

Comparison of three different methods to detect the production of β - lactamase enzyme by *Staphylococci*

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Abstract

Production β -lactamase enzyme is the most common and important mode of exhibiting resistance to β -lactam antibiotics. Manifestation of this enzyme is difficult to demonstrate in routine antibiotic sensitivity testing. Sensitivity to penicillin is not sufficient to indicate whether this antibiotic should be used clinically and main factor compromising the clinical efficacy of these drugs is the production of β -lactamase enzyme. Only feasible method for determination of β -lactam resistance is to demonstrate the physical presence of enzyme. Three different methods (acidometric method, iodometric method and chromogenic cephalosporin method) are available for this. The objective of present study is to compare these 3 methods to detect the production of β -lactamase enzyme in staphylococci isolated from various clinical samples. Susceptibility to penicillin, oxacillin and vancomycin was also determined. Out of total 150 isolates, 114(76%) shows β -lactamase production by chromogenic cephalosporin method, 110(73.3%) by iodometric method and 108(72%) by acidometric method. Penicillin and oxacillin resistance was 74.6% and 53.3% respectively. All isolates are sensitive to vancomycin. Out of three different methods used, chromogenic cephalosporin method is more sensitive (76%) than iodometric method (73.3%) and acidometric method (72%). Results also correlates with penicillin sensitivity in ABST except 2 isolates which shows β -lactamase production by chromogenic cephalosporin method but remains penicillin sensitive in disc diffusion method. In case of agar diffusion tests, there is often insufficient β -lactamase production from the test organism before an inhibitory concentration of antibiotic has diffused from the disc. Only ABST can lead to therapeutic failure sometimes.

Keywords: β -lactamase, three methods, staphylococci.

1. Introduction

The modern era of antibiotics began with the discovery of penicillin by Sir Alexander Fleming [1]. Penicillin was 1st employed as an antibiotic agent in 1941. Since then β -lactam agents are most widely prescribed antibiotics and are important component of empirical therapy [2]. Resistance to β -lactam antibiotics is increasingly common and significant problem. Resistance can be mediated through several mechanisms, out of which production of β -lactamase enzyme is the most important and most common mechanism. Beta lactamases are enzymes

produced by bacteria that inactivates β -lactam drug by opening of β -lactam ring. Penicillin was a drug of choice to treat serious staphylococcal infections. At present over 90% of staphylococcal isolates are resistant to penicillin as a result of production of hydrolytic β -lactamase enzyme [3]. Beta lactamases produced by staphylococci have been stable over several years and have narrow spectrum of activity aimed mainly at penicillin and comes under Bush-Jacoby class 2a. Beta lactamase gene that mediates penicillin resistance in staphylococci is typically located on

plasmid [4]. Strains of *S. aureus* produce up to four different β -lactamase enzyme [5]. Staphylococcal β -lactamases can readily hydrolyze penicillin and penicillin derivatives but cannot effectively hydrolyze cephalosporins or imipenem [6]. Simple laboratory tests of sensitivity to penicillins, cephalosporins and similar compounds may be insufficient to indicate whether these antibiotics should be used. Main factor compromising the clinical efficacy of these drugs is usually the production of a β -lactamase. The manifestation of this enzyme as a resistant mechanism is sometimes difficult to demonstrate in vitro as bacterial concentration needs to be sufficiently high ($>10^6$ cells/ml) and enzyme must be induced before testing. Thus apparent sensitivity in the diagnostic test may fail to reveal a latent and potent resistance mechanism. Only feasible method for the determination of β -lactam resistance may be to demonstrate the physical presence of an active β -lactamase enzyme [7]. Several methods have been developed to detect the production of β -lactamase by bacteria. When β -lactamases hydrolyze benzylpenicillin, penicilloic acid is produced. The acid production has been detected by (1) measuring the change in pH with an indicator dye (acidometric method), (2) exploiting the ability of penicilloic acid to reduce iodine and reverse the formation of blue color when the latter complexes with starch (iodometric method) or by (3) chromogenic cephalosporin method. Out of these, chromogenic cephalosporin method is the most sensitive method and provides very rapid and convenient mode for detection of β -lactamases [7].

2. Material and methods

Total 150 isolates of staphylococci isolated from various clinical samples like blood, pus, urine, sputum, pleural fluid, throat swab, endotracheal secretions are included in study. Staphylococci are identified by gram stain, catalase and coagulase tests [8]. Susceptibility to penicillin, oxacillin and vancomycin was determined by Kirby-Bauer disc diffusion method [9]. Results were interpreted according to CLSI guidelines [10]. All isolates are subjected to 3 methods for β -lactamase detection – acidometric method, iodometric method and chromogenic cephalosporin method.

2.1 Acidometric method:

Whatman No 1 filter paper strips were saturated with penicillin and bromocresol purple, dissolved in NaOH solution. Strips were left to dry in air. Strips were moistened with the sterile distilled water before use. Bacterial growth from an overnight agar culture was applied on the strip and if purple colour changes to yellow in 5 min, the test is positive. Positive reaction indicates the production of β -lactamase enzyme by organism [11].

2.2 Iodometric method:

1 million units of penicillin was dissolved in 1 ml of sterile distilled water and stored at -20°C . Iodine solution was prepared by adding 2.03 gm of iodine and 5.32 gm potassium iodide in 100ml of distilled water. On a glass slide a drop of iodine reagent and a drop of penicillin solution was taken and overnight bacterial growth was mixed with it. Then a drop of 4% starch solution was added to this mixture. If blue color is lost within 10 min, the presence of β -lactamase is inferred. However if blue color persists, the culture was considered to be β -lactamase negative [11].

2.1 Chromogenic cephalosporin method

A more sensitive technique is to measure β -lactamase activity with a chromogenic cephalosporin, nitrocephin. Nitrocephin is normally yellow but when the β -lactam ring is hydrolyzed it turns red. In addition, nitrocephin is most readily hydrolyzed by many β -lactamases. This property makes it the most sensitive detection system. Cefinase disc (paper disc impregnated with nitrocephin) were used for the detection of β -lactamase enzyme, available from BD. Disc was moistened with a drop of distilled water. Bacterial growth from the periphery of the zone of inhibition around the oxacillin disc was taken with sterile applicator stick and smeared on the cefinase disc. Development of red color indicates production of β -lactamase enzyme by test strain. Although color change to red may occur quickly, reaction can take up to 1 hour in case of staphylococci [7].

3. Results

Total 150 isolates of staphylococci were included in the study out of which 76 were *S. aureus* and 74 were Coagulase negative staphylococci (CONS).

Table 1: Results of all three methods tested

Method	No. of isolates shows positive result	<i>S. aureus</i>	CONS	Result in %
Acidometric method	108	60	48	72%
Iodometric method	110	62	48	73.3%
Chromogenic cephalosporin method	114	64	50	76%

112 isolates (74.6%) were resistant to penicillin, 80 isolates (53.3%) were resistant to oxacillin and all isolates were sensitive to vancomycin.

4. Discussion

The problem of β -lactamase first emerged in 1944. In the initial phase the problem was less, but increased with great frequency in 1980s. In the present study, we have used

three different methods to detect the presence of β -lactamase enzyme in staphylococci. From above mentioned table it is clear that chromogenic cephalosporin method is most sensitive method (76%), followed by iodometric method (73.3%) and acidometric method (72%). 2 isolates were penicillin sensitive in disc diffusion test but shows β -lactamase production in chromogenic cephalosporin method. The manifestation of this enzymes as a resistance mechanism is sometimes difficult to demonstrate in vitro as bacterial concentration needs to be sufficiently high ($>10^6$ cells/ml), and enzyme must be induced. In case of agar diffusion tests, there is often insufficient β -lactamase production from the test organism before an inhibitory concentration of antibiotic has diffused from the disc. Thus, apparent sensitivity in the diagnostic test may fail to reveal a latent and potent resistance mechanism. Results of chromogenic cephalosporin method correlate with penicillin resistance.

5. Conclusion

Resistance to β -lactam antibiotics is common and significant problem currently. Most common mechanism of resistance is production of β -lactamase enzyme. Simple laboratory tests of sensitivity to penicillin, cephalosporin and other antibiotics may be insufficient to indicate whether these antibiotics can be effective therapeutically, as manifestation of these enzymes as a resistance mechanism is difficult to demonstrate in vitro. Thus, apparent sensitivity in the diagnostic test may fail to reveal a latent and potent resistance mechanism. In this situation, only feasible method for determination of β -lactam resistance is to demonstrate the physical presence of an active β -lactamase enzyme. Three different methods were used to detect the production of β -lactamase enzyme by staphylococci, out of which chromogenic cephalosporin method is more sensitive (76%) than iodometric method (73.3%) and acidometric method (72%). The result of chromogenic cephalosporin method also correlates with penicillin resistance in disc diffusion method except for 2 isolates which shows production of β -lactamase enzyme but remains penicillin sensitive in disc diffusion method. This can lead to therapeutic failure if tests for β -lactamase detection are not done simultaneously. Thus tests for β -lactamase detection should be done in addition to antimicrobial susceptibility testing to make the treatment clinically more effective and out of all three methods, chromogenic cephalosporin method is more convenient and sensitive method.

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