Journal of Medical Sciences

ISSN 1682-4474

science alert

ANSInet
an open access publisher
http://ansinet.com

Research Paper

JMS (ISSN 1682-4474) is an International, peer-reviewed scientific journal that publishes original article in experimental & clinical medicine and related disciplines such as molecular biology, biochemistry, genetics, biophysics, bio-and medical technology. JMS is issued eight times per year on paper and in electronic format.

For further information about this article or if you need reprints, please contact:

Dr. Mohammad Bokaeian
Department of Microbiology,
Zahedan University of Medical Sciences, P.O. Box 98135-795
Zahedan, Iran

Tel: ++98 541 2440486
Fax: ++98 541 2415081

J. Med. Sci., 7 (1): 150-153
1st January, 2007

Study of Betalactamase Production in Coagulase Positive Staphylococci by Iodometric and Acidometric Methods and Their Antibiotic Resistance Pattern

M. Bokaeian and M.I. Qureshi

Due to importance of coagulase positive staphylococci in pyogenic infections and their increasing rate of resistance to betalactam antibiotics and also important role of betalactamase enzyme in this phenomenon, we accomplished this study for isolation, mesurement of betalactamase enzyme and antibiotic resistance assay by standard methods. Total of 603 different samples obtained from patients with staphylococcal infections were cultured on general and selective media and after 24-48 h incubation at 37°C, coagulase reaction and biochemical tests were used to identification. Betalactamase assays accomplished by acidometric and idometric methods and antibiotic susceptibility of isolated strains were done by standard agar diffusion of Kirby Bauer method. Results revealed out of 603 samples, 38 coagulase positive staphylococci were isolated (6.3%). Betalactamase assay by acidometric and idometric methods were positive in 78.9 and 73.6%, respectively. All of the bacteria were resistant to carbenicillin, ampicillin and amoxycillin but on other hand resistance of other betalactams include as: penicillin (97.3%), oxacillin and methicillin (18.4%), ceftizoxime (17%), cephalothin (15.7%), ceftriaxon (14%), cephalexin (13.1%), cefazolin (7.8%). In accordance with high resistance of isolated coagulase positive staphylococci to carbenicillin, penicillin, ampicillin and amoxycillin and high rate of betalactamase production in these pathogen bacteria that lead to resistance to betalactam antibiotics we suggest the attention of physicians to the role of the bacteria and their proper treatment in the city.

Key words: Coagulase positive staphylococci, betalactamase, resistance

ANSInet
Asian Network for Scientific Information

Department of Microbiology, Zahedan University of Medical Sciences, Zahedan, Iran

INTRODUCTION

Coagulase positive staphylococci include *S.aureus* are important pathoens related to family Micrococcaceae which responsible for more than 80% of pyogenic infections and are the second cause of nosocomial infections (Hindler and Jorgensen, 2000). Infection by coagulase positive staphylococci can prove difficult to treat either because they have an unpredictable sensitivity pattern or because they are resistant to many betalactam antibiotics.

In spite of many antibiotics discovered against them, these bacteria are still of the most important pathogens responsible for problemetics in medicine. Betalactam antibiotics are of the most important antibiotics which are used for treatment of staphylococcal infections nowadays but unfortunately many of coagulase positive staphylococci are resistant to betalactams in different ways (e.g., betalactamase production, intrinsic resistance and resistance due to tolerance) (Waldvogel, 2000).

Of the all above resistance mechanisms, resistance due to betalactamase production is very important and in accordance with estimates about 70-80% of coagulase positive staphylococci seen outside of hospitals and more than 90% of the hospital strains produce this enzyme (Waldvogel, 2000).

Different methods are used for the detection of beta-lactamases namely iodometric, acidometric, chromogenic cephalosporin and microbiological method (Sykes, 1979). The iodometric assay is based on the fact that the intact (active) penicillin molecule does not bind iodine whereas the betalactamase inactivated product penicilloic acid binds iodine. Thus a positive reaction indicates that iodine being bound to penicilloic acid is unavailable for further reaction with starch and so no purple colour develops in testing (Lee and Komarmy, 1981). The acidometric method uses citrate-buffered penicillin and phenol red as a pH indicator. When colonies of beta-lactamase positive organism are added to the solution, the penicilloic acid present results in a drop in pH, causing a color change from red to yellow (Hindler and Jorgensen, 2000).

Many commercial companies produce betalactamase measurement kits which provides different methods and their results are almost same and comparable (Gradus and Silver, 1989) although few studies show different results (Fung and Jang, 1995). Because such kits are not used in our country and betalactamase tests are not done in routine labs, so there are no sufficient information about betalactamase production by coagulase positive staphylococci in Zahedan region. This study was done to measure betalactamase enzyme in these bacteria and to determine susceptibility of the bacteria to betalactam antibiotics.

MATERIALS AND METHODS

In this experimental-diagnostic study, a total of 603 different samples collected from patients of central hospital in Zahedan. All patients were examined by specialist. The samples in our study were as 356 blood, 78 cerebrospinal fluid, 56 sputum, 26 wound exudate, 25 abscess, 22 ear discharge, 14 peritoneal fluid, 10 joint fluid, 10 eye discharge and 6 pleural fluid.

After gram staining, all samples were cultured on blood agar and manitol salt agar. After 48 h incubation at 37°C in aerobic atmosphere, suspected colonies were examed by gram staining and coagulase test (Humphreys, 2002). Betalactamase production in confirmed coagulase positive staphylococci were determined by iodometric (tube test) and acidometric methods (Sonnenwirth and Jarret, 1980) and their antibiotic susceptibility patterns were determined by standard Kirby-Bauer disk diffusion method (Humphreys, 2002).

Antibiotics like Oxacillin (1 μg), Carbenicillin (100 μg), Cephalexin (30 μg), Penicillin (10 U), Cephalothin (30 μg), Ceftriaxon (30 μg), Ceftizoxime (30 μg), Cefazolin (30 μg), Methicillin (1 μg) and Amoxicillin (25 μg) were used.

RESULTS

Thirty eight coagulase positive staphylococcus were isolated (6.3%) from 603 studied samples which include wound exudate (9), sputum (7), abscess (6), ear discharge (5), joint fluid (4), blood (4), eye discharge (2) and cerebrospinal fluid (1) (Table 1).

Betalactamase production test in acidometric and iodometric methods were positive in 78.9 and 73.6%, respectively (Table 2).

Table 1: Coagulase positive staphylococcal growth in 603 studied samples

Culture specimen	Negative	Positive	Total
Blood	352	4	356
Cerebrospinal fluid	77	1	78
Sputum	49	7	56
Wound exudate	17	9	26
Abscess	19	6	25
Ear discharge	17	5	22
Peritoneal fluid	14	0	14
Joint fluid	6	4	10
Eye discharge	8	2	10
Pleural fluid	6	0	6
Total	565	38	603

Table 2: Results of betalactamase test by acidometric and iodometric methods

Result test	Positive	Negative	Total
Acidometery	78.9% (n:30)	21.1% (n:8)	100% (n:38)
Iodometery	73.6% (n:28)	26.4% (n:10)	100% (n:38)

Acidometery detected maximum betalactamase producers in all samples and weak betalactamase producers were detected mainly by acidometery method. Only 7% of betalactamase producers could be detected by one of two methods used whereas 88% positivity was obtained by using both two methods. This shows that a combination of methods is always useful in detecting betalactamase producers.

All of the bacteria were resistant to carbenicillin, ampicillin and amoxycillin but on other hand resistance of other betalactams include as: penicillin (97.3%), oxacillin and methicillin (18.4%), ceftizoxime (17%), cephalothin (15.7%), ceftriaxon (14%), cephalexin (13.1%) and cefazolin (7.8%).

DISCUSSION

Positive cultures, most abundance related to wounds, sputum and less prevalence related to cerebrospinal fluid and eye discharge. In regard to coagulase positive staphylococci, different statistical data observed (Saleao, 1999; Hsueh, 1999). There are different idea about this differentiation which include infection in other parts of body and its transmission to sampling sites, variable rate of carriers, resistance to antimicrobial agents and ability to produce disease in hospital environment (Humphreys, 2002).

In present study, significant difference not seen between the two betalactamase measurement methods ($p<0.05$) which is consistent with the results of other studies that have demonstrated similar results by different methods of betalactamase assay (Gradus and Silver, 1989) but in other study, acidometery have been reported more reliable than iodometery (Nahaee *et al*., 1999).

The time and expense in acidometery were less than iodometery in our study and other studies notify that acidometery is a one-step procedure (Sonnenwirth and Jarett, 1980), nowadays there are also another methods of betalactamase assay (Adeyemi *et al*., 2000; Troillet *et al*., 1998; Chen *et al*., 1994; Ronco *et al*., 1989).

In present study, 84% of the all coagulase positive staphylococci produced betalactamase determined by at least one of the betalactamase assay methods. Different studies show that at least 70% of coagulase positive staphylococci produce the enzyme (Waldvogel, 2000). On the other hand, almost all of coagulase positive staphylococci in our study shows resistance to carbenicillin, ampicillin, amoxycillin and penicillin. Because such antibiotics are susceptible to betalactamase and because of high rate of betalactamase production, these results would be anticipate. Some of the isolated staphylococci were resistant to these antibiotics while their betalactamase test were negative probably due to other mechanisms of resistance (Waldvogel, 2000; Nahaee *et al*., 1999).

18.4% of isolated staphylococci showed resistance to oxacillin and methicillin. Nowadays meticillin resistant *Staphylococcus aureus* (MRSR) have been emerged as problemetic in medicine which its prevalence differs from region to region (Hsueh, 1999; Saleao, 1999; Troillet *et al*., 1998).

The least resistance were observed in cephalothin, ceftriaxon, cephalexin and cefazolin which is probably because of their resistance to betalactamase.

Due to high percentage of resistance of coagulase positive staphylococci to antibiotics such as carbenicillin, ampicillin, amoxycillin and penicillin that correlates with betalactamase production, we suggest use of betalactamase resistant antibiotics such as oxacillin, cloxacillin, cephalothin etc. We also recommend use of betalactamase test (specially acidometery method) before antibiotic susceptibility testing which could save the time and expense and also gains rapid and important information about use of betalactam antibiotics.

ACKNOWLEDGMENTS

We deeply thanks to our colleagues of Microbiology Department of Central Hospital for Technical Assistance.

REFERENCES

Adeyemi Doro, F.A., D.J. Lyon, T.K. Iing and A.F. Cheng, 2000. E test method of antimicrobial susceptibility testing of *Neisseria gonorrhoeae* for routin diagnostic service. Afr. J. Med. Sci., 29: 171-173.

Chen, K.C., L. Chen and L.Y. Lin, 1994. Fluorescent spot test method for specific detection of betalactamase. Anal. Biochem., 219: 53-60.

Fung, C.P. and T.N. Jang, 1995. Antimicrobial susceptibility and betalactamase production of *Moraxella catarrhalis* isolates in Taiwan. J. Formos Med. Assoc., 94: 548-554.

Gradus, M.S. and K.J. Silver, 1989. Comparision of the Quad FERM$^+$ 2h identification system with conventional carbohydrate degradation tests for confirmatory identification of *Neisseria gonorrhoeae*. Sex Transm. Dis., 16: 57-59.

Hindler, J.A. and J.H. Jorgensen, 2000. Procedures in Antimicrobial Suscptibility Testing. In: Textbook of Diagnostic Microbiology. Mahon, C.R. and G. Manuselis (Eds.), 6th Edn. W.B. Saunders, pp: 61-95.

Hsueh, P., 1999. Dissemination of two meticillin resistant *Staphylococcus aureus* clones exhibiting negative staphylase reaction in intensive care unites. J Clin. Microbiol., 37: 504-509.

Humphreys, H., 2002. *Staphylococcus*. In: Medical Microbiology. Greenwood, D., R.C.B. Slack and J.F. Peutherer (Eds.). Churchill Livingstone, Edinburg, pp: 168-173.

Lee, W.S. and L. Komarmy, 1981. Iodometric spots test for detection of betalactamase in *Haemophilus influenzae*. J. Clin. Microbial., 13: 224-225.

Nahaee, M., A. Jalali and S. Nikwash, 1999. Study of two rapid method for betalactamase production assay in ampicillin resistant enterobacteriaceae and its relation with MIC. Tabriz Med. J., 22: 25-27.

Ronco, E., M.L. Migureres and A. Pilippon, 1989. Broad spectrum beta -lactamases and the API ATB 24H system: The need for detection. Pathol. Biol., 37: 549-552.

Saleao, R., 1999. Detection of an archaic clone of *Staphylococcus aureus* with low level resistance to meticillin in a pediatric hospital in portugal and in international samples: Relice of a formerly widely disseminated strain? J. Clin. Microbiol., 37: 1913-20.

Sonnenwirth, A.C. and L. Jarett, 1980. Gradwohl's Clinical Laboratory Methods and Diagnosis. 8th Edn. Philadelphia, Mosby Co., pp: 1959-1960.

Sykes, R.B., 1979. Methods for Detecting Betalactamase. In: Laboratory Methods in Antimicrobial Chemotherapy. David, S.R., P. Ian, W. David and W. Richard (Eds.). Churchill Livingstone, Edinburg, pp: 64-69.

Troillet, N., Y. Carmeli and M. Samore, 1998. Carriage of meticillin resistant *Staphylococcus aureus* at hospital admission. Infect. Control Hosp. Epidemiol., 19: 181-185.

Waldvogel, F.A., 2000. Stapylococci. In: Princples and Practice of Infectious Diseases. Mandel, G.L., R.G. Douglas and R. Dolin (Eds.), 5th Edn. Churchill Livingstone, New York, pp: 1489-1515.