Cefoxitin Disk Diffusion Test - Better Predictor of Methicillin Resistance in Staphylococcus aureus

Dear Editor,

Cefoxitin, a cephemycin, is a more potent inducer of the mecA regulatory system than are the penicillins.[1] Several studies have reported that the Cefoxitin disk diffusion (DD) test is a good alternative method for detection of Methicillin resistance Staphylococcus aureus (MRSA) though Oxacillin is the agent recommended by the CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI).[2,3] This study was undertaken to assess the usefulness of Cefoxitin DD in predicting MRSA.

This study included 155 strains of S. aureus, isolated from various clinical samples in the department of Microbiology, GTB Hospital, Delhi. These isolates were studied to evaluate Cefoxitin DD test for routine detection of MRSA. All the strains were screened for methicillin resistance by Oxacillin (1 µg) and Cefoxitin (30 µg) DD test as per standard guidelines. Zone diameters as recommended by CLSI were read both at 18h and 24 h.

To assess the reproducibility of the Cefoxitin DD method, 10 strains each of MRSA and MSSA (both by Oxacillin and Cefoxitin disk) were taken and the inhibition zone diameters were obtained consecutively on 30 occasions. In addition, five strains of S. aureus which were sensitive by Oxacillin DD and resistant by Oxacillin MIC (between 4-6 µg/ml), Cefoxitin DD and Cefoxitin MIC (>8 µg/ml) were also taken.

The CLSI broth macro dilution (BMD) reference method was used to determine the MIC of Oxacillin and Cefoxitin. (MIC cut off criteria as recommended by CLSI for Oxacillin less than or equal to 2 µg/ml for susceptible and greater than or equal to 4 µg/ml for resistance. Modified breakpoint criteria for Cefoxitin less than or equal to 4 µg/ml for susceptible and greater than or equal to 8 µg/ml for resistance). Isolates that had MIC value of greater than or equal to 4 µg/ml for Oxacillin and greater than or equal to 8 µg/ml for Cefoxitin were taken as Methicillin resistant.

Of the 155 strains which were tested, 48.39% strains were Methicillin resistant by Oxacillin DD method compared to Oxacillin agar screen method which detected 50.32% strains including three strains of S. aureus which had Oxacillin zone diameters of 11-12 mm. These strains were further tested by Cefoxitin DD method. 54.54% strains were found to be resistant. No difference in zone diameters was seen at 18hrs and 24hrs. Cefoxitin MIC’s are shown in Table. No benefit of added salt was noticed.

Staphylococcus aureus is human pathogen with a remarkable propensity for development of antibiotic resistance. Previous CLSI recommendation for detecting MRSA included agar dilution, BMD, DD, the Oxacillin screen test and detection of mecA or product of mecA gene, PBP2a, by PCR and latex agglutination respectively. All these methods used for S. aureus, aside from mecA detection by PCR, are prone to errors. The laboratories that cannot afford to perform the PBP2a, latex agglutination test or do not have access to PCR, need alternative methods for detecting mecA-mediated resistance. Several studies have been done to investigate the utility of Cefoxitin DD for detection of MRSA[2,3] In our study, Oxacillin DD test detected Methicillin resistance in 48.39% strains whereas Cefoxitin DD method detected 54.54% MRSA strains including 10 strains which had intermediate resistance to Oxacillin. The Cefoxitin DD zones were distinct and easy to read. Oxacillin agar screen detected three additional MRSA strains which were not detected by Oxacillin DD method but were detected resistant by Cefoxitin DD test. Moreover, Oxacillin agar screen is cumbersome to perform. Oxacillin MIC detected 52.26% MRSA strains as compared to Cefoxitin MIC, which could detect 54.54% MRSA strains.

References
The use of Cefoxitin MIC test has not been recommended by the CLSI-AST, but its performance was equivalent to Cefoxitin DD when using modified breakpoints of less than or equal to 4 µg/ml for susceptible and greater than or equal to 8 µg/ml for resistance. The results of Cefoxitin DD method were better for isolates with Oxacillin MIC between 4-6 µg/ml (Table). Both Oxacillin DD and Cefoxitin DD gave reproducible results on 30 occasions for 10 MRSA and 10 MSSA strains however, Oxacillin DD gave inconsistent results for five strains with MIC 4-6µg/ml on several occasions in comparison to Cefoxitin DD which gave consistent resistant results for these five intermediate resistant strains. Our study also revealed that low level Oxacillin resistance was detected better by Cefoxitin DD test.

Detecting mecA gene characterization by PCR/PBP2a is recognized as gold standard for detection of MRSA. However, use of PCR assay is generally limited to reference laboratories, especially in developing countries. Our study clearly showed, the substitution of a Cefoxitin DD for an Oxacillin DD test, will result in an easier to read test with greater accuracy for detection of Methicillin resistance in S. aureus.

References

Table: Oxacillin and cefoxitin MIC’s

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Calvarial Tubercular Osteomyelitic Abscess

Dear Editor,

Tuberculosis which is quite common in developing countries is on an upsurge in developed countries in association with immune deficiency syndromes. Reid in 1842, described the first case of calvarial tuberculosis.[1] Raut et al., reported in their study for a decade, 42 cases of calvarial tuberculosis confirming the rarity of tuberculous lesion in skull.[2]

This 29 year-old male, presented to us in April, 2008, with headache and scalp swelling over the left frontal region, treated with over the counter analgesics. The progressive increase in size of the swelling and non-remitting pain brought the patient to us. No history of trauma noted. The patient was afebrile on examination. Examination of the scalp showed a solitary, erythematous, slightly tender, fluctuant, non-pulsatile, non-mobile swelling of size 4 cm diameter. Laboratory investigations showed Hb – 12.6 g%, TLC – 5,000/mm³, DC – N70, L23, E4, M3, ESR- 24mm/hr, Mantoux – 20mm at 72 hours. Routine biochemical parameters were normal. Chest radiograph posterior-anterior view was normal (Fig. 1). A CT scan of brain showed a hyperdense mass lesion of left frontal bone (Figs. 2, 3) of size 3.5cm x 2cm x 2cm located 0.5 cm anterior to left coronal suture, with erosion of both inner and outer tables of the skull and sub-galeal collection. Left fronto-temporal curvilinear incision and evacuation of pus, debridement of granulation tissue and removal of involved bone was performed. Histopathology showed granulomatous lesion with epitheloid cells. The pus was sent for Gram’s staining, fungal staining, acid fast staining, aerobic, fungal and

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