SHORT COMMUNICATION

Reliability of Direct Sensitivity Determination of Blood Cultures

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Antimicrobial susceptibility testing of bacteria isolated from blood is very useful to guide the antibiotic therapy of a septicemic patient.

When susceptibility testing is done by a standard method e.g. CLSI (Clinical and Laboratory Standards Institute), it may take about 24-48 hours to give results. Direct susceptibility test by turbid broth is an accepted method for timely reporting. Although not mentioned in CLSI, but standardization of broth for direct sensitivity is mentioned in BSAC (British Society for Antimicrobial Chemotherapy) methods.[1]

Since 1970's multiple studies have compared the direct versus standard method. Direct susceptibility method is found 94-97% in agreement; yet repeating of sensitivity with standard method has been recommended. Repeating all sensitivity testing is not practical and possible by most of the laboratories, considering the workloads. The routine procedure in most laboratories is to determine direct susceptibility from turbid broth, without repeating the test later.

Johnson *et al.* found direct susceptibility test as both feasible and accurate with only 2.4% minor and 1% major discrepancies as compared with standardized susceptibility testing.[3]

Mirerett also found no "very major" discrepancy and only 0.3% major discrepancy.[4] Doern found 1.6% minor, 1.5% major and 0.1% "very major" discrepancies when he compared the two methods.[5]

It is important to determine where one can or can not rely on direct test, making it practical, feasible as well as acceptable method in the local set-up.

The aim of this study was to evaluate the error in interpreting antimicrobial sensitivity by direct method when compared to standard method and find out if specific antibiotic-organism combination had more discrepancies.

The study was planned to evaluate the error in interpreting antimicrobial sensitivity by direct method when compared to standard method at the Microbiology Laboratory of Liaquat National Hosptial, Karachi. All blood culture samples received at Microbiology Laboratory from 1st July 2006 to 31st August 2006 were included in the study. The cultures growing more than two organisms were excluded.

All samples were inoculated in automated blood culture system "BACTEC 9240" which contained enriched Soybean-Casein Digest broth with CO_2. Once positive, bottles were removed from system; gram staining of the positive broths was done. Susceptibility test was performed from positive broth, on MHA (Mueller-Hinton Agar), with antibiotics panel according to gram stain result. All positive broths were also sub-cultured on blood agar, chocolate agar and McConkey's agar for only gram-negative rods. Direct susceptibility test was done on 140 isolates without any attempt to standardize the turbidity of broth.

In previous studies for direct susceptibility testing, broth was standardized for 0.5 McFarland Standards.[3,4] Practically, we could not standardize blood culture broth density according to organism's inoculums, so positive broth was taken as such. That was easier and practical. Moreover, there was no chance of contaminating the broth.

Next day, the zone sizes of all antibiotics were recorded using measuring scale; and at the same time susceptibility test was repeated from isolated colonies from subcultures, with inoculums prepared of McFarland 0.5 standard.[2] *Staphylococcus aureus* (ATCC 29213); *E.coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were included as quality control strain. Zone sizes were interpreted as 'sensitive'(S), 'resistant'(R) and 'intermediate'(I) according to CLSI recommendation.[6] Two results were compared and recorded.

Minor discrepancy was interpreted if there was a change from resistant or sensitive to intermediate category or vice versa. A discrepancy was considered to be 'major' if the organism was resistant by direct testing but susceptible by the standard method. Discrepancies were considered to be 'very major' when organisms were found to be susceptible by direct testing and resistant by the standardized method.[4]

The errors found in comparing two methods are presented in Table I and II.

Considering interpretation of methicillin resistance in *staphylococci*, by oxacillin disc or cefoxitin disc, no discrepancy was found; when interpretation by two

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methods were compared. This shows that interpreting MRSA by direct method was reliable.

In this study no “very major” discrepancy was found.

Out of a total 1083 combinations, zone diameters by standard method were either equal or greater than direct zone diameter (never smaller).

Most of the discrepancies were in β-lactam/β-lactamase combinations, and aminoglycosides.

While reporting these groups of antibiotics with direct sensitivity test, one should be cautious. These are the major antibiotic used for life-threatening infections. In case of being heavy/lighter standard inoculums or marginal zones, repeating with standard method should be preferred to minimize the chances of error.

Table I: Comparison of interpretive results with direct and standard methods for antimicrobial susceptibility testing of gram-negative rods cultured from blood.

Gram negative rods 810 organisms and antibiotic combinations				
Antibiotics	Enterobac-teriaceae (53)		Non-Enterobac-teriaceae (48)	
	Major	Minor	Major	Minor
Amoxacillin /clavulonic acid	01	02	-	-
Cefepime	-	02	-	01
Cefoperazone/sulbactam	-	11	-	01
Amikacin	02	15	-	-
Ciprofloxacin	-	02	-	01
Imipenam	02	01	01	-
Piperacillin/tazobactam	-	10	-	-
Ceftazidine	-	06	-	01
Total	**05**	**50**	**01**	**04**

Table II: Comparison of interpretive results with direct and standard methods for antimicrobial susceptibility testing of gram-positive cocci cultured from blood.

Gram positive cocci 273 organism antibiotic combinations				
Antibiotics	*Staphylococcs aureus*		Coagulase negative *Staphylococcus*	
	Major	Minor	Major	Minor
Cefoxitin/Oxacillin	-	-	-	-
Vancomycin	-	05	-	-
Erythromycin	-	-	-	-
Gentamicin	-	01	-	-
Ciprofloxacin	-	04	-	02
Clindamicin	01	07	-	-
Fucidic acid	04	-	-	-
Total	**05**	**17**	**00**	**02**

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