Correspondence

Phenotypic screening of resistance mechanism in *Staphylococcus aureus*

Sir,

Apropos article on antibiotic resistance genes by Duran et al. I have the following observations. Though the authors have done an excellent job of genotyping resistance mechanism in *S. aureus*, they failed in phenotypic screening of these isolates. Considering the fact that most laboratories can not afford to perform PCR, phenotypic screening methods remain the backbone for detection of resistance mechanism in clinical isolates.

(1) The incubation temperature for oxacillin disc diffusion (DD) is 33-35°C, not 37°C. Testing at temperatures above 35°C may not detect MRSA.

(2) The reporting of 30 mecA positive oxacillin sensitive *Staphylococcus aureus* (OS-MRSA) must be due to wrong incubation temperature.

(3) Oxacillin DD has a sensitivity of only 91 per cent and specificity of only 58.9 per cent while ceftoxitin DD has sensitivity and specificity of 97.8 and 100 per cent, respectively.

(4) Use of cefoxitin DD method for detection of MRSA would have reduced the number of false negative isolates (OS-MRSA).

(5) Though the authors have genotyped the erythromycin resistance gene but they never made an effort to detect inducible resistance to clindamycin which has got immense clinical significance.

(6) Authors have used 16SrDNA (internal control) and femA (*S. aureus* identification) primers but have not explained the reason for using these.

(7) Antibiotic resistance in *S. aureus* should always be discussed under methicillin resistant and sensitive *S. aureus* (MRSA and MSSA) for better clarity.

(8) Nitrocefin disc test should have been used to test for beta lactamases.

(9) MIC and MIC should have been calculated rather than just mentioning the MIC range.

(10) In Table IV, fourth column, the heading should have been “Number of blaZ PCR negative isolates”.

(11) CLSI has done away with vancomycin DD and recommends only MIC testing. Therefore, the data presented in Table II on vancomycin susceptibility based on DD are not valid.

(12) The authors’ view that phenotypic methods for screening MRSA require at least 24 h for evaluation of results is unfounded as CLSI recommends 16-18 h for cefoxitin DD method.

V. Anil Kumar
Department of Microbiology
Amrita Institute of Medical Sciences
Ponekara
Kochi 682 041, India
vanilkumar@aims.amrita.edu

References


Authors’ response

Sir,

In our study published in the Indian J Med Res, March 2012, disc diffusion susceptibility, and molecular methods to determine of minimum inhibitory concentrations (MIC) were studied. We concluded that multiplex PCR can be used for confirmation of the results obtained by conventional phenotypic methods when needed.

Conventional methods are still widely used. MIC testing is among the often used and sensitive methods as well as the DD test. However, identification and determination of the susceptibility to antibiotics of staphylococci by conventional methods (DD and MIC tests) require a minimum of two-days period, whereas the detection of antibiotic resistance genes by PCR assay can be done within a few hours. The PCR based tests are rapid and reliable methods for antibiotic susceptibility and important to institute appropriate therapy. In our study we emphasized on this.

I would like to thank Anil Kumar for raising questions on our study.

My response is given below:

(1) In Material & Methods section under subtitle “Susceptibility testing”, the incubation time was written as 37 °C by mistake instead of 35 °C. It needs to be corrected as 35 °C.

(2) As reported by author, testing at temperatures above 35°C may not detect MRSA. This is not exactly true. Also, in a study conducted by Skor et al, the influence of incubation time (18 and 24 h) and temperatures (30, 35, 36 and 37°C) on the performance of 10- and 30-µg cefoxitin disks and cefoxitin E test on Mueller-Hinton agar were evaluated for mecA-positive and mecA-negative S. aureus. In this study, the effect of increase in temperature was not significant.

(3) DD was not the method was used in our study, it was one of three methods (DD methods, MIC and molecular methods) used. One of the aims in our study was to compare various methods and emphasize the role of rapid and accurate tests.

(4) Methicillin resistance was determined by three different methods.

(5) It was discussed in discussion section.

(6) All S. aureus isolates (positive for coagulase test) carried the femA gene. Also relevant references were quoted in support.

(7) The idea is solely the opinion of the author. I disagree with the author’s proposal.

(8 & 9) The author may be right, but primary aim was not that. The idea is solely the opinion of the author.

(10) Table IV. Relationship between gentamicin resistance and the present of three resistance genes (aac(6)/aph(2’), aph(3’)-IIIa, ant(4’)-Ia), the heading of the fourth column was given right.

(11) This Table (Table II) was given to demonstrate the accuracy of MIC testing for vancomycin.

(12) According to CLSI criteria, the incubation period must be at least 18 h. Therefore, there was no mistake.

Nizami Duran
Mustafa Kemal University
Medical Faculty
Department of Microbiology & Clinical Microbiology
Antakya-Hatay/Turkey
nizamduran@hotmail.com

References

