, Co-trimoxazole, Amikacin, Amoxy-clavulanic acid, Cefepime, Gentamicin and Ampicillin/Sulbactam. When 311 sample were tested against the 20 antimicrobial agents (a total of 2,542 microorganism-antibiotic combinations) by the Kirby-Bauer disc diffusion method, the overall agreement between the two methods in term of the interpretive categories (susceptible, and resistant) was 96.6%. 12 (0.6%) major errors and 71 (2.8%) minor error have been found by direct method. The major discrepancies were observed for strain of E.coli, Klebsiella spp., and Staphylococcus aureus when testing Ceftriaxone, Penicillin, Nitrofurantion, Ciprofloxacin, Co-trimoxazole, Amikacin, Amoxy-clavulanic acid, Cefepime, Gentamicin, and Ampicillin/Sulbactam.

### Discussion:-

In our study we compared 311 samples of urine, pus, and body fluids. On performing AST and DST on all 311 samples gram positive and gram negative isolates by routine and direct method we found that staphylococcus aureus was found in 91 sample out of 311 in both AST and DST. Klebsiella spp. was identified in 72 Samples in AST and 69 Samples in DST with a similarity of 95.83%, Escherichia coli 132 in AST and 127 in DST with 96.21% accuracy, citrobacter freundii 11 in AST and DST with 100% similarity, proteus mirabilis 6 in AST and DST with 100% accuracy in both the tests. Total 311 samples were used out of which 311 sample were totally identified with microbial agents in AST and 304 samples by DST. Similar to study conducted by Neelima angaali (2017) it was found that a total 57 samples were collected for identification of gram negative isolates by standard susceptibility testing and direct susceptibility testing. It was found that Escherichia coli was identified in 41 samples by Antimicrobial susceptibility testing and in 32 samples direct method with an agreement of 78.04%, Klebsiella spp. in 10 sample by AST and in 8 samples by DST with 80% similarity in results other all isolates in study have been found to on 100% agreement in AST and DST. Total gram negative isolates found to be 57 and 46 in AST and DST respectively with 80.7% similarity alike to our conducted study. [35] Out of 91 gram positive bacteria we found that on performing AST and DST on norfloxacin probability value was 1, which shows that there is no significant difference in results by these two methods. Similarly,

Ciprofloxacin, Cefoxitin, Doxycycline, Amoxy-clavulanic acid, Erythromycin, Clindamycin, Linezolid, Chloramphenicol, Cefuroxime all have a p-value as 1 on performing both the different types of antimicrobial susceptibility testing method which indicates a similarity in their results. Other antimicrobial agent have recorded probability (p) value between 0.85-0.87, which is not statistically significant. Out of 220 gram negative bacteria we found that on performing AST and DST on Norfloxacin probability value was 1, which shows that there is no significant difference in results by these two methods. Similarly, all Doxycycline, Gentamicin, Piperacillin/Tazobactam, Meropenem, Ceftriaxone have a p-value as 1 on performing both the different types of antimicrobial susceptibility testing method which indicates a similarity in their results. Other antimicrobial agent have recorded probability (p) value between 0.85-0.87, which is not statistically significant. Similar to study conducted by At el Raz Nawzad Mohammad (2018): Out of 1940 gram negative bacteria a we found on performing AST and DST on cefpodoxime similarly. Have p-value in their results

other antimicrobial susceptibility testing method which indicates a similarity in their results other antimicrobial agents have recorded probability p value between (0.85-0.87), which is not statistically significant. Meropenem, Amoxicillin-sulfamethoxazole, Gentamicin, Nitrofurantoin, Cefixime, Cefuroxime, Ciprofloxacin, Levofloxacin. [36] When 311 samples were tested against the 20 antimicrobial agents (a total of 2,542 microorganism-antibiotic combinations) by the Kirby-Bauer disc diffusion method, the overall agreement between the two methods in terms of the interpretive categories were 12 (0.6%) major errors caused by the direct method. The major discrepancies were observed for strains of *E. coli*, *Klebsiella* spp., and *Staphylococcus aureus* when testing Ceftriaxone, Penicillin, Nitrofurantoin, Ciprofloxacin, Co-trimoxazole, Amikacin, Amoxy-clavulanic acid, Cefepime, Gentamicin, and Ampicillin/Sulbactam. Similar to study conducted by At el J. Jong (1998): When 146 blood culture containing aerobic GNB were retested against the seven antimicrobial agents (a total of 1,022 microorganism-antibiotic combinations) by the impedance method the overall agreement between them were 11 major errors. The major discrepancies were observed for strains of *E. coli*, *E. cloacae*, *Acinetobacter* spp. and *Stenotrophomonas maltophilia* when testing cefamandole, cefotaxime, or gentamicin, amikacin. [37] We tested 311 samples and a huge number of comparisons have been made, cefoxitin have been found in 10 discrepancies out of 310 samples with 9 minor and 10 major errors and co-trimoxazole with 10 discrepancies in 307 samples with 2 major and 8 minor errors and amoxy-clavulanic acid with 10 discrepancies with 2 major and 8 minor errors have been found after comparing both AST and DST. After testing 311 samples against 20 microbial agents total 2542 microorganism-antibiotic combinations by Kirby-Bauer disc diffusion method overall 12 (0.6%) of major errors have been found and 71 (2.8%) of minor errors were in agreement. As to a study conducted in 1998 by James R Johnson UTI out of the 2,983 individual comparisons between the direct and standard tests, 0.8% represented very major errors, 0.6% represented major errors, 3.1% represented minor errors, and 95.5% were in agreement. [38]

### Conclusion:-

After 1 year of systematic study even though we found that AST and DST both give almost similar results, but keeping in mind major and minor differences in antibiotic sensitivity test. We should consider it only for emergency purposes. In emergency it is observed that DST proved to be more successful because DST provides results within 24 to 32 hours, this providing results faster but it should always be followed by AST for confirmation of results. For OPD or non-critical conditions, AST is only preferred method as it is standardized and CLSI are also based on it. And also DST should always be followed by AST, so method will cost us more. With DST, we have to apply more no. of antibiotic disks to cover all bacteria increasing cost of ABST.

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