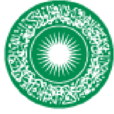

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K Jabeen
Aga Khan University, kausar.jabeen@aku.edu

A Zafar
Aga Khan University, afia.zafar@aku.edu

R Hasan
Aga Khan University, rumina.hasan@aku.edu

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Comparison of Double Disc and Combined Disc Method for the detection of Extended Spectrum ß Lactamases in Enterobacteriaceae

K. Jabeen, A. Zafar, R. Hasan
Department of Pathology, Aga Khan University, Karachi.

Abstract

Objective: To compare double disc approximation and combined disc method for their ability to detect extended spectrum b lactamase (ESBL) production in enterobacteriaceae and determine the percentage of isolates which are falsely reported as sensitive in absence of ESBL detection, in a clinical microbiology laboratory of a tertiary care hospital between September - October 2002.

Methods: Selected isolates were identified according to standard biochemical tests. Disc susceptibility tests were performed according to NCCLS. ESBL detection by combined disc {cefotaxime (30ug) versus cefotaxime plus clavulanate (30+10 ug)} was compared with detection using double discs {amoxy-clavulanic acid (20+10 ug) and aztreonam (30ug) applied 10 mm apart}. Results were interpreted according to NCCLS and analysed on SPSS version 10.

Results: ESBL production was detected in 140 (30%) isolates by combined disc method and 139 (29.5%) by double disc method. There was no significant difference between two methods. Of the ESBL positive isolates 41(29%) gave zone diameters that were within the sensitivity range cutoff and would have been falsely reported as being beta lactam sensitive in absence of ESBL detection.

Conclusion: ESBL detection should be routinely performed in clinical laboratories as false reporting would result in treatment failure despite in vitro sensitivity. No difference was found between the combined disc and double disc methods hence either of two could be used (JPMA 53:534;2003).

Introduction

Extended-spectrum ß-Lactamase (ESBL) producing organisms are a major problem in the area of infectious disease[1] after discovery in 1983.[2,3] These ß-Lactamases can be produced by a variety of Enterobacteriaceae; however, the most common ESBL-producing organisms are Klebsiella species, and Escherichia coli.[4,5] These organisms confer resistant to all ß-Lactam antibiotics except cephamycins and carbapenems.[4,6,7] In addition, ESBL-producing organisms are frequently resistant to many other classes of antibiotics; including aminoglycosides and fluoroquinolones[4,8] thus treatment of these infections is often a therapeutic challenge. ESBL arise by mutations in genes of plasmid mediated beta lactamases (especially TEM and SHV enzymes).[8] A second type of transmissible resistance arises from plasmid acquisition of a chromosomal amp C gene providing resistance to cephamycins as well.[9]

The frequency of ESBL-producing organisms differs significantly by geographic location.[10-12] The SENTRY surveillance program reported the frequency of ESBL-producing K. pneumoniae as 45% in Latin America versus 7% in the United States.[10] Although considerable variation exists in the U.S., with a surveillance study of 15 hospitals in Brooklyn, New York, reporting that 34% of their K. pneumoniae produce ESBL.[11] Frequency of ESBL producing E. coli at hospitals in the SENTRY surveillance program was much lower, 1-8%.[10] In Pakistan, a study performed in Islamabad shows a prevalence of 48% in nosocomial isolates.[13] Another study from Rawalpindi reported 35% ESBL detection in nosocomial isolates.[14]

Detection of ESBL is challenging for the clinical microbiology laboratory.[15] Its presence in the bacterial cell does not always produce a resistant phenotype; some ESBL isolates may appear susceptible to third-generation cephalosporins in vitro. Failure of either MIC or disc test alone to accurately detect the presence of an ESBL has been well documented.[16,17] A proficiency testing project for clinical laboratories participating in the National Nosocomial Infections Surveillance System indicated that as many as 58% of laboratories failed to detect and report ESBL isolates correctly.[18] In some studies, 37% of ESBL producing organisms were misreported[19] whereas in another study only 7 out of 38 laboratories correctly identified these organisms.[20] These data suggest that improvements in the ability of clinical laboratories to detect ESBLs are needed as all ESBL producing organisms should be reported as being resistant to all penicillins, cephalosporins and aztreonam.[21]

National Committee for Clinical Laboratory Standards (NCCLS) and British Society for Antimicrobial and Chemotherapy (BSAC) have made a standardized criterion for screening, confirmatory testing and reporting for these organisms.[21,22] Several ESBL detection test have been proposed based on Kirby Bauer disc diffusion methodology such as the double disc approximation test[23] combined disc method[22] and three-dimensional test.[24] In

addition, MIC based methods in which dilution tests are performed.[25] Several commercial tests (Etest ESBL strips) as well as automated systems (Vitek) are also available for detection of ESBL.[26] None of the tests for phenotypic detection of ESBL are 100% sensitive or specific, however double disc approximation test, combined disc method and broth dilution MIC method are the easiest and cost effective methods for use by many clinical laboratories.[26]

Detection of ESBL was initially performed by double disc approximation test in our laboratory, however there were person-to-person variation in the interpretation of results. We therefore, conducted a study comparing double disc approximation and combined disc method for the detection of ESBL.

Material and Methods

The study was done in a 550-bed tertiary care hospital located in Karachi, Pakistan. The clinical microbiology laboratory receives samples of patients presenting to the hospital as well as outside referrals from other hospitals, clinics and general practitioners across the city.

In this comparative study, samples were taken of patients seen at the AKUH, or those referred from other hospitals, clinics and general practitioners across the city.

Laboratory Methods

All urinary specimens growing members of the family Enterobacteriaceae. These isolates were identified by routine biochemical tests.[27] Disc susceptibility tests were performed by criteria according to NCCLS[21] using Mueller Hinton agar (Oxoid). ESBL detection was done by combined disc method using cefotaxime (30ug) versus cefotaxime plus clavulanate (30+10 ug) (Oxoid) according to NCCLS criteria.[21] Double disc method was performed by using disc containing co-amoxiclavulanic acid (20+10 ug) and aztreonam applied 10 mm apart.[22]

Data analysis was done using SPSS version 10. Frequencies were calculated by using descriptive statistics and expressed as percentages and rate.

Results

During the study period 471 isolates were identified as Enterobacteriaceae. Frequent isolates were E.coli (74%), K. pneumoniae(13%), Enterobacter sp. (9%), Proteus sp. (3%). ESBL production was detected in 140 (30%) isolates by combined disc method and 139 (29.5%) by double disc method. No significant difference was found between the two methods. ESBL production by the organisms studied is shown in Figure 1 exhibiting that frequency of ESPL production was highest in Enterobacter with 34% of 41 Enterobacter tested being ESBL positive. Of the ESBL positive isolates, 41/140(29%) gave zone diameters with cefuroxime, cefixime, cefotaxime and aztreonam that were within the sensitivity range cutoff and would have been falsely reported as being beta lactam sensitive in absence of ESBL detection (Figure 2).

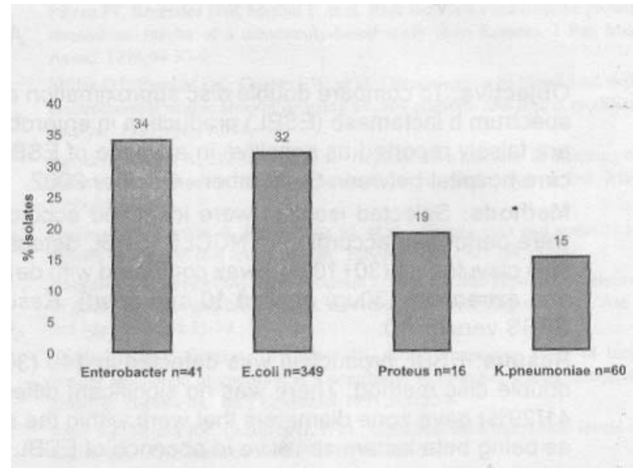

Figure 1. Frequency of ESBL production within individual organism. % isolates represent ESBL positive isolates within individual organism/Total isolates of that organism.

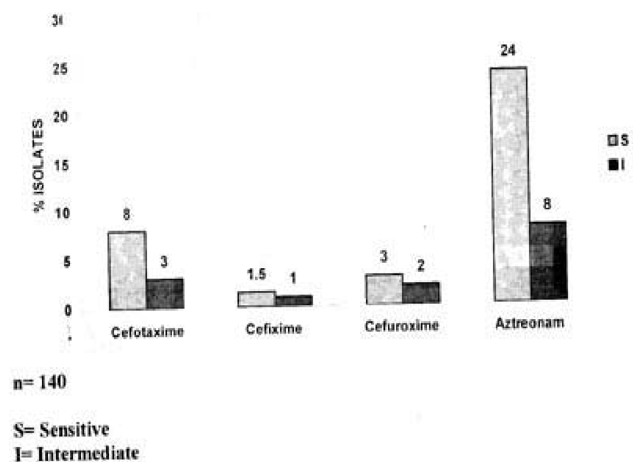

Figure 2. Frequency of isolates that could have been falsely reported to cefotaxime, cefixime, cefuroxime and aztreonam in absence of ESBL detection (n=140).

Discussion

Detection of ESBL is a major challenge for the clinical microbiology laboratory[15] as its detection has major impact on therapy. Moreover, presence of an ESBL also has significant infection control implications.[26]

We have compared two methods for ESBL detection based on Kirby Bauer disc diffusion methodology. Both of these methods were found to be equivalent in detection of ESBL producing isolates. However in the double disc approximation method distance of disc placement is not standardized and it has been suggested that reducing the distance between two discs could increase sensitivity of this

method.[23] Combined disc method is a recently developed method with ability to detect presence of ESBL.[28] A study reported by Zali et al[29] have documented sensitivity of double discs with ceftazidime plus clauvulanic acid as being 86% and with cefotaxime plus clauvulanic acid as being 65% and recommended use of both ceftazidime and cefotaxime combinations to increase the sensitivity upto 93%.[29]

Our results show that in the absence of ESBL detection 29% of isolates would have been reported as being falsely sensitive with cefuroxime, cefixime, cefotaxime and aztreonam. Livermore et all have reported that 37% of ESBL producing organisms were misreported in their study.[19] A study conducted by World Health Organization using disc diffusion technique found that 5.4% of laboratories found an ESBL producing strain to be susceptible to all cephalosporins.[30]

Double disc method needs two discs which are part of routine sensitivity in most laboratories, whereas combined disc method needs only one extra disc in the regular testing battery. Therefore expense of determination of ESBL is minimal. However as use of double disc method needs accurate distance between two discs so there is a need of precision and person to person variation could occur.

We therefore, recommend that determination of ESBL production is necessary to prevent misreporting and hence treatment failure. This detection could be done by either of the two methods, as both of these methods have found to be simple as well as cost effective.

Acknowledgements

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Use of Intravenous anti-D in patients with refractory and relapsed Immune Thrombocytopenic Purpura

R. Sajid, S. N. Adil, Z. Fadoo, S. Sabir, M. Khurshid
Department of Pathology, The Aga Khan University, Karachi.

Abstract

Objective: To determine the response to IV anti-D and its comparison with splenectomy as second line therapy in refractory and relapsed cases of ITP, in the Aga Khan University Hospital, Karachi.

Methods: A total of 23 patients with chronic ITP were treated with either anti-D or splenectomy as second line treatment. The patients were assessed for time to achieve a response to second line treatment, duration of response and adverse events.

Results: There were 12 patients in the anti-D group and 11 in the splenectomy group. The mean platelet count at presentation was 9,000/cumm. The mean age was 8.9 years and 13.0 years and the male to female ratio was 1:1 and 1:1.2 in anti-D and splenectomy group respectively. 54.5% of the patient in the anti-D group responded compared to 81.8% in the splenectomy group. Median time to achieve a response was 7 days in the anti-D group and 1 day in the splenectomy group. Mean time to relapse was 87.8 days in the anti-D group and 55.4 days in the splenectomy group. No adverse events were recorded for any of the infusions of anti-D and none of the patients had more than 0.5 gm /dl fall in the hemoglobin level following anti-D infusion.

Conclusion: It was thus concluded that Anti-D is a relatively safe, convenient and effective therapy for chronic ITP and can be used as a splenectomy sparing agent when treatment is clinically indicated (JPMA 53:537;2003).

Introduction

Immune thrombocytopenic purpura (ITP) is an acquired disease of children and adults defined by a low platelet count in the absence of other clinically apparent causes of thrombocytopenia.[1] It is principally a disorder of increased platelet destruction caused by antiplatelet antibodies.

In 1983 Salama et al reported platelet responses in three of six Rh (D) positive patients treated with 400 to 2500 ug of anti-D (2).Anti-D is a plasma derived immunoglobulin prepared from donors selected for a high titer of Rho (D) antibody. The investigators suggested that the rise in platelet count is due to competitive inhibition of the macrophage binding of platelets by preferential sequestration of immunoglobulin coated red blood cells.[2]

Important observations from published reports include evidence of a dose response relationship, reproducibility of responses, and efficacy of anti-D in Rh (D) positive but not in Rh (D) negative subjects. Similarly splenectomized patients had minimal or no responses.[3] The lower cost of anti-D plus the ease of administration make anti-D therapy an attractive option as splenectomy sparing therapy and as maintenance therapy in patients with chronic ITP.[4]

Patients and Methods

This was a case series study which was carried out from January 2000 to October 2002.

This study was conducted upon diagnosed patients with Immune Thrombocytopenic Purpura. All patients of ITP received Anti-D were included in one group and patients who underwent splenectomy were included in the second group. Patients with established diagnosis of ITP were included in the study. All patients who had persistently low platelet counts for at least six months , were blood group Rh positive (anti-D group) had not undergone splenectomy and were not receiving other forms of therapy were included in the study. Post splenectomy patients and patients with blood group other than Rh positive were excluded.

Patients were eligible for inclusion if they had a diagnosis of acute or chronic ITP, had not undergone splenectomy, their blood group was Rh positive (anti-D group). All 23 patients included in the study fulfilled the criteria mentioned above. All patients were analyzed for demographic features including age, sex, age at the time of diagnosis, clinical features at presentation, response to initial treatment, rise in platelet counts following second line treatment (IV anti-D) and persistence of response for 3 months. Response was defined as an increase in platelet count to >50,000/cumm or doubling of pretreatment platelet counts. Any adverse events with anti-D or splenectomy were also recorded.

Anti-D (WinRho) was given at a dose of 50mg/kg. The anti-D preparation was diluted in physiologic normal saline and infused intravenously during a 15-30 minute period on an outpatient basis. Patients were followed at weekly intervals and any adverse events were recorded. Complete blood count was performed at each outpatient