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RESEARCH ARTICLE
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PREVALENCE OF MULTI DRUG RESISTANT GRAM-NEGATIVE BACTERIA AND ITS SUSCEPTIBILITY PATTERN ISOLATED FROM ADMITTED PATIENTS IN A HOSPITAL OF CENTRAL INDIA; AN EMERGING GLOBAL PROBLEM

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Abstract

Introduction: The multi-drug resistant gram-negative bacterial infections are the principle threats to the critically ill patients. Multidrug-resistant Gram-negative rods (MDR-GNR) are emerging as a major challenge to human health. The prevalence of drug-resistant cases is increasing globally. However, the impact of these infections on the patient's clinical outcome has not yet been clearly evaluated.

Aim: To determine the incidence and associated clinical outcome of multi-drug resistant gram-negative bacterial infections.

Materials and Methods: This record-based retrospective cross-sectional study was designed to analyse MDR-GNB positive cases at a tertiary care hospital of central India. MLB Medical college with attached. Hospital is a 700-bed referral tertiary care centre. An increase of MDR-GNB was seen from September 2022 to June 2023 in the hospital. A retrospective analysis of blood culture GNB-positive samples was performed to evaluate MDR-GNB-positive cases at admission.

Results: The total number of positive blood cultures in September 2022 to June 2023, November to January 2023 and February to April 2023 were 236, 186 and 206, respectively, with 76.83%,80.% and 71.83% GNB-positive. Total MDR-GNB-positive cases were 27.08%, 34.98% and 32.65%,

respectively, and amongst these MDR-GNB, 24%, 32% and 5% where positive at time of admission to the hospital. The MDR-GNB were *Escherichia coli*, *Klebsiella*, *Acinetobacter*, *Pseudomonas* and *Enterobacter*, *Klebsiella pneumoniae*, *Acinetobacter* spp., *Pseudomonas aeruginosa* and *Escherichia coli* have alarming degrees of antimicrobial resistance and are associated with high mortality and morbidity.

Conclusion: The incidence of multi-drug resistant gram-negative bacterial infections was remarkably high in our hospitals and showed a significant association with healthcare-associated infections and in-hospital-mortality.

Key words: Multi drug resistant, Pulmonary infiltrate, Excessive use of antibiotics.

Introduction

Multidrug-resistant Gram-negative bacterial infections are emerging as a major challenge to clinicians, especially intensive care units for neonates, infants, children, young adults as well as for adults. Bacteraemia may be associated with high level of morbidity and mortality. Emperical antibiotic therapy without confirming susceptibility pattern may be life threatening, increases the hospital stay , increases morbidity and in many cases it may leads to death.

Its an emerging health issue globally and commonly associated organism are *Klebsiella pneumoniae, Acinetobacter* baumannii, *Pseudomonas aeruginosa* and *Escherichia coli*. The global scenario shows that gram-positive infections are common in the developed countries ICUs [1]. However, multidrug-resistant gram-negative bacteria (MDR-GNB) infections dominate in the Asia-Pacific region [2, 3]. Among MDR-GNB, extended-spectrum beta-lactamases (ESBL) organisms, carbapenemase producing enterobacteriaceae, carbapenem-resistant *Acinetobacter species*, multidrug- resistant *Pseudomonas aeruginosa* are the major culprits. Unfortunately, new antibacterial agents have not been developed in pace with the growth of multidrug-resistant (MDR) organisms [4].

As per the World Health Organization (WHO) fact sheet, infections by antimicrobial-resistant organisms can result in failure of treatment, increased cost of medical treatment, increased hospitalization stay and increased socioeconomic burden [5]. Infections caused by multidrug-resistant Gram-negative bacteria (MDR-GNB) have been reported to have increased significantly worldwide in recent years. The increase in MDR-GNB cases has become a serious challenge for health care professionals. Excessive use of antibiotics including the use without treatment indication is believed to be one of the major factors accelerating the spread of antibiotic resistance. A survey by Van Boeckel et al. on total antibiotic sales from 2000 to 2010 in 71 countries indicated that India is the country with the highest consumption of antibiotics (approx. 13 billion standard units, i.e. pill/capsule/or ampoule), followed by China (approx. 10 billion standard units) and the United States (more than 6 billion standard units). Consumption of antibiotics increased by 36% in this time period. Brazil, Russia, India, China and South Africa accounted for 76% of this increase [6,7].

Material and methods

This study was conducted at Government Prakash Chandra Sethi Hospital, Indore it is multispecialty, referral secondary care centre. It was observed that the MDR-GNB cases were rising from September 2022 to June 2023 in the hospital. However, there was no outbreak. The study was designed to analyze the prevalence and epidemiology of increase in MDR-GNB cases. Cases resistant to carbapenem, third and fourth generation cephalosporins were considered as MDR-GNB cases.

Study design

A retrospective, cross-sectional study design was used for the research. A retrospective analysis of data of all hospitalized patients from September 2022 to June 2023 was done for presence of MDR-GNB-positive blood culture samples seen at the time of admission to hospital. As a part of routine

practice, blood culture was drawn within 24 h for all the patients admitted with sepsis. Routine conventional Method of blood culture was used for antibiotic susceptibility and bacterial identification.

Data review procedure

Records of hospitalized patients from September 2022 to June 2023 were reviewed stepwise with the focus on results of blood culture analysis. Blood culture data showing positive MDR-GNB was separated and analyzed further for the type of GNB.

Statistical analysis

Data were entered in the MS Excel 2007 and analyzed with STATA version 14.

MICROBIOLOGICAL PROCEDURES

Pathogenic bacteria isolated from the clinical specimens from the various wards and ICU,NICU,SNCU,PICU etc. were further characterized by conventional biochemical tests to identify the specific GNB by using standard microbiologic methods [08]. Antibiotic susceptibility test of GNB strains was done by the Kirby Bauer disc diffusion method on Mueller Hinton agar (MHA) as per the Clinical Laboratory Standard Institute (CLSI) guidelines [09]. Antibiotics of following concentrations were used: ampicillin (10µg), amikacin (30µg), gentamycin (10µg), tobramycin(10µg), ciprofloxacin (5µg), levofloxacin (5µg), chloramphenicol (30µg), co-trimoxazole (25µg), ceftazidime (30µg), cefotaxime (30µg), cefepime (30µg), piperacillin (100µg), carbenicillin (100µg.), piperacillin-tazobactam (100/10µg), imipenem (10µg), tigecycline (30µg), polymyxin B (300unit), and colistin sulphate (10µg) from Hi Media Laboratories, India. Disk zone diameters were interpreted according to the CLSI 2017 recommendations. Quality control for culture plates and antibiotic susceptibility was performed using *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. All the strains were subjected to various phenotypic methods for the screening and confirmation of the beta lactamases. Strains showing decreased sensitivity to ceftazidime/ cefotaxime were considered as screen positive for ESBL production and were subjected to the following confirmatory phenotypic tests as per the CLSI guidelines [10].

- ESBL- A difference in the zone size of 5 mm between ceftazidime and ceftazidime+ clavulanic acid and cefotaxime and cefotaxime+clavulanic acid discs was considered as confirmed ESBL producer [10].
- Carbapenemase- The screen positive for carbapenemase production was considered for strains showing resistance to carbapenems. A positive modified hodge test (MHT) with appearance of clover leaf at the streaking line was considered as carbapenemase producer as per the CLSI guidelines .A difference in the zone size of 7 mm between Imipenem and Imienem+ EDTA disc in the EDTA disk synergy test was considered as MBL producer [10].

RESULT

A total 710 patient's samples were analyzed which included blood 174 ,body fluids 64 ,urine 232 pus 108 ,sputum 35,and Other samples(Vaginal Swab ,Catheter tip, Peritoneal Fluid, ET Suction Tip) 97 (Table 1). Total 362 samples were positive for growth of the MDR organisms. Mostly MDR organism isolated from urine samples around 89,followed by blood samples, around 58 isolates from pus and around 45 from different body fluids i.e. Ascitic fluid, synovial fluid, pleural fluid from patients admitted in intensive care units.

On culturing of 97 various catheters like ET Tube, Umbilical catheter tip, Foley's catheter & samples of Vaginal swab around 72 isolates were obtained.

Table.1 Sample profile from which MDR organisms isolated

S. No.	Sample	Isolates	MDR Isolates
1.	Blood	**174**	**72**

2.	Urine	**232**	**89**
3.	Pus	**108**	**58**
4.	Body fluids	**64**	**45**
5.	Sputum	**35**	**26**
6.	Other samples(Vaginal Swab ,Catheter tip, Peritoneal Fluid, ET Suction Tip)	**97**	**72**

From above given 710 isolates around 362 (52%) are Multi drug resistance organisms were isolated, out of which Escherichia coli was most pravelent organism followed by Citrobacter *spp*. and Klebsiella *spp.*

Among non-fermenters Pseudomonas *aeruginosa* is most prevalent followed by Acinetobacter *spp.* Around 38.8% of Pseudomonas are multidrug resistant shown in Table-2.

Among antibiotics ampicillin shows highest number of resistance pattern i.e. in E.coli. followed by Klebsiella.

Table 2: Pattern of isolates Organisms Isolates

S. No.	Organism	Isolates	MDR Isolates	Percentage
1.	Escherichia coli	**256**	**159**	**62.10 %**
2.	Klebsiella spp	**124**	**52**	**41 %**
3.	Citrobacter spp	**180**	**98**	**54.4 %**
4.	Enterobacter spp	**09**	**02**	**2.2 %**
5.	Proteus spp.	**13**	**03**	**2.3 %**
6.	Pseudomonas spp.	**108**	**42**	**38.8 %**
7.	Other Non-fermenting GNB	**20**	**06**	**3 %**

Table 3: Number of gram negative bacteria resistant to given antimicrobial agent.

Antibiotic	E.coli	Klebsiella	Citrobacter	Proteus	Pseudomonas
Amikacin	**115(72%)**	**39(75%)**	**77(78%)**	**2(66%)**	**33(78%)**
Ampicillin	**146(91%)**	**44(84%)**	**67(68%)**	**3(100%)**	**17(40%)**
Ciprofloxacin	**76(47%)**	**24(46%)**	**52(53%)**	**1(33%)**	**27(64%)**
Chloramphenicol	**55(34%)**	**34(65%)**	**82(83%)**	**2(66%)**	**18(42%)**
Carbenicillin	**58(36%)**	**22(42%)**	**24(25%)**	**2(66%)**	**08(19%)**
Cefepime	**82(51%)**	**42(80%)**	**46(47%)**	**2(66%)**	**32(76%)**
Cefotaxime	**75(47%)**	**32(61%)**	**72(73%)**	**1(33%)**	**26(61%)**
Co-Trimoxazole	**37(23%)**	**42(80%)**	**52(53%)**	**1(33%)**	**22(52%)**
Ceftazidime	**67(42%)**	**25(46%)**	**84(85%)**	**1(33%)**	**29(69%)**
Colistin Sulphate	**39(24%)**	**22(42%)**	**39(40%)**	**1(33%)**	**07(16%)**
Gentamicin	**42(26%)**	**34(65%)**	**46(47%)**	**1(33%)**	**22(52%)**
Imipenem	**36(22)**	**12(23%)**	**26(27%)**	**1(33%)**	**08(19%)**
Levofloxacin	**58(36)**	**43(81%)**	**51(52%)**	**1(33%)**	**27(64%)**
Tobramycin	**23(14%)**	**18(34%)**	**26(27%)**	**1(33%)**	**10(27%)**
Tigecycline	**12(7.5%)**	**22(42%)**	**23(24%)**	**00**	**08(19%)**
Piperacillin-Tazobactam	**82(51%)**	**42(80%)**	**72(73%)**	**2(66%)**	**27(64%)**
Polymyxin B	**42(26%)**	**28(53%)**	**32(33%)**	**1(33%)**	**32(76%)**

Discussion

Antimicrobial resistance is increasing worldwide, threatening global public health and the effective prevention and treatment of infections [8]. The U.S. National Healthcare Safety Network reported

increasing occurrence of MDR-GNB (*E. coli, K. pneumoniae, Enterobacter* spp., etc.) amongst which more than 60% were *Acinetobacter* spp. Similarly, in Europe the European Antimicrobial Resistance Surveillance Network reported recognizable resistance trends for Gram-negative bacteria, with highest levels of resistance reported for *Acinetobacter* spp., followed by *E. coli* and *K. pneumoniae* [2]. Reports from various studies from hospitals in India suggest that the prevalence of ESBL-producing GNB range between 19% and 60%, and that of carbapenem-resistant GNB between 5.3% and 59% [6]. In Mumbai, west India, the prevalence of drug-resistant Enterobacteriaceae was about 18.5% [9]. The prevalence of ESBL- and carbapenemase-producers in Kolkata was estimated to be 70% and 39%, respectively [10]. In south India, the occurrence of drug-resistant GNB was 53% of isolates from patients with community-acquired bacteraemia caused by *E. coli* and *Klebsiella* spp. [11]. This indicates the increased burden of MDR-GNB globally including India. In the present study, it was revealed that the numbers of blood culture-positive cases in hospital were increasing consecutively in the years 2012–2014, amongst which the GNB-positive cases were more than 70%, showing a similar trend of increase in GNB-positive cases as reported globally. *Acinetobacter*, *Klebsiella*, *E. coli* and *Enterobacter* are the most common MDR-GNB isolated from neonatal septicaemia at tertiary hospitals [12]. Similarly, *E. coli* and *Klebsiella* are the most predominant organisms causing urinary tract infection (UTI) in children. There are several studies showing *E. coli* as the significant pathogen causing UTI [13], [14]. Fifty-three percent of Gram-negative organisms isolated from children were found to be multidrug-resistant [15]. Mandell et al. reported that Gram-negative bacteria were the predominant cause of UTI when compared with Gram-positive bacteria [16]. Our present study also supported the findings by various studies that *E. coli, Klebsiella, Acinetobacter* and *Pseudomonas/Enterobacter* were the GNB which were the most prominent cause of infections in hospitalized patients; however, there was a greater incidence of *Acinetobacter* and *Klebsiella* MDR-GNB amongst all other GNB. *Pseudomonas* spp. and *Acinetobacter* spp. are the most common organisms isolated from ICU in south India and Delhi [17]. Javeri et al. also reported *Acinetobacter* as the second most common isolate in ICU of tertiary care centres in Ahmadabad [18] indicating the wide spread of MDR-GNB throughout India, supporting our findings. Overall, MDR-GNB cases increased during the period of 2021–2022. The number of MDR-GNB *Klebsiella* and *Acinetobacter* increased in consecutive years over the study period, indicating the need for analysis of MDR-GNB cases at primary and secondary care units to prevent the further spread of resistance.

Conclusion

MDR-GNB blood cultures positive at admission increased from July 2021 to June 2023 and hence there is an urgent need for possible contact isolation of all patients coming from primary and secondary to tertiary health care centres to be made compulsory until screening to rule out MDR-GNB has been performed, to prevent spread of MDR organisms in the hospital. The study findings will be part of a strict Antibiotic Stewardship (AMS) programme and also indicate that AMS should begin at primary and secondary health care centres to prevent antimicrobial resistance.

Funding

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Conflict of interest

The authors declare no conflict of interest.

Ethical approval

Not required

Limitations

Patients who were in Incubation Period of nosocomial infections on discharge, who manifests it after discharge, were not covered in current study. Contribution of their load to current study prevalence is unknown.

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