ORIGINAL ARTICLE

DETECTION OF COLISTIN RESISTANCE AMONG CARBAPENEM RESISTANT GRAM NEGATIVE BACILLI IN SEPTICAEMIC PATIENTS AT A TERITARY CARE HOSPITAL

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ABSTRACT

Introduction: Colistin belongs to the family of Polymyxins, cationic polypeptides, with broad-spectrum activity against Gram-negative bacteria. In spite of their toxicity, Polymyxins have important therapeutic options in many hospitals. Carbapenem-resistance occurs in Enterobacteriaceae due to the enzyme carbapenemase produced by them. Most of the Carbapenem resistant gram negative bacteria are found to be resistant to Colistin as well. The colistin resistance in Gram-negative bacteria is because of modifications of lipid A by addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and/or phosphoethanolamine (PEtn) to reduce the net LPS negative charge (affinity).

Aim & Objectives: To isolate carbapenem resistant Gram Negative Bacilli (GNB) from blood samples collected from septicemic patients and to detect Colistin resistance among them.

Materials and Methods: This cross sectional prospective study was conducted for a period of 2 years from July 2015 to May 2017.1108 blood samples were collected from hospitalized patients with septicaemia and Carbapenem resistance was detected by disk diffusion method. The isolates that showed the zone size of less than 19mm to Imipenem were considered to be resistant and were tested for Colistin resistance. Colistin resistance was determined by MIC as per Standard Guidelines.

Result: Out of 1108 blood samples, 106 gram negative bacilli were isolated. In that, 22 isolates were Carbapenem resistant and 4 isolates were Colistin resistant of which 1 (25%) was *Klebsiella* species, 2 [50%] *Pseudomonas* species and 1 [25%] *Acinetobacter* species.

Keywords: Colistin resistance, Septicaemia, Carbapenemase, Gram negative bacilli, Modified Hodge Test, Microbroth dilution method.

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INTRODUCTION: In 21st century antimicrobial resistance is

recognized as most serious global threat to human health. The increasing emergence of Carbapenemase- producing Gram negative bacilli strains that are resistant to all β-lactams, fluoroquinolones and aminoglycosides has led to renewed interest in Polymyxin antibiotics as therapeutic agents[1,2].

Colistin is a broad spectrum antibiotic which act against most of the multidrug resistant gram negative bacteria. The drug belongs to the family of Polymyxins, which is a cationic polypeptides. [3]Polymyxin E (Colistin) and Polymyxin B are two types which are currently in clinical use, which differ only by one amino acid from each other and have comparable biological activity[4]. Colistin is a rapidly acting bactericidal agent, for all Gram-negative bacteria, that will interact with the lipid A moiety of lipopolysaccharide (LPS) that causes disruption of the outer membrane.[5] Two forms of Colistins are available for commercial purpose: Colistin sulfate and Colistimethate sodium. Most of the patients admitted in medical intensive care units with nosocomial infections, caused by *Acinetobacter baumannii* are multidrug resistant except for Colistin.[6]

Currently most of the Gram negative bacilli are becoming resistant to colistin by various mechanisms. One of them is modifications of lipid A by addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and/orphosphoethanolamine (PEtn) which reduces the net lipopolysaccharide negative charge (affinity), thereby increasing resistance to polymyxins[7].

AIM & OBJECTIVES:

1. To isolate Carbapenem resistant GNB from blood samples by Modified Hodge test.
2. To screen Colistin resistance by using Broth Microdilution test.

MATERIALS AND METHODS:

This cross sectional prospective study was conducted for a period of 2 years from July 2015 to May 2017.

SPECIMEN COLLECTION AND PROCESSING:

Blood sample were collected from hospitalized patients with septicemia. 5-10ml of blood was collected under sterile aseptic precautions by venipuncture, either from a peripheral site or central, arterial line and inoculated in the 50-100ml of BHI broth. Blood collected was immediately transported to the laboratory and incubated at 37°C in BHI broth. The broth was observed daily for macroscopic evidence of growth (Turbidity or Hemolysis). Once turbidity was observed after incubation at 37°C, subculture was done on MacConkey agar and Blood agar plates for further identification[8].

IDENTIFICATION OF PATHOGEN:

The plates with growth were processed and organism identified by colony morphology, Gram staining, biochemical reactions like Catalase test, Oxidase test, Motility test, Oxidative-Fermentative test, Indole test, Citrateutilization test, Urease test stand Triple sugar iron test.

ANTIBIOTIC SUSCEPTIBILITY TESTING:

Antimicrobial susceptibility testing was done on Mueller-Hinton agar plates by Kirby- Bauer's disc diffusion method using commercially available discs (obtained from Hi-media laboratories limited, Mumbai, India) as per Clinical and Laboratory Standards Institute (CLSI) guidelines[9].

The antibiotic discs namely Amoxycillin / Clavulanic acid (20/10ug) Amikacin (30ug), Gentamicin (30ug), Ampicillin

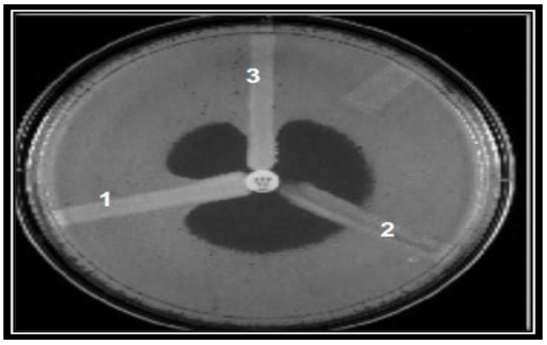
(10ug), Co-trimoxazole (25ug), Ceftazidime (30ug), Cefotaxime (30ug), Cefepime (30ug), Imipenem (10ug) were included in this study.

PHENOTYPIC CONFIRMATION:

All strains resistant to Imipenem were subjected to Modified Hodge Test to detect Carbapenemase production.

CONFIRMATORY TEST FOR CARBAPENEM RESISTANT GNB:

Figure 1: Modified Hodge Test



PROCEDURE:

We should Prepare a 0.5 McFarland dilution of the E.coli strain ATCC 25922 in 5 ml of broth. Dilute it with 1:10 by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of broth. Streak a lawn culture of the 1:10 dilution of E.coli strain ATCC 25922 to a Mueller Hinton agar (MHA) plate and allow to dry for 5 minutes. Place a 10 µg Meropenem or Imepenem susceptibility disc in the center of the test area. Then, streak the test organism in a straight line starting from the edge of the disc to the edge of the plate. With this method, we can test up to four organisms on a same plate by using one drug. Incubate it for overnight at 35 degree C ± 20 degree C in ambient air for 16–24 hours.

INTERPRETATION:

POSITIVE: After 24 hrs, a clover leaf-like indentation of *Escherichia coli* along the test organism growth streak indicates Carbapenemase production.

NEGATIVE: No clover leaf-like indentation of *Escherichia coli* indicates negative test.

MIC FOR COLISTIN BY BROTH MICRODILUTION:

The Minimal Inhibitory Concentration of an antimicrobial agent gives a quantitative estimate of the susceptibility. It is defined as the lowest concentration of antimicrobial agent required to inhibit the growth of the organism. All the Carbapenem resistant GNB were subjected to Colistin MIC by Broth Microdilution as per CLSI. Broth Microdilution (BMD) is the most standard method for Colistin antibiotic susceptibility testing[10]. Disc diffusion and gradient diffusion methods are unacceptable. Broth Microdilution test can be used to test the susceptibility of microorganisms to multiple antibiotics at once[11]. The susceptible range for Colistin is ≤ 0.06 to > 64µg/L. The strains which shows >64µg/L are considered as resistant.

PROCEDURE:

The bacterial suspension of each Colistin resistance isolate was tested with a turbidity equivalent to that of a 0.5 McFarland standard. Then, the organisms were inoculated into a liquid growth medium in the presence of different concentrations of an antimicrobial agent of Colistin along with control organisms in 96-well Microtitre plate[12]. The plates were then incubated at 37 degree C for 18-22 hours.

INTERPRETATION:

The MIC was read as the lowest concentration without visible growth. The range for Colistin is ≤0.06 to >64µg/L.

RESULT:

Out of 1108 blood samples, 106 gram negative bacilli were isolated. In that, 22 isolates were Carbapenem resistant and 4

isolates were Colistin resistant of which 1 [25%] were *Klebsiella* species, 2 [50%] *Pseudomonas* species and 1 [25%] *Acinetobacter* species.

Figure 2: Total number of Gram negative bacilli isolated from the blood samples

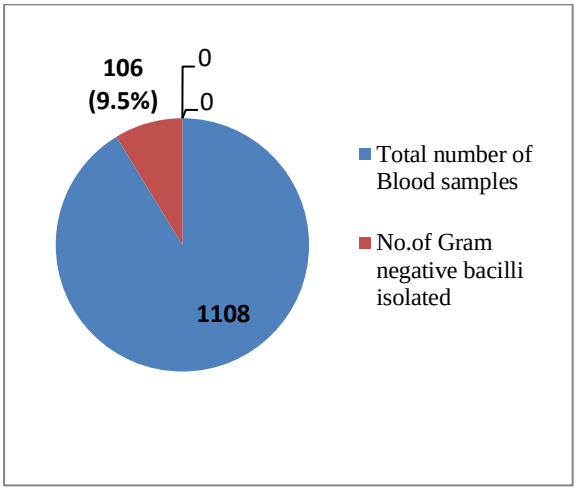


Figure 3: Carbapenem Resistant among Gram negative bacilli

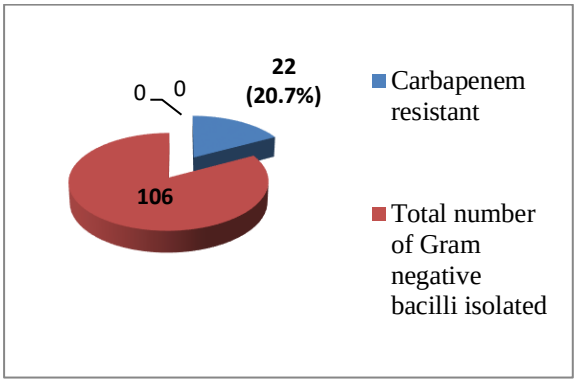


Figure 4: Colistin Resistant among Carbapenem Resistant Strains

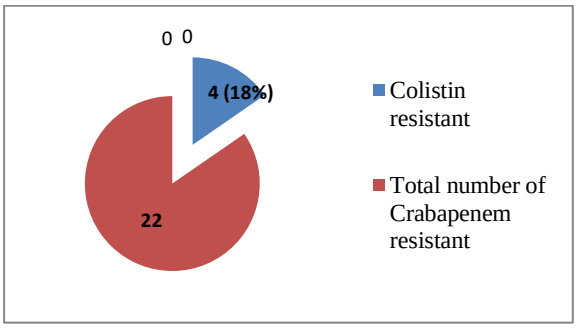
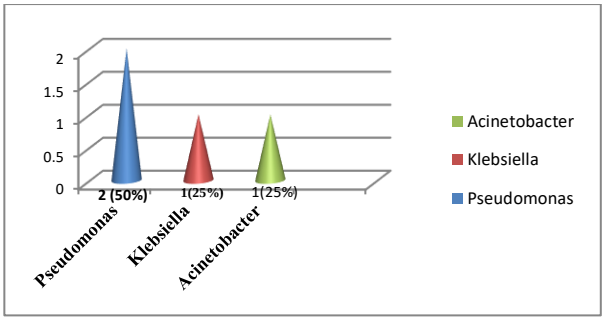


Figure 5: Colistin resistant Gram negative bacilli



DISCUSSION:

It was identified from our study that the predominant age group of patients in whom Colistin-resistant strains were identified is from 31-60 years and this age-range also showed expressive resistance to Carbapenems whereas in a study conducted by Silvana Oliveria dos Santos et al the predominant age group of patients in whom Colistin-resistant strains were identified was 41-69 years old and this age-range also showed expressive resistance to Carbapenems.[13]Our study shows all carbapenem resistant Gram negative bacilli were also resistant to other antibiotics such as Amikacin, Amoxicillin / Clavulanic acid, Co-trimoxazole, Ampicillin, Cefotaxime, Ceftazidime, Cefepime, Gentamicin where as in Mai M. Zafer et al showed that more than half of Colistin resistance strains in multidrug-resistant (Penicillin, Cephalosporin, Carbapenem, Aminoglycoside, Cotrimoxazole, and Fluoroquinolones) Gram negative bacilli isolates were obtained from blood stream infections.[14]

It was found in our study that Colistin resistant was present in 4 (3.7%) isolates out of 22 Carbapenem resistance Gram negative strains. In the study of Olaitan AO et al Colistin-resistant gram negative strains was reported in the frequencies of 5.8% and 6.6% [15] Currently most of the Gram negative bacilli are becoming resistant to Colistin by various

mechanisms. One of them is modifications of lipid A by addition of 4-amino-4-deoxy-L-arabinose (L-Ara4N) and/or Phosphoethanolamine (PEtn) which reduces the net lipopolysaccharide negative charge (affinity), thereby increasing resistance to Polymyxins.

May be patients in our study group have been already treated with Colistin and that could be the reason for Colistin resistance. The Colistin resistant gram negative bacteria are found to have already obtained resistance to Carbapenemsbecause of that we have chosen Polymyxins as last resort. But unfortunately, among these strains there is increasing trend of Colistin resistance that further complicates the issue. Our study aims to create awareness to the clinicians in our hospital to avoid the usage of higher antibiotics, which is different from other study. And Colistin is the last drug to be used in the antibiotic pipeline and the clinicians should have a limitation for the usage of Colistin drug to avoid resistance and to follow the Antibiotic stewardship program.

CONCLUSION:

The emergence of drug resistance in microorganisms is a serious problem and several strategies have been proposed to tackle it. Prevention of drug resistance in microorganisms should be the ultimate solution and vaccines also suggested as a strategy that can be used to slow down the emergence of drug resistance by decreasing the infection rate and hence antibiotic usage[16]. But unfortunately, we are far from this ideal. Many broad surveillance programs[17]and education of pharmacists, clinicians, veterinarians and the public regarding the wide spread of antimicrobial resistance and the outcome of antibiotic misuse should have a significant impact[18]. We should restrict the use of higher antibiotics, especially Cephalosporins and to follow the therapeutic guidelines.

At risk, people should be given more care to prevent Colistin resistance such as advanced age, underlying diseases and severity of illness, inter-institutional transfer of the patient, especially prolonged hospital stay, gastrointestinal surgery or organ transplantation, exposure to invasive devices like central venous catheters and others, long term exposure to antibiotics especially Cephalosporins.

Combined use of the Colistin and Carbapenem may provide good therapeutic options for infection caused by carbapenem-resistant Gram Negative Bacilli and warrants further investigations.

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