Advancing antimicrobial susceptibility testing in body fluid-associated infections: a focus on direct testing for timely and targeted therapeutic interventions

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Summary

Background: timely Antimicrobial Susceptibility Testing (AST) is critical in managing infections, particularly with the rise of multidrug-resistant organisms. This study evaluates the efficacy of Direct AST (D-AST) on body fluids in reducing turnaround time compared to Routine AST (R-AST).

Materials and Methods: over six months, 43 monobacterial body fluid samples were analyzed using both D-AST and R-AST at a tertiary care hospital. D-AST was performed on the same day of sample receipt, while R-AST followed Clinical and Laboratory Standards Institute (CLSI) guidelines. Concordance between the methods was statistically assessed.

Results: D-AST showed high concordance with R-AST for most antibiotics ($p>0.05$), with a significant reduction in turnaround time (D-AST: 24-36 hours; R-AST: 48-72 hours). For Gram-negative organisms, discrepancies were noted for amikacin sensitivity in *Escherichia coli* (D-AST: 76.19%; R-AST: 90.4%) and ciprofloxacin in *Klebsiella pneumoniae* (D-AST: 80%; R-AST: 100%). Gram-positive organisms showed 100% concordance for key antibiotics like linezolid and teicoplanin.

Conclusions: D-AST is a reliable, rapid alternative to R-AST, enabling earlier therapeutic decisions and reducing empirical antibiotic use. It shows promise in improving outcomes and combating antimicrobial resistance, particularly in resource-limited settings. Further standardization is needed for broader application.

Introduction

The possibility of encountering an infection caused by multidrug-resistant organisms has increased tremendously in recent times [1,11]. This increased risk of clinical treatment failure emphasizes the role of Antimicrobial Susceptibility Testing (AST) [13]. The identification of clinically significant bacteria in the laboratory and the performance of AST provide necessary information for the accurate management of patients suffering from bacterial infections [5]. However, results are only available 48-72 h after sampling, as bacteria must be cultured before AST can be performed. Meanwhile, the patients must receive empirical antibiotics. The unpredictable susceptibility of the causative bacteria to antibiotic agents can lead to inadequate therapy and urges the empiric use of antibiotics. De-escalation of treatment is practiced only after results from AST are available, which has immediate as well as long-term consequences, such as the emergence of multidrug-resistant

microorganisms and an increased risk of superinfections, morbidity, mortality, and costs [2].

The most commonly used methods for AST testing in developing countries are conventional phenotypic methods based on culturing on agar (e.g. disk diffusion tests) [6]. Disk diffusion offers several advantages, as it is cheap, flexible, allows growth visibility, and accurate determination of pure or mixed cultures. Another possibility is to perform Direct Antibiotic Susceptibility Testing (D-AST), which aims at a shortened turnaround time. D-AST determines the antibiotic sensitivity for the whole sample instead of individual cultured colonies, enabling an assessment of the population's susceptibility to antibiotics [2].

Peritoneal Dialysis (PD) is routinely performed in most tertiary care hospitals and is associated with reduced costs in most countries and improved quality of life [4]. The major complication of PD is peritonitis, which leads to significant morbidity, mortality, and healthcare costs [7]. PD peritonitis is mostly caused by bacterial infections, with Gram-positive organisms responsible for >65% of cases [12]. In about 20% of cases, no organisms can be found in the culture [10]. Even in culture-positive peritonitis, empirical therapy is found to be inadequate in 2% of Gram-positive and 8% of Gram-negative cases [8]. Similarly, in bacterial pleural effusion, it is difficult to find the causative agent on the culture of the pleural fluid sample. As further decisions on antibiotic therapy rely on the availability of microbiological culture and antibiotic susceptibility results, this information may take several days (or may not be available at all in culture-negative cases). This leaves clinicians reliant on extended empirical therapies, which further contribute to antimicrobial resistance, and loss of normal commensal flora [8].

The Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) provide breakpoints for D-AST using the disc diffusion method, but only for blood culture. This is because of the predominantly monomicrobial infection and the feasibility of inoculum standardization of the blood specimens [14]. In CLSI, the guidelines are available only for Enterobacterales, *Pseudomonas aeruginosa,* and Acinetobacter species. Also, these breakpoints are available for a limited number of antibiotics in CLSI guidelines [3,9]. In EUCAST, the method is validated for the following species: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis, Enterococcus faecium*, and *Acinetobacter baumannii*. In the lack of available D-AST breakpoints for other body fluid samples, the timely therapeutic management of such cases is often delayed. In the present study, we have compared D-AST results for other body fluid samples with conventional AST technique to assess its utility in early, targeted therapeutic management of these cases.

Materials and Methods

Sample inclusion

This study included biological fluid samples (pleural fluid, ascitic fluid, and peritoneal fluid) collected over a six-month duration between January 2023 and June 2023 during working days in the morning (9 a.m.-4 p.m.) at the Microbiology Department of a 1500-bedded hospital. These samples were submitted to the Bacteriology lab in sterile tubes without any chemical preservatives for routine culture and antimicrobial susceptibility testing. The samples were processed without any further delay at the lab. Following the standard protocol, direct Gram staining, and culture were performed. The antimicrobial susceptibility testing was performed as per the latest CLSI guidelines [3,8]. Samples with insufficient volume (less than 1 mL) or excessive viscosity (data not recorded) were excluded from this study. Samples were excluded from the study if routine culture showed more than one type of growth.

Rapid identification procedure

Direct Gram-stained smear from the sample was analyzed. If all the bacteria present in the smear showed uniform morphology & stain reaction, then that sample was assumed to contain monobacterial infection, and a D-AST was performed from that biological fluid sample on the same day of sample receiving. Four drops of the sample were dispensed onto an MHA plate. The sample was uniformly spread across the entire surface using a sterile cotton swab. This step was repeated twice after rotating the MHA plate by 60 degrees each time. Antimicrobial discs were dispensed onto the surface of the inoculated MHA plate after 3-5 minutes and pressed down gently. The inoculated plate was incubated overnight at 37°C. The antibiotics tested for Gram-negative and Gram-positive organisms have been depicted in Table 1 and Table 2, respectively. D-AST was done in addition to the routine standard antimicrobial susceptibility testing from cultured bacterial isolates on the subsequent day. Routine AST was performed using the disc diffusion method according to CLSI. D-AST was not performed on samples having different bacterial types on Gram staining. Cut-off points for standard cultured drug-bug combinations from CLSI guidelines were extrapolated to direct antimicrobial susceptibility testing in the absence of available breakpoints.

Statistical analysis

The p-value for comparing direct and routine testing methods was calculated for each antibiotic-agent combination. While minor differences were observed, they were not statistically significant ($p>0.05$), indicating a high concordance between direct and routine AST for most antibiotics tested.

Results

Organisms isolated

Out of 67 body fluid samples, 43 samples were monobacterial. D-AST was performed for these 43 monobacterial biological fluid samples. Gram-negative organisms accounted for the majority of isolates (n=37), while Gram-positive organisms constituted a smaller proportion (n=6). The detailed distribution of isolates is provided in Figure 1.

Comparison of antimicrobial susceptibility

Gram-Negative organisms

The D-AST method demonstrated comparable results to R-AST for most antibiotics, with notable differences observed for specific agents. For example, *Escherichia coli* showed 76.19% sensitivity to amikacin in direct testing compared to 90.4% in routine testing. Similarly, *Klebsiella pneumoniae* and *Klebsiella oxytoca* exhibited consistent results between both methods, with 100% sensitivity to amikacin and meropenem. However, discrepancies were noted for ciprofloxacin, where DST indicated 80% sensitivity in *Klebsiella pneumoniae*, compared to 100% in routine testing (Table 1).

Gram-Positive organisms

Among Gram-positive isolates, *Staphylococcus aureus* showed identical sensitivity patterns for linezolid and teicoplanin across both testing methods (100%). However, for erythromycin and clin-

damycin, sensitivity remained low (25%) in both methods. For *Enterococcus faecalis*, sensitivity to chloramphenicol and linezolid was consistent between the two methods (100%) (Table 2).

The average time from sample receipt to antimicrobial susceptibility reporting was significantly reduced using D-AST compared to routine methods. D-AST provided results approximately 24 hours earlier, with an average turnaround time of 24-36 hours compared to 48-72 hours for routine testing.

Discussion

This study highlights the potential of D-AST in body fluid-associated infections to significantly improve diagnostic efficiency and clinical outcomes. By enabling faster identification of bacterial isolates and their susceptibility profiles, D-AST demonstrates a promising alternative to traditional methods, which often require 48-72 hours for results.

Table 1. Comparison of antimicrobial susceptibility pattern of Gram-negative bacteria in pleural fluid samples between direct and routine testing.

Antibiotic		*E. coli*	*P. aeruginosa*	*C. freundii*	*C. koseri spp.*	*Acinetobacter*	*K. pneumoniae*	*K. oxytoca*
Cotrimoxazole	D-AST	100	-	-	-	-	-	-
	R-AST	100	-	-	-	-	-	-
Amikacin	D-AST	76.19	-	100	0	100	100	100
	R-AST	90.4	-	100	0	100	100	100
Ceftazidime	D-AST	14.28	100	50	0	0	20	0
	R-AST	19.04	100	50	0	0	40	0
Imipenem	D-AST	33.33	0	50	0	100	80	100
	R-AST	28.57	100	50	0	0	80	100
Meropenem	D-AST	80.95	-	100	0	100	100	100
	R-AST	76.19	-	100	0	100	100	100
Piperacillin- tazobactam	D-AST	38.09	-	75	0	100	100	66.66
	R-AST	57.14	-	75	0	0	100	66.66
Gentamicin	D-AST	100	100	75	-	-	100	100
	R-AST	100	100	75	-	-	100	100
Ciprofloxacin	D-AST	33.33	100	75	0	0	80	0
	R-AST	42.85	100	75	0	0	100	66.66
Tetracycline	D-AST	90.47	-	-	-	-	-	-
	R-AST	90.47	-	-	-	-	-	-
Cefotaxime	D-AST	80.95	-	-	-	-	75	-
	R-AST	80.95	-	-	-	-	75	-

D-AST, Direct Antimicrobial Susceptibility Testing; R-AST, Routine Antimicrobial Susceptibility Testing.

Table 2. Comparison of antimicrobial susceptibility pattern of Gram-positive bacteria in pleural fluid samples between direct and routine testing.

Antibiotic	AST method	Staphylococcus aureus	Enterococcus faecalis
Cefoxitin	D-AST	0	-
	R-AST	0	-
Linezolid	D-AST	75	100
	R-AST	75	100
Teicoplanin	D-AST	-	100
	R-AST	-	100
Chloramphenicol	D-AST	75	100
	R-AST	75	100
Cefoxitin	D-AST	0	-
	R-AST	0	-
Erythromycin	D-AST	25	0
	R-AST	25	0
Clindamycin	D-AST	25	0
	R-AST	25	0
Cotrimoxazole	D-AST	50	-
	R-AST	50	-
Ciprofloxacin	D-AST	25	-
	R-AST	25	-
Tetracycline	D-AST	100	0
	R-AST	100	0

D-AST, Direct Antimicrobial Susceptibility Testing; R-AST, Routine Antimicrobial Susceptibility Testing.

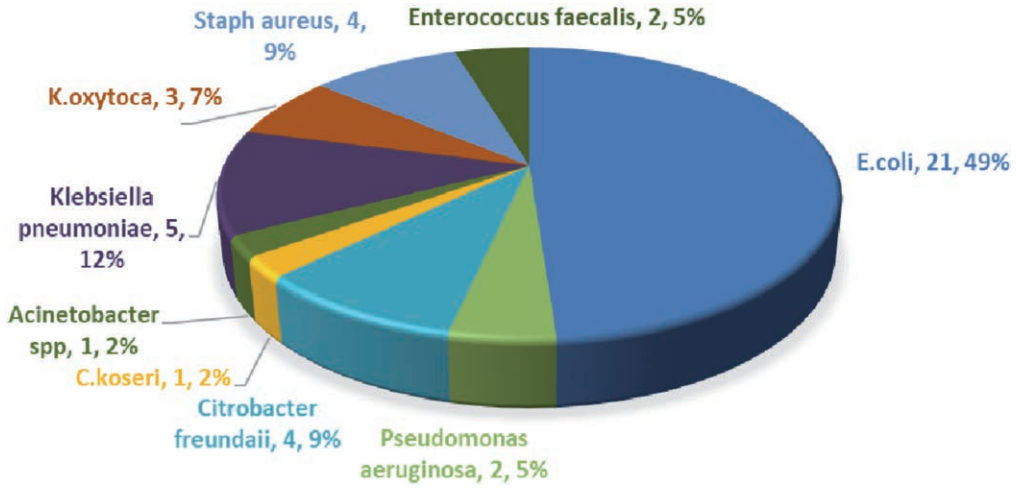

Figure 1. Organisms isolated from body fluid samples.

The study findings emphasize a high concordance between D-AST and routine AST methods for most antibiotics, as evidenced by statistical analyses showing no significant differences ($p>0.05$). Notably, D-AST reduced the turnaround time by approximately 24 hours compared to routine testing, which is critical for conditions like peritoneal dialysis-associated peritonitis and bacterial pleural effusion. In these conditions, delays in appropriate therapy are associated with increased morbidity, mortality, and the development of Antimicrobial Resistance (AMR) [1,11].

Among Gram-negative organisms, direct antimicrobial susceptibility testing provided a reliable guide for timely, effective therapeutic interventions for most drug-bug combinations. However, discrepancies noted for antibiotics such as amikacin sensitivity for *Escherichia coli and* ciprofloxacin in *Klebsiella pneumoniae* underscore the need for further standardization and validation of D-AST protocols [12].

For Gram-positive organisms, D-AST demonstrated reliable results for critical antibiotics like linezolid and teicoplanin, with 100% concordance observed across both methods for *Staphylococcus aureus* and *Enterococcus faecalis*. These findings align with the CLSI guidelines for direct testing of specific organisms and antibiotics [9]. However, low sensitivity rates for erythromycin and clindamycin in both methods suggest the limited utility of D-AST for these antibiotics.

The adoption of D-AST in clinical practice could have significant implications for antimicrobial stewardship. By reducing reliance on extended empirical therapy, D-AST can help minimize the misuse of broad-spectrum antibiotics, thereby reducing the risk of AMR and preserving the efficacy of existing antimicrobial agents [5,13]. This is particularly important in resource-limited settings, where the burden of multidrug-resistant organisms is disproportionately high [6]. Furthermore, rapid identification of resistant pathogens enables earlier de-escalation of inappropriate antibiotics, which is critical in reducing hospital-associated infections and healthcare costs [10].

Limitations and future directions

Despite its advantages, the study has several limitations. The analysis was confined to a single tertiary care hospital, which may limit the generalizability of findings to other settings. Furthermore, the reliance of this study on manual methods for D-AST highlights the need for automated platforms to enhance reproducibility and scalability. Finally, the lack of CLSI and EUCAST breakpoints for D-AST of many antibiotics and organisms restricts its widespread application [14].

Future research should focus on expanding the scope of D-AST to include a broader range of pathogens and clinical samples, such as pleural and peritoneal fluids. Additionally, the development of standardized guidelines and automated systems for D-AST could further enhance its diagnostic utility and clinical impact.

Conclusions

This study demonstrates the potential of D-AST as a reliable and efficient reinforcement to R-AST of body fluids from associated infections. By reducing turnaround times and supporting timely & targeted therapy, D-AST has the potential to improve patient outcomes and mitigate the global challenge of AMR. Further validation and standardization are essential to facilitate its integration into routine clinical practice.

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