Abstract

Introduction: The emergence of antibiotic-resistant organisms is a major public health concern, particularly in hospitals and other health care settings. Carbapenem are a group of β-lactam antimicrobial agents with an exceptionally broad spectrum of activity. They are used as a last resort drug against many multi-drug resistant (MDR) microorganisms such as extended-spectrum beta-lactamase (ESBL), Metallo-β-lactamase (MBL), and AmpC β-lactamase enzyme-producing gram-negative bacilli. Aim of the study: The aim of this study was to ascertain the prevalence of MBL-producing gram-negative bacilli in a tertiary care hospital. Materials & Methodology: This Cross-sectional study was carried out in the Department of Microbiology, Chittagong Medical College and Hospital, Chittagong. During the period of July 2015 to June 2016 after approval of the protocol by the ethical review committee of Chittagong Medical College. Total 220 samples were collected from both sexes and different age groups. The specimens were collected and processed according to the standard methodology. Non-molecular methods were used for the detection of Metallo-Beta-Lactamases producing isolates. Results: MBL producing organisms were 100% resistant to Imipenem and Ceftazidime, 98% resistant to Cefotaxime, Cefepime and Amoxicillin-Clavulanic acid, 94% resistant to Ceftriaxone and Ciprofloxacin, 86% resistant to Gentamicin. 84% resistant to Cotrimoxazole and Aztreonam, 78% resistant to Amikacin and Netilmicin, 34% resistant to Piperacillin-Tazobactam and 22% resistant to Colistin. Conclusions: The high rate of Metallo-Beta-Lactamases producing gram-negative bacteria in this study emphasizes the need for active surveillance in the microbiology laboratories for the detection of these resistant strains and also stresses the judicious use of Carbapenems to prevent the spread of the resistant organisms. The non-molecular method was used to detect MBL. Keywords: Metallo-Beta-Lactamase, gram-negative bacilli, Antibiotic-resistant.

INTRODUCTION

Metallo-β-lactamase (MBL) encoding genes have been reported all over the world in clinically important pathogens, such as Pseudomonas spp., netobacter spp. and members of the Enterobacteriaceae family [1]. MBLS spread easily on plasmids and the acquired resistance mechanisms are attained by bacteria through mutations or mechanisms of horizontal gene transfer such as transformation, conjugation, transduction, transposon and insertion sequence common region (ISCR) elements. The increasing rates of antibiotic resistance are a major cause for concern in both gram-negative bacilli and isolates of the Enterobacteriaceae family. B-lactams have been the mainstay of treatment for serious infections. Most active of these are the carbapenems, which are advocated for use for the treatment of infections caused by extended-spectrum-β-lactamase (ESBL)- producing Enterobacteriaceae, particularly Escherichia coli and Klebsiella pneumonia & non-fermenters, particularly Pseudomonas spp. and Acinetobacter spp[2]. In India MBL producing Porsginosa was first reported by Navaneeth et al. [3]. Out of 450 clinical isolates of
gram-negative bacilli, there were 27(6%) isolates resistant to Imipenem and 3rd generation of Cephalosporin. Moreover, the prevalence of MBL production in Pseudomonas spp. (9.92%) consequently followed by Klebsiella spp. (7.26%), similarly Acinetobacter spp. (7.14%) and at the same way E. coli (287%). Fam, N et al. [4], in Egypt showed prevalence of MBL production among the gram-negative isolates were 10% among them 50% of Pseudomonas spp. isolates were MBL producer. Bhongle, NN et al. [5] showed, the prevalence of MBL in Pseudomonas spp. was 10-30% among various clinical samples. Five different types of MBLs whose prevalence are increasing rapidly are IMP, VIM, SPM, GIM and SIM [6]. Among these, IMP and VIM are the most predominant [7]. With the global increase in the occurrence and types of MBLs, early detection is crucial, the benefits of which include timely implementation of strict infection control practices and treatment with alternative antimicrobials [8]. Molecular techniques are available to detect MBL producers [9]. In Australia MBL, producing gram negative organisms had emerged in the year 2005. The resistance gene, blaIMP [4], appeared highly mobile; this outbreak involved 5 different gram-negative genera from patients with close epidemiological links [10]. Pitout et al. in Canada [11] showed, 46% MBL producer among all Pseudomonas spp which were resistant to Carbapenem. Carbapenems and cephalosporin/inhibitor combinations are being used as the “last resort” in these infections since the last few years. Therefore, the aim of this study was to ascertain the prevalence of MBL-producing gram-negative bacilli in a tertiary care hospital.

OBJECTIVES

The objective of this conservative cross-sectional study was to see the prevalence of Gram-negative bacteria among patients in a tertiary care hospital.

MATERIALS & METHODOLOGY

This Cross-Sectional study was carried out in the Department of Microbiology, Chittagong Medical College and Hospital, Chittagong. During the period of July 2015 to June 2016 after approval of the protocol by the ethical review committee of Chittagong Medical College. Samples were collected from patients admitted to the Intensive Care Unit (ICU), Chittagong Medical College and Hospital, Chittagong. Total 220 samples were collected from both sexes and different age groups; informed written consent was duly taken. The categories of patients were included in this study were, the patients with infected wounds & the patients with urinary catheterization Infected fur patients. Community-acquired infections were excluded in this study.

Isolation and identification

Identification of organisms was done on the basis of their colony morphology, staining characteristics, pigment production, oxidase reaction, citrate utilization, hydrogen sulphide production, and other relevant biochemical tests as per standard laboratory methods of identification. Prior to the above test for detection of the urinary pathogen from the plate, colony count was done by calibrated loop (0.01ml) method. The number of colonies. Grown were counted and interpreted as CFU/ml of urine by multiplying the colonies grown by 100. Colony counts more than or equal to 10^3 CFU/ml were taken as significant. Bacteriuria [12].

Antimicrobial susceptibility test

All the Pseudomonas spp., Ecoli, Klebsiella spp., and Acinetobacter spp. isolates from Chittagong Medical College were tested for antimicrobial susceptibility testing by a disc diffusion method using the Kirby–Bauer technique (Bauer et al. 1996) and as per the recommendations of the CLSI, 2012. For Enterobacteriaceae and Acinetobacter species Amoxyccillin-Clavulanic Acid (AMC), Ciprofloxacin (CIP), Cotrimoxazole (SXT), Amikacin (AK), Colistin (CT), Gentamycin (GN), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Netilmicin (NET), Imipenem (IPM), Cefepime (FEP), Aztreonam (AT) and Piperacillin-Tazobactam (TZP) were used. For Pseudomonas species Amoxyccillin-Clavulanic Acid (AMC), Ciprofloxacin (CIP), Cotrimoxazole (SXT), Amikacin (AK), Colistin (CT), Gentamycin (GN), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX), Netilmicin (NET), Imipenem (IPM), Cefepime (FEP), Aztreonam (AT) and Piperacillin-Tazobactam (TZP) were used. Staphyllococcus aureus and Coagulase-negative Staphylococcus were tested against Amoxyccillin-Clavulanic Acid (AMC), Cotrimoxazole (SXT), Oxacillin (OX), Ciprofloxacin (CIP), Gentamycin (GN), Amikacin (AK), Vancomycin (VA), Ceftriaxone (CRO), Ceftazidime (CAZ), Cefotaxime (CTX) and Imipenem (IPM). For Enterococci species Amoxyccillin-Clavulanic acid (AMC), Gentamycin (GN), Vancomycin (VA), Ciprofloxacin (CIP). Ceftriaxone (CRO), Ceftazidime (CAZ). Cefotaxime (CTX), Amikacin (AK) and Imipenem (IPM) were used.

Metallo β Lactamase detection

All the Imipenem resistant Pseudomonas spp., E.coli, Klebsiella spp., and Acinetobacter spp. will be tested for detection of MBL by following methods:
1. Double Disc Synergy Test (DDST)
2. Combined Disc Synergy Test (CDST)

Imipenem-EDTA Double Disc Synergy Test (DDST)

The Imipenem-EDTA Double Disc Synergy Test was performed as described by Lee et al. 0.1 M EDTA solution was prepared by dissolving 18.61g of disodium EDTA in 100ml of distilled water and adjusting it to pH 8.0 by using NaOH [13]. The mixture was sterilized by autoclaving. Direct colony suspension of test organism adjusted to match 0.5 McFarland
turbidity was prepared and inoculated onto Mueller-Hinton agar plate as recommended by the National Committee for Clinical Laboratory Standards. One Imipenem (10 µg) disc was placed 10 mm apart from edge to edge from a blank disc containing 10 µl of 0.1 M EDTA (750µg). The inhibition zone of the Imipenem and EDTA disc were compared after 16 to 18 hours of incubation at 37°C. Enhancement of the zone of inhibition in the area between imipenem disc (10 mm) and blank disc containing EDTA was interpreted as a positive result [14].

**Imipenem-EDTA Combined Disc Synergy Test (CDST)**

The Imipenem-EDTA Combined Disc Synergy Test was performed as described by Yong et al. Test organisms were inoculated onto plates with Mueller Hinton agar as recommended by NCCLS. Two 10 µg Imipenem discs were placed on the plate and appropriate amounts of 10 ul of EDTA solution were added to one of them to obtain the desired concentration (750 µg). The inhibition zones of the Imipenem and Imipenem EDTA discs were compared after 16 to 18 hours of incubation at 37°C. In the Combined Disc Test, the increase in inhibition zone with the Imipenem and EDTA disc was 27 mm than the Imipenem disc alone, it was considered as MBL positive [14].

**DATA COLLECTION**

Data collection was done by using a structural questionnaire comprised of general information, history of getting antibiotics, clinical findings and checklists.

**DATA ANALYSIS PLAN**

Data was analyzed by using computer software SPSS (Statistical Package for Social Sciences) v. 20.0 for Windows. Data was collected, recorded, edited, and analyzed in a predesigned datasheet. The result of the experiment was recorded systematically and statistical analysis was performed by Chi-Square test.

**RESULTS**

From the study patients, the age group and sex distribution of 220 cases of them 70(31.8%) were male and 150(68.2%) were female. The male and female ratio was 1:2. The highest 83(37.7%) cases were from 21-30 years age group followed by 33(15.0%) cases from 31-40 years, 31(14.1%) cases from 11-20 years, 27(12.3%) cases were from 41-50 years, 16(7.3%) cases were from 51-60 years, 15(6.8%) cases were from > 60 years and ≤ 10 years [Table-I]. In Table II the distribution of gram-negative bacterial isolates was studied. Among the 220(100%) isolates 197(89.5%) were gram-negative bacterial isolates of which the majority were Klebsiella species 86 (39.1%) followed by E.coli 50 (22.7%). Pseudomonas species 49(22.3%), Acinetobacter species 7(3.2%), Proteus species 5(2.3%). This table also showed that among the 172(100%) wound swab and pus majority were Klebsiella species 76(44.2%) followed by Pseudomonas species 36(20.9%), E. coli species 28 (16.3%), Acinetobacter species 7(4.1%), Proteus species 3(1.7%). Among the 48 (100%) urinary isolates majority were E. coli 22(45.8%) followed by Pseudomonas species 13(27.1%), Klebsiella species 10(20.8%). Proteus species 2(4.2%) and Acinetobacter species nil. This table also showed the distribution of gram-positive bacterial isolates. Among the 220 (100%) isolates 23(10.5%) were gram-positive bacteria of which Staphylococcus aureus was 12(5.4%), Coagulase-negative Staphylococcus 10(4.5%), Enterococcus fecalis 1(0.4%). Staphylococcus aureus 12(7.0%) and Coagulase-negative Staphylococcus 10(5.8%) were isolated only from wound swab and pus. No Enterococcus fecalis was isolated from wound swab and pus but 1(2.1%) Enterococcus fecalis was isolated only from urine samples. No Staphylococcus aureus and Coagulase-negative Staphylococcus were found in urine. All bacterial isolates were tested for antimicrobial sensitivity by Kirby Bauer disc diffusion technique against different antimicrobial agents. Table III showed the antibiogram of gram-negative isolates where E coli showed 90% sensitivity to Colistin followed by 84% Piperacillin-Tazobactam, Imipenem 72%, Gentamicin 70%, both Amikacin and Netilmicin 66%, Aztreonam 64% but 70% resistant to Cefotaxime followed by Amoxicillin-Clavulanic acid 68%, Cotrimoxazole and Ceftazidime 56%, Ceftriazone 54%, Ciprofloxacin 52%. Klebsiella species showed 91% were sensitive to Colistin followed by 86% to Piperacillin-Tazobactam, Netilmicin 76%, Imipenem 72%, Gentamycin, Ciprofloxacin and Cefepime 48%, Aztreonam 44% but 77% resistant to Cefazidime followed by Amoxicillin-Clavulanic acid 66%, Cefotaxime 64%, Cotrimoxazole 58%, Amikacin and Ceftriaxone 57% each. Pseudomonas species were 94% were sensitive to Piperacillin-Tazobactam followed by Colistin 86%, Amikacin 65%, Imipenem 61%, Netilmicin 57%, Ceftriaxone 47% but 76% resistant to Ceftazidime, Cefotaxime 73%, Amoxicillin-Clavulanic acid 69%, Cotrimoxazole 67%. Aztreonam 63%. Proteus species were 100% sensitive to Colistin followed by 80% sensitive to Amikacin, Gentamycin, Cefepime and Aztreonam each. 60% sensitive to Imipenem, Ceftazidime, Ciprofloxacin, Cefotaxime, Piperacillin-Tazobactam but 80% resistant to Ceftriaxone, Cotrimoxazole, 60% resistant to Amoxicillin -Clavulanic acid and Netilmicin. Acinetobacter species were 86% sensitive to Colistin, 29% sensitive to Piperacillin Tazobactam and Aztreonam followed by Ciprofloxacin and Cotrimoxazole 14% but 100% resistant to Imipenem, Ceftazidime, Amoxicillin-Clavulanic acid, Ceftriaxone. Cefepime, Gentamycin, Amikacin, Netilmicin each. Antibiotic-resistant pattern of MBL producing gram-negative bacilli was shown in Table IV. MBL producing organism were 100% resistant to Imipenem and Ceftazidime, 98% resistant to Cefotaxime, Cefepime and Amoxicillin-Clavulanic acid, 94% resistant to Ceftriaxone and Ciprofloxacin, 86%..
and resitance to Gentamicin, 84% resistant to Cotrimoxazole and Aztreonam, 78% resistant to Amikacin and Netilmicin, 34% resistant to Piperacillin-Tazobactam and 22% resistant to Colistin.

Table-I: Distribution of culture positive samples according to age and sex (N=220)

| Age of patients | Male | Female | Total |
|-----------------|------|--------|-------|
| ≤10 yrs         | 10   | 4.5    | 5     | 2.3  | 15 (6.8%) |
| 11-20 yrs       | 10   | 4.5    | 21    | 9.5  | 31 (14.1%) |
| 21-30 yrs       | 10   | 4.5    | 73    | 33.2 | 83 (37.7%) |
| 31-40 yrs       | 10   | 4.5    | 23    | 10.4 | 33 (15.0%) |
| 41-50 yrs       | 7    | 3.1    | 20    | 9.1  | 27 (12.3%) |
| 51-60 yrs       | 7    | 3.1    | 9     | 4.1  | 16 (7.3%)  |
| >60 yrs         | 6    | 2.7    | 9     | 4.1  | 15 (6.8%)  |
| Total           | 70   | 31.8   | 150   | 68.2 | 220 (100.0%) |

Table-II: Distribution of gram negative & gram-positive bacteria among the total isolates (N=220)

| Number of bacterial species | Wound swab and pus N(%) | Urine N(%) | Total number of bacteria N(%) |
|-----------------------------|--------------------------|------------|-----------------------------|
| Klebsiella spp.             | 76 (44.2)                | 10 (20.8)  | 86 (39.1)                  |
| E.coli                      | 28 (16.3)                | 22 (16.3)  | 50 (22.7)                  |
| Pseudomonas spp.            | 36 (20.3)                | 13 (27.1)  | 49 (22.3)                  |
| Acinetobacter spp.          | 07 (4.1)                 | 0 (0.0)    | 07 (3.2)                   |
| Proteus spp.                | 03 (1.7)                 | 02 (4.2)   | 05 (2.3)                   |
| Gram Negative Total         | 150 (87.2)               | 47 (97.9)  | 197 (89.5)                 |
| Staphylococcus aureus       | 12 (7.0)                 | 0 (0.0)    | 12 (5.4)                   |
| Coagulase negative Staphylococcus | 10 (5.8)       | 0 (0.0)    | 10 (4.5)                   |
| Enterococcus foecalis       | 0 (0.0)                  | 1 (2.1)    | 01 (10.5)                  |
| Gram Positive Total         | 22 (12.8)                | 1 (2.1)    | 23 (10.5)                  |
| Grand Total                 | 172 (100.0)              | 48 (100)   | 220 (100.0)                |

Table-III: Antimicrobial sensitivity pattern of isolated gram-negative organism (N=197)

| Name of Antimicrobial agent | Sensitivity pattern | E. coli (n=50) | Klebsiella spp. (n=86) | Pseudomonas spp. (n=49) | Proteus spp. (n=6) | Acinetobacter spp. (n=7) |
|-----------------------------|---------------------|----------------|------------------------|------------------------|--------------------|--------------------------|
| Imipenem                    | S                   | 36 (72)        | 62 (72)                | 30 (61)                | 3 (60)             | 0 (0)                    |
| Ceftaxidime                 | S                   | 14 (28)        | 24 (28)                | 19 (39)                | 2 (40)             | 7 (100)                  |
| Amikacin                    | S                   | 28 (56)        | 66 (77)                | 37 (76)                | 2 (40)             | 7 (100)                  |
| Gentamycin                  | S                   | 33 (66)        | 37 (43)                | 32 (65)                | 4 (80)             | 0 (0)                    |
| Ciprofloxacin               | S                   | 17 (34)        | 49 (57)                | 17 (35)                | 1 (20)             | 7 (100)                  |
| Cotrimoxazole               | S                   | 22 (44)        | 26 (42)                | 16 (33)                | 1 (20)             | 1 (14)                   |
| Ceftriaxone                 | S                   | 28 (56)        | 50 (58)                | 33 (67)                | 4 (80)             | 6 (86)                   |
| Cefotaxime                  | S                   | 23 (46)        | 37 (43)                | 23 (47)                | 4 (20)             | 0 (0)                    |
| Cefepime                    | S                   | 27 (54)        | 49 (57)                | 26 (53)                | 1 (80)             | 7 (100)                  |
| Piperacillin-Tazobactam     | S                   | 15 (30)        | 28 (36)                | 14 (27)                | 3 (60)             | 0 (0)                    |
| Aztreonam                   | S                   | 35 (70)        | 58 (64)                | 36 (73)                | 2 (40)             | 7 (100)                  |
| Amoxicillin Clavulanic acid | S                   | 29 (58)        | 40 (48)                | 21 (43)                | 4 (80)             | 0 (0)                    |
| Netilmicin                  | S                   | 21 (42)        | 46 (52)                | 28 (57)                | 1 (20)             | 7 (100)                  |
| Colistin                    | R                   | 42 (84)        | 74 (86)                | 46 (94)                | 3 (60)             | 2 (29)                   |
| Brave                       | R                   | 18 (36)        | 48 (56)                | 31 (63)                | 1 (20)             | 5 (71)                   |
| Bravacel                    | R                   | 16 (32)        | 29 (34)                | 15 (31)                | 3 (40)             | 0 (0)                    |
| Amoxicillin-Clavulanic acid | S                   | 34 (68)        | 57 (66)                | 34 (69)                | 2 (60)             | 7 (100)                  |
| Netilmicin                  | S                   | 33 (66)        | 39 (76)                | 28 (57)                | 2 (40)             | 0 (0)                    |
| Bravacel                    | R                   | 17 (34)        | 27 (24)                | 21 (43)                | 3 (60)             | 7 (100)                  |
| Colistin                    | R                   | 45 (90)        | 78 (91)                | 42 (86)                | 5 (100)            | 6 (86)                   |
| Resistance                  | R                   | 5 (10)         | 8 (9)                  | 7 (14)                 | 0 (0)              | 1 (14)                   |
Table-IV: Results of antibiotic resistant pattern of MBL producing gram negative

| Name of antibiotic          | MBL producer |
|-----------------------------|--------------|
| Imipenem                    | 50 (100%)    |
| Ceftazidime                 | 50 (100%)    |
| Cefotaxime                  | 49 (98%)     |
| Cefepime                    | 49 (98%)     |
| Amoxicillin-Clavulanic acid | 49 (98%)     |
| Ceftriaxone                 | 47 (97%)     |
| Ciprofloxacin               | 47 (97%)     |
| Gentamicin                  | 43 (86%)     |
| Aztreonam                   | 34 (84%)     |
| Cotrimoxazole               | 34 (84%)     |
| Amikacin                    | 39 (78%)     |
| Netilmicin                  | 39 (78%)     |
| Piperacillin-Tazobactam     | 17 (34%)     |
| Colistin                    | 11 (22%)     |

DISCUSSION

Carbapenems are effective therapeutic agents against highly resistant pathogens such as Pseudomonas spp. and Acinetobacter spp. The spread of this resistance among these pathogens and transfer to other gram-negative bacteria would seriously restrict therapeutic options. The occurrence of an MBL. Positive isolates in a hospital setting pose a therapeutic problem, as well as a serious concern for infection control management. The accurate identification and reporting of MBL. Producing bacteria will aid infection control practitioners in preventing the spread of these multidrug-resistant isolates [15].

From the study patients, the age group and sex distribution of 220 cases of them 70(31.8%) were male and 150(68.2%) were female. The male and female ratio was 1:2.1. The highest 83(37.7%) cases were from 21-30 years age group followed by 33(15.0%) cases from 31-40 years, 31(14.1%) cases from 11-20 years, 27(12.3%) cases were from 41-50 years, 16(7.3%) cases were from 51-60 years, 15(6.8%) cases were from > 60 years and ≤ 10 years.

The distribution of gram-negative bacterial isolates was studied. Among the 220(100%) isolates 197(89.5%) were gram-negative bacterial isolates of which the majority were Klebsiella species 86 (39.1%) followed by E.coli 50 (22.7%), Pseudomonas species 49(22.3%), Acinetobacter species 7(3.2%), Proteus species 5(2.3%). From the 172(100%) wound swab and pus majorly were Klebsiella species 76(44.2%) followed by Pseudomonas species 36(20.9%), E. coli species 28 (16.3%), Acinetobacter species 7(4.1%), Proteus species 3(1.7%). Among the 48 (100%) urinary isolates majority were E. coli 22(45.8%) followed by Pseudomonas species 13(27.1%), Klebsiella species 10(20.8%). Proteus species 2(4.2%) and Acinetobacter species nil. Also found is the distribution of gram-positive bacterial isolates. Among the 220 (100%) isolates 23(10.5%) were gram-positive bacteria of which Staphylococcus aureus was 12(5.4%), Coagulase-negative Staphylococcus 10(4.5%), Enterococcus fecalis 1(0.4%). Staphylococcus aureus 12(7.0%) and Coagulase-negative Staphylococcus 10(5.8%) were isolated only from wound swab and pus. No Enterococcus fecalis was isolated from wound swab and pus but 1(2.1%) Enterococcus fecalis was isolated only from urine samples. No Staphylococcus aureus and Coagulase negative Staphylococcus were found in urine. All bacterial isolates were tested for antimicrobial sensitivity by Kirby Bauer disc diffusion technique against different antimicrobial agents. Pondel. K et al. [16] found 50% of Pseudomonas aeruginosa was the predominant microorganism isolated from the wound swab and Staphylococcus aureus was the only gram positive organism isolated. But out of 48(100%) culture positive urine samples gram negative bacilli were 47(97.9%), among them E.coli 22(45.8%) was highest followed by Pseudomonas spp. 13(27.1%), Klebsiella spp. 10(20.8%) and Proteus spp. 2(4.2%), and only one (2.1%) gram positive Enterococcus fecalis was isolated. Guentzel et al. [17] found E.coli was the highest 25% followed by Pseudomonas spp.11%, Klebsiella spp. 8%, Proteus spp. 5% which correlates with this study.

In our study the susceptibility pattern of clinical isolates of Pseudomonas spp. showed in higher sensitivity to Piperacillin-Tazobactam 94% followed by Colistin 86%, Amikacin 65%, Netilmicin 57% but higher resistance to Ceftazidime 76%, Cefotaxime 73%
Amoxicillin Clavulanic acid 69%, Cotrimoxazole 67%, Aztreonam 63%, Gentamicin 59%, Cefepime 57%, Ciprofloxacin 55%, Ceftriaxone 53% and Imipenem 39%. In one study, Anwar, S et al. [18], Bangladesh, showed 40,1% Pseudomonas spp were resistant to Imipenem and Noyal et al. [19] in India showed 31.1% Imipenem resistant in another study. Kumar, R et al. [20] showed higher sensitivity to Colistin (97%), followed by Cefazidime 78%, Imipenem 68% and Ciprofloxacin 59%. Shammugam et al. [21] in India showed almost 70% of isolated Pseudomonas spp. was resistant to Cefotaxime followed by Cefazidime 68%, Ciprofloxacin 56%, Amikacin 38% and Piperacillin-Tazobactam 25% resistant. The problem of MBL producing strains were originally confined to Pseudomonas spp. and Acinetobacter spp.

In our study, E.coli showed higher sensitivity to Colistin 90%. Piperacillin-Tazobactam 84% followed by Imipenem 72%, Gentamicin 70%, Amikacin and Netilmicin 66%, Aztreonam 6-4% and resistant to commonly used drugs like Cefotaxime 70%, Amoxicillin clavulanic acid 68%, both Cotrimoxazole and Cefazidime 56 % Ceftriaxone 54%, Ciprofloxacin 52% and Cefepime 42%. Bora, A et al. [22] in Nepal found E.coli were 100% sensitive to Imipenem followed by Piperacillin-Tazobactam 67.1%, Gentamicin 62.1%. Amikacin, and 60.5% and highly resistant to Cefotaxime 68.5% followed by Aztreonum 67.5%, Cefazidime 67.1%, Ceftriaxone 64.8%, Cefepime 62%, Cotrimoxazole 50.4%. Ciprofloxacin 46.7%. These findings are similar to ours, except high level resistance to fourth generation of Cephalosporin Cefepime, Aztreonam as well as to the B-lactam/ß-lactamase inhibitor Piperacillin-Tazobactam. They also found all isolates of E.coli were 100% sensitive to both Imipenem and Colistin whereas in our study, we found 90% E.coli were sensitive to Colistin.

In our study, 77% Klebsiella spp. were resistant to Cefazidime followed by Netilmicin 76%, Amoxicillin clavulanic acid 66%, Cefotaxime 64%, both Ceftriaxone and Amikacin 57%, Aztreonam 56% but highly sensitive to Colistin 91%, Piperacillin-Tazobactam 86%, Netilmicin 76%, Imipenem 72%, Gentamicin, Cefepime and Ciprofloxacin 48%, 1 Cotrimoxazole 42%. Bora, A et al. [22] in Nepal found, 70.3% were resistant to Cefazidime followed by Cefotaxime 68.6%, Aztreonam 67.6% and Ceftriaxone 66.5% but sensitive to Colistin 100%, Imipenem 78.9%, Amikacin 65.9%, Piperacillin Tazobactam 61.1%, Gentamycin 58.9%, Ciprofloxacin 55.1% and Cotrimoxazole 53.5%. The antimicrobial agents are losing their efficacy because of the spread of resistant organism due to indiscriminate use of antibiotic, lack of awareness, patient noncompliance and unhygienic condition.

Antibiotic resistant pattern of MBL. Producing gram negative bacilli was shown in our study. MBL producing organisms were 100% resistant to Imipenem and Cefazidime. 98% resistant to Cefotaxime, Cefepime and Amoxicillin-Clavulanic acid, 94% resistant to Ceftriaxone and Ciprofloxacin, 86% resistant to Gentamicin, 84% resistant to Cotrimoxazole and Aztreonam, 78% resistant to Amikacin and Netilmicin but 66% sensitive to Piperacillin-Tazobactam and 78% sensitive to Colistin. In India, Patel, D et al. [23] showed in her study, MBL producing organism were 100% resistant to Imipenem, Cefazidime, Amikacin, Ciprofloxacin, Cotrimoxazole and Ceftriaxone, 68% resistant to Gentamicin, Aztreonam, Netilmicin but 54% sensitive to Piperacillin Tazobactam, 100% sensitive to Colistin which was closely similar to our study.

In Bangladesh, treatment was guided by the antibiotic resistance pattern. Many MBL strains were resistant to all antibiotics except Colistin. Colistin was an older antibiotic that has not been used much in recent decades, because it was somewhat more toxic than other antibiotics. A few MBL strains have been sensitive to Tigecycline. A few strains have also been sensitive to Aztreonam available. Combination of two antibiotics such as Imipenem and Amikacin. Piperacillin and Amikacin synergistically inhibit NDM-1 producing bacteria. Piperacillin and Imipenem have shown antagonism in vitro when given together. As a single agent, none of the three antibiotics (Tigecycline, Meropenem and Colistin) showed bactericidal concentration for some Carbapenemase producing strains, but Tigecycline and Colistin, when given together produced bactericidal effect. (Shamsuzzaman, M et al. 2011). Colistin could be a drug of choice in Carbapenem resistant gram negative infection but it should be used when no other drugs are effective (Saini, M et al. 2016).

LIMITATIONS OF THE STUDY

The present study has the sample size of the study was small due to time and resource constraints, only 90 samples were collected. The study was conducted in one tertiary care hospital in Bangladesh, so the findings may not represent the situation of the whole country.

CONCLUSION

The high rate of Metallo-Beta-Lactamases producing gram-negative bacteria in this study emphasizes the need for active surveillance in the microbiology laboratories for the detection of these resistant strains and also stresses the judicious use of Carbapenems to prevent the spread of the resistant organism. The non-molecular method was used to detect MBL. There is a need for a simple and accurate test for MBL detection to prevent the spreading of infection with nosocomial strain in hospital settings. E-test and PCR are other methods for MBL detection but due to their high cost, not feasible in routine laboratory practice. Controversies exist regarding the choice of...
optimal laboratory methods because the two tests are almost similar. So, both tests can be used as an alternative method. Microbiology laboratories must be prepared for screening of MBL-producing isolates by a low-cost, convenient, and sensitive procedure. In addition, routine surveillance of MBL-producing bacteria is crucial for establishing appropriate empirical antimicrobial therapy and restraining their spread in a hospital environment.

REFERENCE

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