INTERNATIONAL JOURNAL OF PHARMACEUTICAL RESEARCH AND BIO-SCIENCE

PROFILE OF GRAM NEGATIVE ORGANISMS CAUSING BSIS IN ICUS AND CHARACTERIZATION OF THESE ISOLATES INTO ESBL, AMPC AND CARBAPENAMASE (MBL) PRODUCERS.

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Accepted Date: 11/06/2016; Published Date: 27/06/2016

Abstract: **Background:** An alarming rise in the rates of the antibiotic resistance has now become a serious and an increasingly common public health concern, with severe implications, especially in the intensive care units. A variety of β-lactamases which include ESBLs, AmpC β-lactamases and metallo-βlactamases, have emerged as the most worrisome mechanism of resistance among the gram negative bacteria, which pose a therapeutic challenge to the health care settings. **Materials and Methods:** The present study was aimed at knowing the prevalence of various β-lactamases in the gram negative isolates which were obtained from ICU patients. A total 221 gram negative isolates from 3016 blood samples, received over a period of one year were processed for their identification and were characterized for β-lactamase production. **Results:** Among the 221 isolates, the β-lactamase production was observed in 111 isolates. 52 (23.7%) Gram negative isolates were ESBL producers and same number of the isolates were found to be AmpC producers, followed by 29 (13.1%) carbapenamases (MBL/ KPC) producers the major ESBL, carbapenamase and AmpC producer was Klebsiella pneumoniae. The co-production of the ESBL/ carbapenamase / AmpC β- lactamases was also observed among the Gram negative isolates. **Conclusion:** The high prevalence of the β- lactamases in the ICU isolates emphasizes the need for a continuous surveillance in the ICUs to detect the resistant strains, strict guidelines for the antibiotic therapy and the implementation of infection control measures to reduce the increasing burden of antibiotic resistance.

Keywords: β-lactamases, ESBL, AmpC β-lactamases, carbapenamases, BSIs, Intensive care units (ICUs)

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PAPER-QR CODE

Access Online On:

www.ijprbs.com

How to Cite This Article:

Rama Gupta, IJPRBS, 2016; Volume 5(3): 142-152

INTRODUCTION

Bloodstream infection (BSI) is a leading, preventable infectious complication in critically ill patients and has a negative impact on patient's outcome.[1] A wide range of bacteria have been described as causative agents of BSIs including Gram negative bacteria such as Escherichia coli, Pseudomonas aeruginosa, Acinetobacterbaumannii, Klebsiellapneumoniae, Neisseria meningitidis, Haemophilus influenza and Gram positive bacteria such as coagulase negative staphylococci (CONS), Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus agalactiae, and Enterococcus faecium.[2-6] Morbidity and mortality among patients infected with Gram-negative bacilli are higher than among those patients who have Gram-positive cocci causing bacteraemia.[7-11] The surveillance of bloodstream pathogens in a hospital is important in monitoring the spectrum of microorganisms that invade bloodstream and such data is often used to determine empiric antibiotic therapy and to alert clinicians to emerging pathogens that may pose a threat to community.[5] One of the more alarming recent trends in infectious diseases is the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community acquired infections. Numerous classes of antimicrobial agents have become less effective as a result of the emergence of antimicrobial resistance, often as result of selective pressure of antimicrobial usage.[12] The infections caused by drug resistant organisms are more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics. The incidence of infections by resistant organisms due to production of various enzymes like extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases and metallo-β-lactamases (MBLs) or carbapenemases by Gram negative bacteria has increased in recent years. Their detection is of paramount importance as these strains are more prevalent than currently recognized and constitute a serious threat to currently available antibiotics.

Therefore, the present study was undertaken to know the profile of Gram Negative organisms causing BSIs in ICUs and characterization of these isolates into ESBL, AmpC and Carbapenamase (MBL) producers.

MATERIAL AND METHODS

The present study was a prospective study conducted in the Department of Microbiology, Dayanand Medical College and Hospital, Ludhiana. This was conducted for a period of one year from 1st March 2014 to 28th February 2015.

Sample Processing: Pre inoculated BACTEC 9240 or BacT/Alert blood culture bottles were received in the microbiology lab. Blood culture bottle received were loaded in the Bactec or Bac-T/Alert microbial detection system and incubated for a maximum period of 7 days or till the bottle was indicated positive by the system. [13] Smears were prepared from the positive blood

culture bottles and Gram staining of the smears was done. Simultaneously the sample from all the positive bottles was sub cultured on blood agar and MacConkey's agar plates. The plates were incubated at 370C for 18-24 hours.

Identification & Antimicrobial susceptibility testing:

Growth was identified by the colony characteristics, Gram staining and the VITEK 2 system using GN ID cards. For AST of Gram negative organisms, AST-GN280 card were usedt for lactose fermenters and AST-GN281 for non lactose fermenters. ATCC strains of Escherichia coli 25922 and Pseudomonas aeruginosa 27853 were used as quality controls. In order to evaluate MIC results, CLSI (Clinical & Laboratory Standards Institute) recommendations were used. [14]

Screening for ESBL, AmpC and carbapenemases (MBL/KPC) production in Gram negative bacteria[14]

The antimicrobial discs used for screening procedure were cefotaxime and ceftazidime (for ESBLs), cefoxitin (for AmpC β-lactamase) and ertapenem (for carbapenemase). Then zones of inhibition were measured and compared with the Performance Standards of Antimicrobial Disc Susceptibility Test provided by CLSI. [14]

ENZYME	ANTIMICROBIAL DISC	ZONE SIZE INDICATING RESISTANCE
ESBLs	**1. Ceftazidime** **2. Cefotaxime**	**≤ 22 mm** **≤ 26 mm**
AmpC β-lactamases	**Cefoxitin**	**< 18 mm**
Carbapenemases **MBL**	**Ertapenem (10µg)** **Meropenem (10µg)**	**19-21 mm** **16-21 mm**

Probable ESBL/AmpC or carbapenemase (MBL/KPC) producer were subjected to confirmatory tests.

CONFIRMATORY TESTS

ESBL- Double Disc Diffusion Test [14] (DDDT)

The lawn culture of test organism was done on MHA. The discs of ceftazidime alone (30 µg) and in combination with clavulanic acid (10 µg) were applied on the plate. The discs were placed in such a way that the centre to centre distance between the discs was minimum 30 mm. The MHA plate were incubated at 35°C for 24 hours. An expansion of zone of inhibition ≥ 5 mm around the combination disc was considered a positive result.

AmpC -AmpC Disc Test [15]

A lawn culture of E.coli ATCC 25922 was prepared on a MHA plate and a cefoxitin disc (30 µg) will be placed on the plate. AmpC disc (filter paper disc containing Tris-EDTA) was moistened with 20 µl of sterile saline & inoculated with colonies of test organism. This disc was then placed beside the cefoxitin disc (almost touching) with the inoculated side facing downwards. The MHA plate was incubated at 35°C for 24 hours. If there was flattening or indentation of cefoxitin inhibition zone, it was considered as an AmpC producer.

Carbapenamase/Metallo-β-lactamase (MBL) Modified Hodge Test (MHT) [14, 16]

A 0.5 McFarland standard suspension of E. coli ATCC 25922 was prepared in saline, and was diluted 1:10 in saline. A lawn culture was prepared on MHA plate as for the routine disc diffusion method. The plates were allowed to dry for 3-10 minutes. One 10µg ertapenem disc was placed at the centre of the plate. Then using a 10µl loop, 3-5 colonies of the test organism were picked and inoculated in a straight line out from the edge of the disc. The streak was 20-25 mm in length. The plate was incubated at 35-37°C for 24 hrs.Enhanced growth around the test organism at the intersection of the streak and the zone of inhibition was considered positive for carbapenemases (MBL/KPC).

OBSERVATIONS AND RESULTS

This prospective study was conducted over a period of one year (March 2014- Feb 2015) in the Department of Microbiology, DMCH, Ludhiana.

From a total of 3016 blood samples received for culture, from various ICUs during the study period, out of the total 286 isolates, 221 Gram negative isolates were obtained. All these isolates were identified and antimicrobial susceptibility pattern was determined by VITEK 2 system. Among the Gram negative isolates, the most common organism isolated was K.pneumoniae (87, 39.3%), followed by A.baumannii (68, 30.8%), E.coli (31, 14%) and P.aeruginosa (21, 9.5%) (Figure -1) isolates were further characterized for production of extended spectrum β lactamases (ESBL), AmpC β lactamases (AmpC) and carbapenemases (MBL/KPC).

Out of total 221 Gram negative isolates, 117 (52.9%) were probable ESBL producers and 52 (23.7%) were confirmed ESBL producers. 36.2% (80) of the isolates, were probable AmpC producers, whereas only 52 (23.7%) isolates were confirmed AmpC producers. Further, 134 (60.6%) Gram negative isolates were probable carbapenemases (MBL/KPC) producers and very few, 29 (13.1%) could be confirmed as carbapenemases (MBL/KPC) producers. (Figure-1). In all, among the 221 Gram negative isolates, the β lactamase production was detected in 111 (50.3%) isolates. (Figure-2) 28.7% of K. pneumoniae, 25.8% of E. coli, 23.8% of P.aeruginosa and

20.6% of Acinetobacter spp. isolates were confirmed ESBL producers, whereas AmpC production was observed in. 29.9% of K. pneumoniae, 29.4% of Acinetobacter spp., 14.3% of P.aeruginosa and 9.7% of E. coli isolates. Similarly, 21.8% of K. pneumoniae, 10.3% of Acinetobacter spp., 9.5% of P.aeruginosa and 3.2% of E. coli isolates were confirmed carbapenemases (MBL/KPC) producers. (Figure-3)

The co-production of ESBL/ carbapenamase, carbapenamase /AmpC and ESBL/AmpC β - lactamases was observed in 35 (15.8%) Gram Negative isolates. The ESBL/carbapenamase and ESBL/ AmpC co production was detected in 17 (7.7%) isolates each, while AmpC/ carbapenemase, co-production was seen in 1 (0.5%) isolate only. None of the isolate showed simultaneous production of ESBL/ AmpC/carbapenemase. (Figure-4)

Figure-1: Distribution of Gram negative organisms (n=221) isolated from blood samples

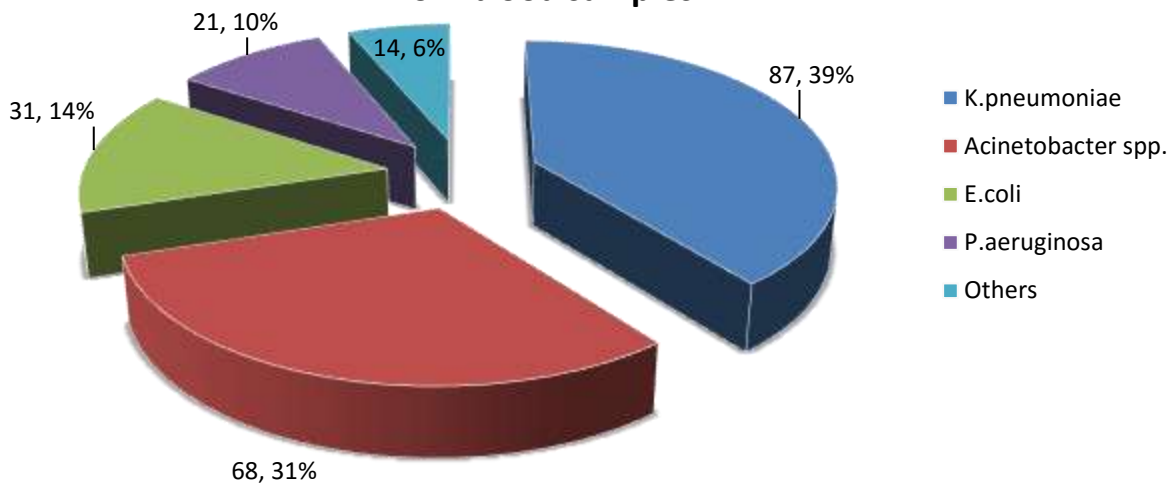
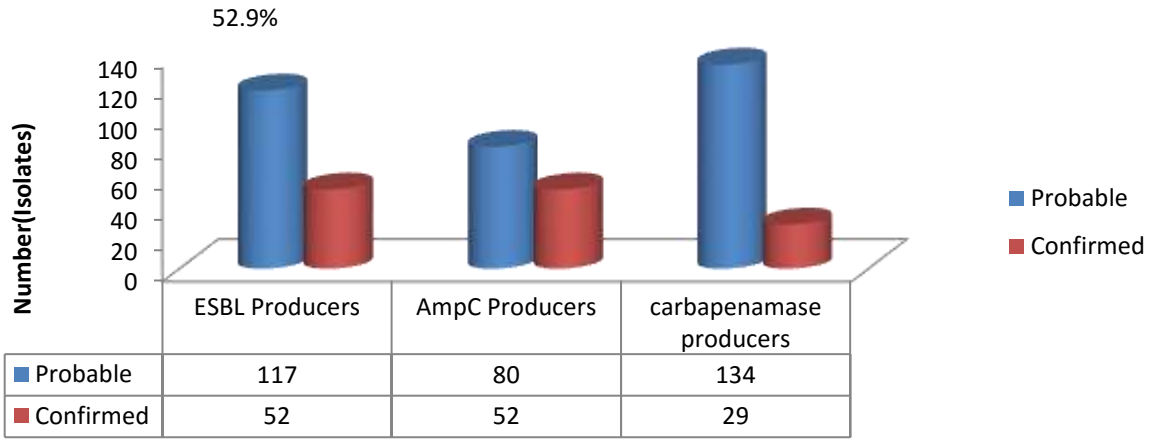
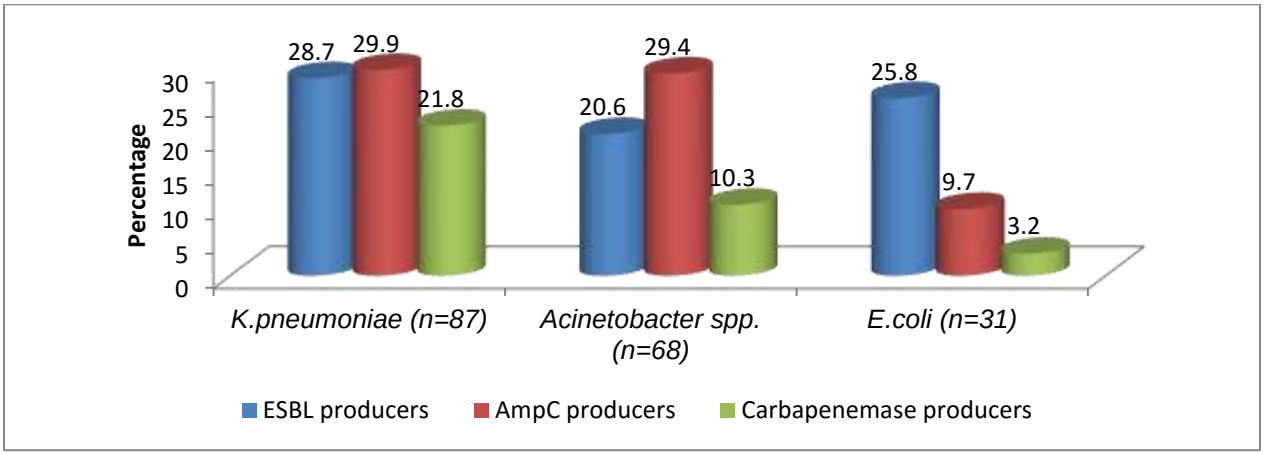


Figure-2: Distribution of various β-lactamases among Gram Negative Isolates (N=221)



	ESBL Producers	AmpC Producers	carbapenamase producers
Probable	117	80	134
Confirmed	52	52	29

Figure 3: Comparison of ESBL, AmpC & Carbapenemases (MBL) producers among common Gram negative isolates



DISCUSSION

This prospective study was done in the department of Microbiology and ICUs in which a total of 282 cases of positive blood culture were included. Microbiological profile in these cases was studied.

Bloodstream infections are an important cause of morbidity and mortality in critically ill patients resulting in prolonged hospitalization, frequent diagnostic testing, greater prescription of antibiotics and increased health care expenses. Critically ill patients are at increased risk of acquiring nosocomial bloodstream infections because of their underlying disease process and because of invasive devices such as central venous catheters. ICUs are important breeding grounds for development and spread of antibiotic resistant bacteria because of exposure of such patients to heavy antibiotics. Frequent contact with hospital staff further increases risk of cross infection.

Bloodstream infections in the ICUs occur because of the frequent interventions and more frequent use of IV lines and handling by hospital personnel. So the type of organisms which are colonizing the IV lines, or which are present over the hands of hospital personnel get transferred to the patients and thus predominant organism varies from place to place depending on environmental and geographical locations. In developing countries, many hospitals are not well equipped with isolation facilities and it is of high probability that prevalent organisms in hospitals in these countries may be different from those in developed countries.

In the present study, a total of 221 gram negative isolates and K. pneumoniae was the most common isolate (30.4%) followed by A. baumannii (23.1%) and E. coli (10.9%). Similar findings have been observed by various authors reporting K.pneumoniae as the most common Gram negative bacteria isolated from septicemia patients.[17,18-22] However, Mehta et al has reported A.baumannii and Pseudomonas aeruginosa, respectively as the commonest Gram negative isolates from blood samples.[6]

Prescription of antibiotics to control infection is the basic treatment offered, but drug resistance to multiple classes of drugs is becoming another huge problem associated with organisms isolated from the ICUs. The pattern of drug resistance keeps on changing frequently with use and with withdrawl of drugs. So the resistance pattern of all organisms isolated from ICUs can serve as good guide for future empiric therapy. BSI caused by multidrug-resistant gram negative bacilli specifically by β lactamase producers have been reported increasingly in the intensive-care units and are associated with incresed morbidity and mortality. [23] The numerous β- lactamases are encoded either by the chromosomal genes or by the transferable genes which are located on the plasmids or the transposons.[24] These enzymes are produced by all members of Enterobacteriaceae and other gram negative bacilli with varying frequency [25-26]

In the present study, the prevalence of various β lactamases in the gram negative bacteria, which included the Enterobactericeae and the nonfermenters was 50.3%, which was alarmingly high. However Obroy et al has reported a very high (70.7%) prevalence of β lactamases among Gram negative isolates.

In the present study, ESBL production was 23.5%, found to be significantly lower than that reported in the previous studies (39.8 – 74%). [27-31] However, Laghawe et al, has reported 19.67% ESBL producers among the Gram negative isolatesfrom ICU patients. [32] It has been documented that the prevalence of the ESBLs among the clinical isolates varies from country to country and institution to institution within the same country. Further, a higher prevalence of ESBL producers among E.coli (41-63.6%) and K.pneumoniae (40-66.7%). Isolates has been reported by various studies from India. [33-34] In our study, only 28.7% of K.pneumoniae and 25.8% of E.coli were found to beESBL producers.

In our study, AmpC production was seen in 23.5% of Gram negative isolates, which is higher than that reported in previous studies. It was 17.3% in Kolkata [35]and 22% in a study which was done by Bandekar et al.,[27] on burn patients, whereas a study which was done by Obroi et al showed only 5.4% AmpC producing Gram negative isolates. AmpC production was high in K.pneumoniae (29.9%) and A.baumannii (29.4%) in the present study similar to study done by

Subha et al in which maximum AmpC production was seen in Klebsiella (24.1%) and E.coli (37%).[36]

The only β-lactams which were active against the AmpC and the ESBL producers were the carbapenems, however, recently, the resistance to the carbapenems has been increasing, which is mostly due to the production of the metallo β-lactamases. In the index study, Carbapenemase (MBL/KPC) production was seen 13.1% of Gram negative isolates with maximum production seen in K.pneumoniae (21.8%) followed by A.baumannii (10.3%). Our findings were in concordance with the previous studies. [25,37]

The coexistence of different classes of β-lactamases in a single bacterial isolate may pose diagnostic and treatment challenges The ESBL/carbapenamase and ESBL/ AmpC co production was detected in 17 (7.7%) isolates each, while AmpC/ carbapenemase, co production was seen in 1 (0.5%) isolate only. However, another study from North India has reported coproduction of ESBL/ MBL in 8.79% isolates, AmpC / MBL co production in 3.67% isolates and AmpC/ ESBL co production in 6.59% isolates.[obroi] A study by Arora et al has reported the AmpC/MBL coproduction in 46.6% isolates and the ESBL/ AmpC co production in 3.3% isolates [33]

The increase in the prevalence of the β-lactamase producing isolates may be indicating the increasing trend of more and more isolates acquiring the resistance mechanisms and narrowing down the treatment options available for empiric therapy against infectious bugs. Therefore, early detection in routine laboratory, immediate infection control and antibiotic stewardship programs should be implemented in order to limit the spread of β-lactamase-producing organisms.

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