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Original Research Article

STUDY OF METALLO BETA LACTAMASE PRODUCTION IN CLINICAL ISOLATES OF DIFFERENT GRAM NEGATIVE BACTERIA AT A TERTIARY CARE CENTER IN BIHAR

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Abstract

Background: This study was designed to investigate the prevalence of ESBLs producing organisms among wound infections which would guide clinicians and microbiologists for proper handling of these pathogens & prevent unnecessary use of antibiotics. **Materials and Methods:** This cross-sectional study was carried out in the Department of Microbiology, JLNMC, Bhagalpur, Bihar during the period of March 2021 to February 2022. Samples were collected after taking informed written consent from patients admitted in different wards of the hospital. A sterile technique was applied to aspirate or collect pus or wound swab from abscess or wound infection, either by disposable syringe or by sterile swab stick and inoculated in Blood agar and Mac Conkey agar media. After overnight incubation at 37°C, plates were checked for presence of any suspected pathogens. Identification of organisms was done as per standard laboratory methods of identification and antimicrobial sensitivity of the isolates were tested against different antibiotics. ESBL detection: The method recommended by Clinical Laboratories Standard Institute (CLSI) requires a two-step approach of initially screening for ESBL production and then performing confirmatory tests on screen positive isolates. **Result:** Among the 94 bacterial isolates 75 (79.8%) were gram negative and 19 (20.2%) were Gram-positive. Out of gram-negative species, majority were Klebsiella species 18(24.0%) followed by E. Coli 18(24.0%) and Pseudomonas species 16(21.3%). **Conclusion:** Indiscriminate use of antibiotics especially 3rd generation cephalosporin's and monobactams should be avoided. The regular detection of ESBLs producing organisms should be carried out in every laboratory.

INTRODUCTION

Antimicrobial resistance is emerging as one of the major public health threats at the beginning of the 21st century worldwide. The wide spread use and in some cases, misuse of antimicrobials in all health care settings over the past several decades has been cited as a contributing factor in the development of drug resistance in virtually all bacterial species. The accelerated emergence of antibiotic resistance among the prevalent pathogens is the most serious threat to the management of infectious diseases.[1] Infections by extended spectrum beta lactamases (ESBLs) producing organisms are causing significant diagnostic and therapeutic problems in affected patients.[2] ESBLs are mutant forms of β-lactamases enzymes coded by genes located on transferable plasmids, which can easily spread form one organism to another. The ESBL producing organisms are often multi drug resistant, as the plasmids producing ESBLs can carry resistance to other antibiotics.[3] Extended spectrum β-lactamases producing bacteria produce Extended Spectrum β-Lactamases (ESBLs) enzymes that mediate resistance to extended spectrum (Third generation) cephalosporins and monobactams but do not affect cephamycins (e.g. Cefoxitin and cefotatan) or carbapenems and are inhibited by β-lactamase inhibitors such as clavulanate, sulbactam and tazobactam.[1,4,5]

The ESBL producing bacteria are increasingly becoming a major threat for patients in the hospital, long term care facilities and community. The increasing drug resistance among these bacteria has made therapy difficult and has led to a greater use of expensive broad-spectrum antibiotics.[6] Inappropriate antibiotic selection in infections caused by these organisms is associated with

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treatment failures, poor clinical outcomes, prolonged hospital stay, increased morbidity, mortality and health care costs. The widespread use of the third generation cephalosporins and aztreonam is believed to be the major cause of the mutations in these enzymes.[7] Drug resistance of this form is often difficult to recognize using conventional antimicrobial susceptibility methods. Failure to identify ESBL producing organisms also contributes to their uncontrolled spread. Therefore, identification of the resistant phenotypes is important, particularly in developing countries where there is excessive use of antibiotics and lack of adequate antimicrobial resistance surveillance.[8,9] This study was designed to investigate the prevalence of ESBLs producing organisms among wound infections which would guide clinicians and microbiologists for proper handling of these pathogens & prevent unnecessary use of antibiotics..

MATERIALS AND METHODS

This cross-sectional study was carried out in the Department of Microbiology, JLNMC, Bhagalpur, Bihar during the period of March 2021 to February 2022. Samples were collected after taking informed written consent from patients admitted in different wards of the hospital.

Inclusion Criteria

The following categories of patients were included in this study:

i. Patients with infected wound.
ii. Infected burn patients.

Exclusion Criteria

Newly admitted burn/surgical cases.

A sterile technique was applied to aspirate or collect pus or wound swab from abscess or wound infection, either by disposable syringe or by sterile swab stick and inoculated in Blood agar and Mac Conkey agar media. After overnight incubation at 37°C, plates were checked for presence of any suspected pathogens. Identification of organisms was done as per standard laboratory methods of identification and antimicrobial sensitivity of the isolates were tested against different antibiotics.

ESBL detection: The method recommended by Clinical Laboratories Standard Institute (CLSI) requires a two-step approach of initially screening for ESBL production and then performing confirmatory tests on screen positive isolates.[10]

Screening for ESBL producers by dilution method Agar dilution method: The screening for ESBL producers was done by agar dilution method as was recommended by CLSI. Any of the isolated organisms found to be grown at this stated screening antibiotics concentration (That is Minimum Inhibitory Concentration [(MIC)] of third generation cephalosporin's, namely ceftriaxone, ceftazidime and cefotaxime >2µg/ml) according to CLSI, 2007 was considered as possible ESBL producers and spelled for the confirmatory tests. The use of more than one antimicrobial agent for screening improves the sensitivity of detection.[10]

Detection of ESBLs by the confirmatory tests

Phenotypic confirmatory test: Confirmation of the ESBL producing isolates was done by the phenotypic confirmatory test according to CLSI recommendation. In this test, third generation cephalosporin i.e. ceftazidime (30 µg) and cefotaxime (30 µg) disc alone and in combination with clavulanic acid (10µg) were used. Ceftazidime, cefotaxime discs were placed on one side and ceftazidime, cefotaxime discs combined with clavulanic acid (30/10 µg) were placed on other side of the inoculated plate. After overnight incubation at 37 C, diameter of zone of inhibition was measured. A 5 mm or more increases in diameter of zone of inhibition for ceftazidime and cefotaxime tested in combination with clavulanic acid versus its zone when ceftazidime and cefotaxime tested alone confirms a ESBLs producing organism.[10]

Reference strain for quality control used for ESBL detection E. coli BB-32327 (CTX-M9) was used as positive control and E. coli ATCC (American Type Culture Collection) 25922 was used as negative control of ESBL detection test.

RESULTS

A total 103 samples of wound swab & pus from different patients were studied, of which 94 (91.3%) bacterial strains were isolated & 9(8.7%) found no growth.

Among the 94 bacterial isolates 75 (79.8%) were gram negative and 19 (20.2%) were Gram-positive. Out of gram-negative species, majority were Klebsiella species 18(24.0%) followed by E. Coli 18(24.0%) and Pseudomonas species 16(21.3%) [Table 1].

Table 1: Distribution of sample based on bacterial species from the isolates (N = 94)

Organism	Number (Percentage)
Gram positive species (N = 19)	
1. Staphylococcus aureus	16 (17.0%)
2. Staphylococci spp	3 (3.2%)
Gram negative species (N = 75)	
1. Klebsiella species	22 (23.4%)
2. E. coli	21 (22.3%)
3. Pseudomonas species	19 (20.2%)
4. Proteus species	13 (19.8%)

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Total 75 isolated gram-negative bacteria were screened for suspected ESBLs producers on the basis of MIC ESBL breakpoints by agar dilution method, out of which 71 (94.7%) were found suspected ESBLs producers and rest 4 (5.3%) gave negative result.

Screening positive 71 suspected ESBLs producing bacteria were subjected to phenotypic confirmatory test, 48 (67.6%) were found as confirmed ESBL producers & 23 (32.4%) showed negative result.

Out of 75 gram-negative bacteria 48 (67.6%) were found to ESBLs producer. Higher rate of ESBLs was observed in Klebsiella species 18 (81.8%) out of 22, followed by E. Coli 16 (76.2%) out of 21, Pseudomonas species 10 (52.6%) out of 19& Proteus species4 (30.8%) out of 13.

DISCUSSION

In the present study, 94 (91.3%) bacteria were isolated from 103 wound swab & pus samples from different patients.Among the 94 bacterial isolates 75 (79.8%) were gramnegative and 19 (20.2%) were Gram-positive [Table 1]. Similar to present study, Rahman found 90% gram-negative & 10% gram-positive isolates.[11] Amongst the isolates, Klebsiella species (23.4%) was the predominant organism, followed by E. Coli (22.3%) Pseudomonas species (20.2%) Staphylococcus aureus (17.0%) Proteus species (19.8%) and Coagulase-negative staphylococci (3.2%). Sule et al. in Nigeria also found Klebsiella species (25.3%) as the most common bacterial isolates from wound swab.[12] In contrast to present study Rahman et al. of Dhaka, found Staphylococcus aureus (39.4%) was the principal organism, followed by Klebsiella species (21.1%) E. Coli (11.2%) Proteus species (8.4%) and Pseudomonas species (5.6%) in surgical wound.[13] These different findings may be due to that etiological agent may however, vary from country to country, from hospital to hospital and from one community to another.[14]

In the present study, we found 71 (94.7%) suspected ESBLs producers from 75 gram-negative isolates, based on Minimum Inhibitory Concentration (MIC) ESBLs screening breakpoints. As using more than one antibiotic increase the sensitivity, we used three third generation cephalosporins (Ceftriaxone, ceftazidime & cefotaxime) for the screening. Our finding is closely related to that of Metri et al. in North Karnataka, India, who found 91.74% suspected ESBLs producers by screening test.[7] When these 71 screening positive isolates were subjected to the confirmatory tests, 48 (67.6%) were confirmed as ESBL producers by phenotypic confirmatory test.

The prevalence of ESBLs producing organisms in the present study were found to be 67.5%, which is higher than that of Alim 23.2% and Rahman 30.9% both in BSMMU but lower than that of Biswas of BSMMU 80% and Yasmin of Mymensingh 71.30%.[11,15,16,17] Ullah et al. in Pakistan found 58.7% ESBL producers.[18] The prevalence of ESBLs producers in India ranges from 6.6% to 91%, in Europe from 23-25% for Klebsiella species and 5.4% for E. Coli and in United States from 0 to 25%, depending on the institution.[3,7]

The variation on ESBL positivity might be due to the number of isolates studied, variation in institution to institution & geographic location.[5,8] The prevalence of ESBL production is high in the referral centers and the intensive care unites where the patients are referred from the peripheral centers and where the antibiotic use is profuse.[7] The uncontrolled use of 3rd generation cephalosporins at the hospital could be a leading contributory factor to the high ESBLs prevalence.[19]

ESBLs are most commonly recognized in Klebsiella species and E. Coli and most prevalent in Klebsiella pneumoniae.[20,21] We also found Klebsiella species as the leading ESBLs producers followed by E. Coli and Pseudomonas species and then by Proteus species in our study, which correlates with those of Rahman & Alim both in BSMMU, Yasmin of Mymensingh who also found Klebsiella species as the most common ESBL producers.[11,15,17] The high occurrence of ESBLs in Klebsiella species is of great concern since infections caused by this bacterium are very common and resistance of the organism may be due to the presence of capsule & multidrug resistance efflux pump, easy spreading nature, pathogenic and efficient at acquiring and disseminating resistance plasmid and production of endotoxin, carbapenemases, which make it more resistant.[22]

CONCLUSION

Existing of extended spectrum β-lactameses in bacteria and their potential multidrug resistance will create serious problem in the future as their continuous mutation and limited therapeutic option. Indiscriminate use of antibiotics especially 3rd generation cephalosporins and monobactams should be avoided. The regular detection of ESBLs producing organisms should be carried out in every laboratory.

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