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Original Research Article

Characterization of antimicrobial resistance mechanisms of multidrug resistant Gram negative bacterial wound infections and their clinical epidemiology from a tertiary care hospital in Karnataka, India

Anusha Gopinathan[1]*, Chiranjay Mukhopadhyay[2], Vandana K. E.[2]

[1]Department of Microbiology, Amrita Institute of Medical Sciences, Amrita University, Kochi, Kerala, India
[2]Department of Microbiology, Kasturba Medical College, Manipal, Karnataka, India

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***Correspondence:**
Dr. Anusha Gopinathan,
E-mail: anusha.gopinathan@gmail.com

ABSTRACT

Background: Extended spectrum beta lactamases, AmpC and Metallo-betalactamases in GNB isolates are a common occurrence in most Indian hospitals. The presence of these antimicrobial resistance mechanisms contributes to prolonged hospital stay, poor quality of life, increased morbidity and mortality among patients with these infections. The aim of the study was to analyse the antimicrobial resistance mechanisms of multidrug resistant Gram negative bacterial wound infection and their clinical epidemiology.
Methods: A prospective study was conducted for one year among 100 patients of Kasturba Medical College, Manipal admitted with MDR GNB wound infections. The antibiogram and phenotypic resistance mechanisms of the bacterial isolate from these infections were identified using phenyl boronic acid and ethyl diacetate. The empirical therapy, specific therapy and clinical outcome of the patients were also analyzed.
Results: Out of 100 study patients, 152 MDR GNB isolates were obtained. 73% patients were admitted in the surgical wards. 43% patients had diabetes. Ulcers (27%) and abscess (25%) were the most common diagnosis. *Escherichia coli* (39%), *Klebsiella pneumoniae* (24%) and *Pseudomonas aeruginosa* (19%) were the most common isolates. Maximum number of ESBL was seen among Enteric Gram negative bacilli (36%), MBL was seen among *Pseudomonas aeruginosa* and *Acinetobacter species* (55% each), AmpC was seen among enteric GNB (10%) and Acinetobacter species (18%). Cefaperazone sulbactam, amikacin and meropenem were the most common antibiotics given as specific therapy. Clinical response was observed among 93% patients.
Conclusions: The determination of the antimicrobial resistance mechanisms of GNB isolates from wound infections plays a major role in establishing an antibiotic policy for the treatment of these infections.

Keywords: AmpC, ESBL, MBL, MDR GNB

INTRODUCTION

Resistance among Gram negative bacteria (GNB) has become a societal issue. It affects the lives and livelihoods of patients and threatens to endanger health delivery programmes. Much attention and emphasis was given in the past on the risk posed by Gram positive bacterial infections such as MRSA (methicillin resistant *Staphylococcus*) and VRSA (Vancomycin resistant *Staphylococcus aureus*) but the limelight has now shifted upon GNB with the rise in resistance, especially multidrug and colistin resistance among these organisms. Multidrug resistant GNB are resistant to three or more classes of antibiotics. It is a common occurrence in most hospitals across India. A study from North India quotes 25% MDR GNB among isolates obtained from neonates

with septicaemia. 37% MDR strains were reported from another study performed at Wardha, North India.[1,2] 74% ESBL (Extended spectrum betalactamase producer) were seen among Gram negative bacilli isolated from skin and soft tissue infections in a study from South India.[3] The grave prognosis caused by MDR GNB infections on immunocompromised individuals such as patients with prolonged neutropenia and chemotherapy mucositis is a well-known fact.[4] The present study was conducted to identify MDR GNB from skin and soft tissue infections at Kasturba Medical College, Manipal, Karnataka, India over a period of one year and to analyse these isolates for the production of MBL, ESBL and AmpC enzymes. The present study evaluates the bacterial aetiology, antibiogram of wound infections with special reference to phenotypic characterization of ESBL, AmpC and MBL resistance mechanisms.

METHODS

This study was conducted between 2011-2012 at Kasturba Medical College, Manipal, Karnataka, India. 100 patients with multi drug resistant Gram negative bacterial skin and soft tissue infections were randomly selected from surgical, dermatological, medicine and burns wards and intensive care units. Swabs, tissue biopsy, curettage, pus were collected from the patients according to the nature of the skin and soft tissue infection. Samples were not refrigerated before or during transport. The bacterial aetiology of the skin and soft tissue infections were identified using Gram stain, bacterial culture of the sample onto 5% sheep blood agar and MacConkey agar plates and using a battery of biochemical tests. Antimicrobial susceptibility testing was performed using Kirby Bauer disc diffusion method on Mueller Hinton agar plates according to CLSI 2011 guidelines.[5]

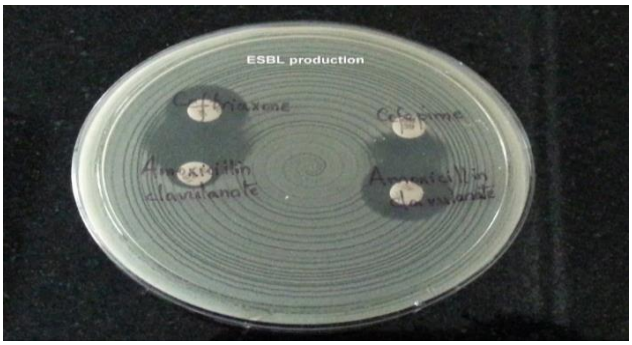
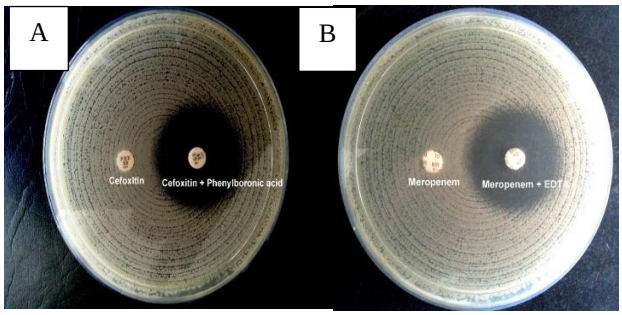


Figure 1: Kirby Bauer disc diffusion method of antimicrobial susceptibility testing of *Escherichia coli* showing ESBL production.

Gram negative bacterial isolates which were resistant to three or more classes of antimicrobial agents were classified as Multi drug resistant bacteria. Double disc approximation method using amoxicillin clavulanate (20/10µg) and cefepime (30µg); ticarcillin clavulanate (75/10µg) and cefepime (30µg); ticarcillin clavulanate (75/10µg) and aztreonam (30µg) was used to identify ESBL producer (Figure 1).[5] 10µl of 0.5 M EDTA along with meropenem disc (10µg) was used to identify metallobetalactamse production as per Franklin C et al (Figure 2).[6] 20 µl of phenyl boronic acid was used to identify AmpC betalactamase according to Couldron et al.[7]



Interpretation: AmpC betalactamase producing Escherichia coli.
Interpretation: MBL producing Escherichia coli.

Figure 2: Phenotypic method of detection of AmpC betalactamase (A) and MBL (B) in *Escherichia coli* combined disc method.

RESULTS

100 patients with skin and soft tissue infections were admitted in the study. 152 MDR Gram negative isolates were obtained from these patients. The demographic profile of the patients is provided in Table 1.

Table 1: Demographic profile of the study patients.

Demographic profile	Number (%)
Sex	
Male	76 (76)
Female	24 (24)
Age (years)	
18-30	9 (9)
31-45	33 (33)
46-60	24 (24)
>61	34 (34)
Patient Location	
Intensive care unit (ICU)	8 (8)
Medical wards	19 (19)
Surgical wards	73 (73)
Underlying diseases	
Respiratory disease	11 (11)
Renal pathology	6 (6)
Nil	41 (41)
Risk factors	
Diabetes Mellitus	43 (43)
Cancer/ Immunosuppression	3 (3)
HIV	1 (1)
Peripheral vascular disease and varicose veins	2 (2)

Mean age of the patients was 51 years with a standard deviation (SD) of ±15 years. Table 2 shows the different types of skin and soft tissue infections seen in these patients.

Table 2: Types of skin and soft tissue infections among the study patients (N=100).

Type of infection	Number (Percentage)
Abscess	25 (25)
Ulcer	27 (27)
Surgical site infection	20 (20)
Cellulitis	8 (8)
Necrotizing fasciitis	12 (12)
Burns	8 (8)

The mean duration of hospital stay among the study patients were 6 days (abscess), 18 days (surgical site infection), 7 days (ulcer), 12 days (cellulitis), 7 days (necrotizing fasciitis) and 10 days (burns).

The bacteria responsible for these skin and soft tissue infections were *Escherichia coli* (39%), *Klebsiella pneumonia* (24%), *Pseudomonas aeruginosa* (19%), *Acinetobacter spp* (7%), *Enterobacter spp* (5%), *Citrobacter spp* (3%), *Proteus spp* (1%) and Gram negative non-fermenters (1%). Among these polymicrobial infections were seen in 46% study patients were the distribution was as follows *Escherichia coli* and *Klebsiella pneumonia* (54%); *Escherichia coli* and *Pseudomonas aeruginosa* (37%); *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (9%).

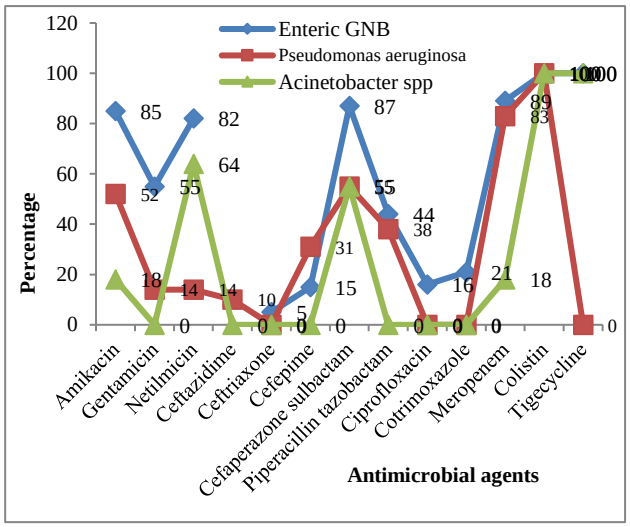


Figure 3: Antimicrobial susceptibility among MDR Gram negative bacilli (N=100).

The antimicrobial susceptibility patterns of the isolates are shown in Figure 1. The antimicrobial resistance mechanisms among the isolates are as shown in Figure 2. Among the 100 study patients, response to therapy was seen in 93 (93%). 6 patients were lost to follow up and one expired.

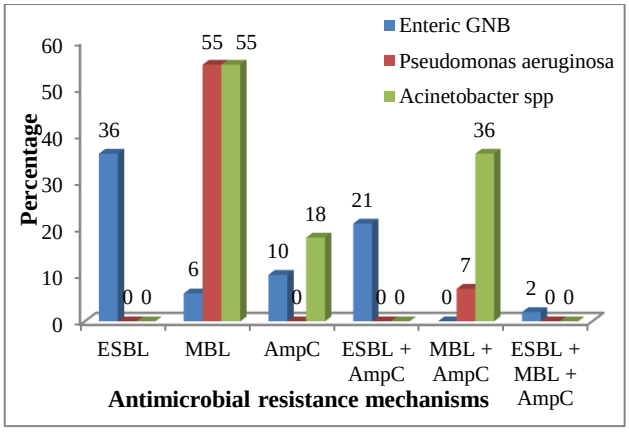


Figure 4: Antimicrobial resistance mechanisms in MDR Gram negative bacilli (N=152).

DISCUSSION

MDR Gram negative infections, one of the most feared infections among doctors across the globe has become rampant in our country. It causes immense human suffering, loss of productivity and in most cases even death. Wound infections namely cellulitis, diabetic foot infections, burns, abscesses, surgical site infections and necrotizing fasciitis increase in incidence proportionately with rise in co-morbid illnesses such as diabetes mellitus, immunosuppression due to HIV (Human immunodeficiency disease), malignancy, chemotherapy and renal failure to name a few.

In the present study, among the study patient's majority were males (76, 76%) and above 61 years of age (34, 34%). This predominance of males in the study is because males in our society seek more medical attention compared to females. A similar case was seen in a study from North India on diabetic foot ulcers.[8] Most study patients were admitted in the surgical wards (73, 73%) as compared to intensive care units (ICU) (8, 8%). Ulcer (27, 27%) and abscess (25, 25%) were the most common presentation in this study which does not require intensive care treatment. This was also seen in a previous study where only 9.1% cases had ICU admission.[9]

The risk factors associated with wound infections are diabetes, chronic disease, immunosuppressive drugs, malnutrition, age more than 60 years, intravenous drug misuse, peripheral vascular disease, renal failure, underlying malignancy and obesity.[10] Diabetes mellitus (43, 43%) was the leading risk factor in this study. A prospective study on soft tissue infections in US reported 7.1 days as the mean hospital stay duration (ranging from 5.8 days in abscess to 8.1 days in surgical site infections).[11] This was found the same in this study.

In this study enteric Gram negative bacilli (63%) was the most common isolate cultured from patients with skin and soft tissue infections followed by *Pseudomonas aeruginosa* (19%) whereas in other studies *Pseudomonas*

aeruginosa was the leading aetiological agent.[12,13] Polymicrobial infections contributed to 46% of wound infections in this study. This is a common occurrence as seen in other studies as diabetic wound infections which are polymicrobial in nature formed the majority of infections in this study.[14,15]

Antimicrobial resistance was significantly high in this study. This is a common scenario seen across hospital around the globe. Indiscriminate use of antibiotics, inappropriate dose and duration of antibiotics, lack of compliance to the hospital antibiotic policy, lack of awareness among the prescribing clinicians and patients about antibiotic resistance, lack of stepping down to lower generation antibiotics once the patient is out of critical care identified as reasons for this high incidence of antibiotic resistance in this study.[16]

An Indian study on soft tissue infections documented 72.37% ESBL poducers among *E. coli*, 68.79% among *Acinetobacter spp* and 58.9% among *Pseudomonas spp*.[17] In this study, ESBL, MBL and AmpC enzymes were produced by majority of the isolates. These enzymes prolong the duration of treatment among patients with these infections. ESBL detection is done in most laboratories across India. But so is not the case with AmpC betalactamase and MBL detection. AmpC and MBL detection are important as the production of these enzymes confer resistance to cephalosporins and betalactam and betalactam inhibitors which are commonly used as first line therapy for the treatment of wound infections. This implies that patients with infections caused by enzyme producing MDR GNB and treated with betalactams will have persistent infections with or without complications.

There was clinical response in 93% patients in this study. Studies done in other countries also paint the same picture.[11,14] This study was conducted in a tertiary health care setup with good nursing care and drug compliance. These may be the reasons for the good outome seen in most of the patients.

CONCLUSION

The alarming rise in MDR GNB infections is a man-made disaster. Poor knowledge of antibiotics, peer pressure, fear of bad outcome in patients and lack of strict legislation against injudious usage of antibiotics are the root cause of this disaster. Identifying the epidemiology of the bacteria resident in the wards and ICU of hospitals is of prime importance in order to tailor empirical therapy so that we may help better the quality of life of the patients.

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