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EARLY ANTIMICROBIAL SUSCEPTIBILITY RESULTS FROM GROWTH OF POSITIVE BLOOD CULTURE BY DISK DIFFUSION METHOD SAVING TIME FOR CRITICALLY ILL HOSPITALIZED PATIENTS

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Abstract

Due to lack of proper and rapid identification of responsible pathogens, broad spectrum antibiotics are being overused in critically ill patients which are additional dilemma to development of antimicrobial resistance. The current study was conducted to perform antimicrobial susceptibility of positive blood culture by disk diffusion method and focused on reducing the 18 to 24 h of incubation interval to 6 hours and comparison of this result with standard disk diffusion testing performed on standard 24 hours growth. Antimicrobial susceptibility testing of identified isolates was performed as per guidelines on Muller Hinton agar plates. The susceptibility plates were examined at two intervals, first after 6 hours of incubation then after 24 hours of incubation and susceptibility results of isolates were noted and compared.

INTRODUCTION

Human health is facing a major threat in form of emerging antimicrobial resistance and key intervention to combat this challenge is rapid detection of resistance along with antimicrobial stewardship (van et al., 2020). Antimicrobial resistance is leading to increased morbidity and mortality which is not limited to specific regions but rising dangerously in all parts of world, causing million deaths across the world (Murray, 2022).

Due to lack of proper and rapid identification of responsible pathogens, broad spectrum antibiotics are being overused in critically ill patients which are additional dilemma to development of antimicrobial resistance (Fahim et al., 2021). According to World health Organization report published in year 2022, antibiotic resistance is developing rapidly in countries where antibiotics are sold without prescription and used prophylactically in livestock farming or used as growth promoting substances. Hence causes of antimicrobial resistance are becoming complex and multifaceted (WHO).

The susceptibility reports are furnished not less than 48 hours which is leading to prolonged use or overuse of broad-spectrum antibiotics, leaving behind poor patient outcome and increased risk of antibiotic resistance (Benkova et al., 2020)

Disk diffusion method for measuring antimicrobial susceptibility is simple, reliable, cost effective and reproducible, which was standardized by Bauer et al. in 1966. As per CLSI guidelines, inoculums for disk diffusion test should be

prepared from colonies on a non-selective agar plate that have been incubated for 18-24 hours and before reading and interpretation of results, the plates are incubated for additional 16-24 hours (Cockerill et al 2012).

The incubation period for disk diffusion testing can be shortened without significant impact on susceptibility results (Chandrasekaran et al 2018). Timely and reliable susceptibility results facilitate antimicrobial stewardship programs to change and focus empiric antibiotic therapy (Perillaud et al 2019). The current study aims to perform antimicrobial susceptibility of positive blood culture by disk diffusion method and focus on reducing the 18 to 24 h of incubation interval to 6 hours and compare those results with standard disk diffusion testing performed on standard 24 hours growth. This study will help in early and appropriate treatment with antimicrobial agents. Such studies are crucial particularly for countries like ours where antimicrobial resistance is spreading rapidly leaving behind fewer options of available antibiotics.

MATERIALS AND METHODOLOGY:

This comparative study was conducted at Microbiology Department of Combined Military Hospital, Multan which is 1600 bedded Teaching hospital receiving patients of all categories and ages. Blood samples of hospitalized patients were received in Microbiology Department for culture and sensitivity and were included in the study. Exclusion criteria included those patients who were on antibiotic, duplicate samples of same patient within 24 hours and samples from outpatient department.

In Microbiology Department a total of 554 blood samples were received from hospitalized patients of various clinical departments from January 2024 to June 2024. Positive Blood Cultures from BACTEC system were processed further according to standard microbiological practice which includes subculturing on Blood agar, MacConkey agar and Chocolate agar. In this study, 55 consecutive, non-repeated isolates were further processed for final identification and susceptibility. After 24 hours of incubation at 37C, the culture plates were examined for growth and further processed for final identification and susceptibility as per recommended guidelines.

Antimicrobial susceptibility testing of isolates was then performed on Muller Hinton agar plates. The susceptibility plates were examined at two intervals, first after 6 hours of incubation then after 24 hours of incubation and susceptibility results of isolates were noted and compared.

RESULTS:

A total of 554 blood samples were received in Microbiology Department, out of which 55 samples were signaled positive on BACTEC system. Figure shows that out of total received samples, 9.2% (n=55) were positive for any growth.

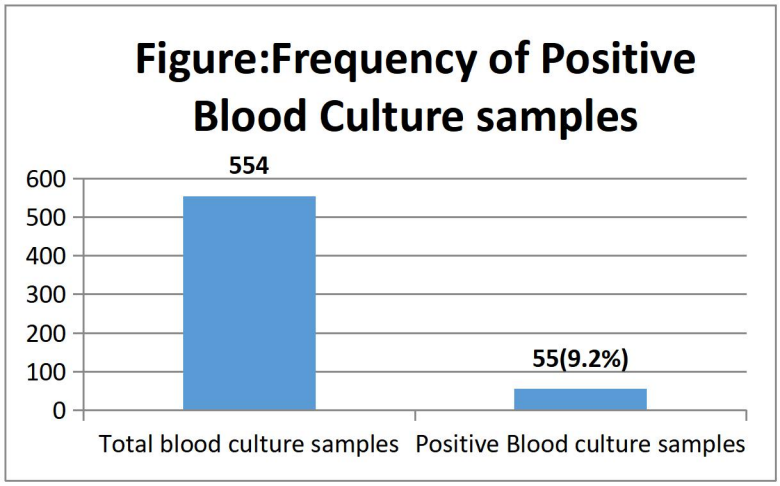

Table 1: Frequency of various isolates from positive blood cultures {n=55 (9.2%)

S.No	Name of isolates	Number of isolates	Frequency of isolates
1.	Citrobacter freundii	7	12.7%
2.	Klebsiella pneumoniae	14	25.4%
3.	Acinetobacter baumanii	6	10.9%
4.	Staphylococcus species	13	23.6%
5.	Salmonella typhi	5	9%
6.	Pseudomonas aeruginosa	2	3%
7.	E.coli	8	14.5%
8.	Total	55	100%

The above table shows the frequency of various isolates identified from positive blood culture samples (n=55)

Table 2: Comparison of susceptibility results of various isolates at two intervals

S.No	Name of isolates	Number of isolates	Susceptibility after 6 hours incubation		Susceptibility after 24 hours incubation	
			Sensitive	Resistant	Sensitive	Resistant
1.	*Citrobacter freundii*	7	1	6	1	6
2.	*Klebsiella pneumoniae*	14	4	10	4	10
3.	*Acinetobacter baumanii*	6	1	5	2	4
4.	*Staphylococcus species*	13	5	8	3	10
5.	*Salmonella typhi*	5	2	3	2	3
6.	*Pseudomonas aeruginosa*	2	1	1	1	1
7.	*E.coli*	8	2	6	2	6
8.	Total	55(9.2%)	16(30%)	39(70%)	15(28%)	40(72%)

This table shows the comparison of 6 hours and 24 hours susceptibility patterns of various organisms isolated from positive blood culture samples received from various clinical wards of admitted patients.

DISCUSSION:

Early growth interpretation for disk diffusion testing is a simple and accurate method of antimicrobial susceptibility testing that can reduce time to results by as much as 18 h while adding no additional cost to the testing method (Saymour et al., 2017)

In this study 55 isolates from positive blood culture on BACTEC were included and their susceptibility was interpreted at 6 hours incubation and at 24 hours of incubation.

Figure 1 shows that 9.2 % blood culture samples were positive for any growth. The frequency of positive blood cultures was 8.2 % in a study conducted by Shaikh in Karachi, Pakistan. In table 1, frequency of various organisms isolated from positive blood cultures is shown. The results are comparable to study conducted in a teaching hospital by Ejaz in year 2020.

In results the table 2 shows that at 6 hours incubation, 39 (70%) out of 55 isolates were resistant to their respective CLSI antimicrobial panels and 16 (30%) isolates were sensitive. *Citrobacter freundii, klebsiella pneumonia, Salmonella typhi, pseudomonas aeruginosa and E.coli* showed 100% commitment at 6 hours and 24 hours zone diameters by disk diffusion methods where the growth inhibition and non-inhibition zones by antimicrobial disks was same at both 6 and 24 hours results interpretation. However, *Staphylococcus species* (including MRSA and MRSE) and *Acinetobacter baumanii* showed variation in zone diameters in disks of cefoxitin,clindamycin and erythromycin and Doxycylcine,Colistin respectively.

Our results show correlation with other studies done at various places of world (Webber et al., 2022).

The study concludes that reading susceptibility tests from blood cultures after 6 h of incubation are as reliable as compared to 24-h results and produced 2 % difference in results in few isolates. The reduced incubation time is promising and there was no major difference in results.

This approach should be considered by laboratories that are receiving samples from critical wards like Intensive care units, Neonatal ICU, Pead's ICU as it will save time and early susceptibility reports furnished to patients will be ultimately leading to better clinical outcome.

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