



MULTI-DRUG RESISTANT GRAM-NEGATIVE BACILLI IMPLICATED IN LOWER RESPIRATORY TRACT INFECTIONS AT A TERTIARY CARE HOSPITAL IN BIHAR, INDIA

Saroj Kumari	Tutor, Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga Bihar
Md Shabbir Azad*	Tutor, Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga Bihar *Corresponding Author
Ramshanker Prasad	Associate Professor and Head of Department, Department of Microbiology, Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga Bihar

ABSTRACT **Background:** Lower respiratory tract infections (LRTI) are leading cause of illness and death in children and adults across the world and the emergence of multidrug resistant gram-negative organism (MDRO) as causative organism is an issue of increasing concern. **Aims & Objectives:** This study was focused on obtaining a comprehensive insight into prevalence of multi drug resistance organisms (MDROs) among LRTI causing gram negative bacilli. **Materials and Methods:** A total of 1144 respiratory samples (sputum, broncho-alveolar lavage fluid and endotracheal aspirate) were processed for microscopy, culture and susceptibility testing following standard laboratory protocols. Multidrug resistant gram-negative bacilli causing lower respiratory tract infections were studied for their causation of disease. **Results:** A total of 349 gram-negative pathogens were isolated from respiratory samples during the study period. Among these 213 (61%) gram-negative pathogens were found to be multidrug resistant. Although the percentage of multi drug resistance was higher among *Escherichia coli* (88% MDRO) and *Acinetobacter baumannii* (80% MDRO), the predominant multidrug resistant gram-negative bacilli isolated were *Klebsiella pneumoniae* 119 (34%) and *Pseudomonas aeruginosa* 108 (30%). Among MDROs, other isolates were *Citrobacter freundii*, *Enterobacter cloacae*, *Proteus mirabilis* and *Providentia* spp. **Conclusion:** A large majority of pathogenic gram-negative bacilli isolated were found to be the multidrug resistant. Regular antibiotic resistance surveillance are indispensable in the effective management of lower respiratory tract infections and can reduce morbidity and mortality associated with MDROs.

KEYWORDS : Multidrug resistance gram-negative bacilli, lower respiratory tract infection, tertiary care hospital

INTRODUCTION

Respiratory tract infections are some of the most common causes of human illness and the leading cause of death from infectious diseases worldwide.¹ Lower respiratory tract infection (LRTI) is a broad description of a group of disease entities, encompassing acute bronchitis, pneumonia and exacerbations of chronic lung disease. Lower respiratory tract infections are acute illness (present for 21 days or less), usually with cough as the main symptom, accompanied by other lower respiratory tract symptom (sputum production, dyspnoea, wheeze or chest discomfort/pain) and no alternative explanation (e.g. sinusitis or asthma).²

Community-acquired pneumonia (CAP), nosocomial pneumonia and acute and chronic bronchial infections in patients with chronic obstructive pulmonary disease (COPD) and bronchiectasis are among the common respiratory infections. A large proportion of gram-negative bacteria (GNB) are the causative pathogens of these lower respiratory tract infections. Several studies have reported an increased rate of GNB as causal agents in recent years, especially in hospital-acquired infections and also in patients coming from the community.³

The main problem concerning the treatment of gram-negative bacterial infections is their related antibiotic resistance, reported as multidrug resistant (MDR), extensively drug resistant (XDR) and pan-drug resistant (PDR). Different definitions for MDR bacteria have being used in the literature. The European Centre for Disease Prevention and Control (ECDC) and the Centres for Disease Control and Prevention (CDC) joined in 2011 to standardize the terminology used to describe acquired resistant profiles in *Staphylococcus aureus*, *Enterococcus* spp., *Enterobacteriaceae* (other than *Salmonella* and *Shigella*), *P. aeruginosa* and *Acinetobacter* spp., all related to healthcare-associated infections. In the definitions proposed, non-susceptibility refers to either, resistant or intermediate result from in vitro antimicrobial susceptibility test. MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories. In addition, extensively drug resistant (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories and pan-drug resistance (PDR) as non-susceptibility to all agents in all antimicrobial categories.⁴

The most frequent MDR or potentially drug-resistant GNB isolated in respiratory infections are *Pseudomonas aeruginosa*, *Klebsiella*

pneumoniae, *Escherichia coli*, *Acinetobacter baumannii*, *Stenotrophomonas maltophilia* and other *Enterobacteriaceae* spp.⁵

P. aeruginosa is a ubiquitous microorganism that is the leading cause of opportunistic human infections.⁶ Treatment failure due to the development of resistance is indeed a frequent outcome in patients suffering from *P. aeruginosa* infections. Due to its ability to induce changes in genes, it generates mutants that are resistant to clinical concentrations of all antimicrobial agents used for therapy.⁷ Another important feature is its capacity to produce biofilms to evade antibiotic effect.⁸

P. aeruginosa is responsible for severe nosocomial infections, especially in the intensive care units (ICU).^{9,10} However, it has also been described in patients with pneumonia coming from the community¹¹ and it is well recognized as a major cause of chronic respiratory infections in patients with bronchiectasis and COPD.^{12,13}

Escherichia coli, *Klebsiella pneumoniae* and other *Enterobacteriaceae* spp. are responsible for different respiratory infections acquired in the hospital setting, although some studies have also reported the presence of these GNB in non-nosocomial infections.³ They have the property of acquiring resistance to different antibiotic resulting in treatment failures. They are also capable to produce extended-spectrum β -lactamases (ESBL), including carbapenemases that can hydrolyse most β -lactams, including the carbapenems.^{14,15}

Acinetobacter baumannii is an opportunistic pathogen that is frequently involved in outbreaks of infection, occurring mostly in the ICU. *A. baumannii* have been described as a cause of respiratory infections following hospitalization in patients with severe illness. MDR isolates of *A. baumannii* have been reported increasingly during the last years, especially against carbapenems, and have been related to poor clinical outcomes.¹⁶

The emergence of GNB antibiotic resistance and the lack of novel antibiotics in the development pipeline represent one of the biggest respiratory threats in the recent years. Hence this study was planned to identify MDR gram-negative bacilli causing LRTIs, to correlated their isolation with the causation of disease.

MATERIALS & METHODS

This prospective study was conducted for a period of one year from July 2022 to June 2023, at Darbhanga Medical College and Hospital, Laheriasarai, Darbhanga Bihar, a tertiary care hospital. A total of 1144 respiratory samples (sputum, broncho-alveolar lavage fluid and endotracheal aspirate) from both out-patient and in-patient suspected of LRTI were processed for microscopy, culture and susceptibility testing following standard laboratory protocols.

The quality of specimens was evaluated based on Gram stain findings, followed by culture and susceptibility testing. All sputum gram stains were read under oil immersion objective (x100) and evaluated according to the Bartlett criteria. Specimens were scored 0, +1, or +2 according to the number of leukocytes seen per field and 0, -1, and -2 according to the number of squamous epithelial cells seen per field. Specimens with total scores of zero or less were considered inadequate and heavily contaminated with oropharyngeal flora. Those containing greater than 25 leucocytes and fewer than 10 squamous epithelial cells per field were optimal specimens and further processed.¹⁷

Samples were subjected to gram's staining and then sub-cultured onto 5% sheep blood agar, chocolate agar and MacConkey agar plates and incubated. Biochemical tests were performed on culture isolates following standard laboratory protocols. Gram negative bacilli were identified by performing various biochemical tests.¹⁸

Antibiotic sensitivity testing was performed on Mueller Hinton agar (MHA) plates by modified Kirby Bauer disk diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines 2021.¹⁹

For quality control, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *K. pneumoniae* ATCC 700603 were used.

MDR gram-negative bacilli were defined as isolates showing resistance to at least 3 different antimicrobial groups.⁴ Clinically isolates were considered pathogenic if there was presence of fever (temperature >38°C), raised leucocyte count ($>12.0 \times 10^9$ cells/L), presence of purulent sputum, positive chest auscultatory findings and radiological findings of chest infection.

Statistical Analysis:

Data analysis was done using Statistical Package for Social Sciences (SPSS) software version 25.0. The level of significance for tests was set at $P < 0.05$. Microsoft excel were used for totaling, percentage and frequency.

RESULTS

A total of 349 gram-negative pathogens were isolated from 1144 respiratory samples [sputum (n= 932), broncho-alveolar lavage fluid (n= 119) and endotracheal aspirates (n= 93)]. 233 GNB pathogens were isolated from sputum samples, 44 GNB pathogens from broncho-alveolar lavage (BAL) fluid and 72 that from endotracheal aspirates (ETA). Mixed GNB pathogens were isolated predominantly from ETA samples from the patients on ventilatory life support in ICU. *Klebsiella* spp. 119 (34%) was the most common gram-negative bacilli (GNB) followed by *Pseudomonas* spp. 108 (31%), *Acinetobacter* spp. 51 (14.6%) and *Escherichia coli* 51 (14.6%). Other GNB isolates were *Citrobacter* spp. 9 (2.6%), *Enterobacter* spp. 6 (1.7%) and *Proteus* spp. 4 (1.14). *Providentia* spp. isolated was only one in number.

Among these gram-negative pathogens, 213 (61%) GNB were found to be multidrug resistant. [Figure 1]

Escherichia coli 45 (88%) and *Acinetobacter* spp. 41 (80.4%) were the predominant MDROs. [Figure 2]

Other MDR gram-negative bacilli isolates were *Klebsiella* spp. 80 (67%), *Pseudomonas* spp. 35 (32.4%), *Citrobacter* spp. 6 (66.7%), *Proteus* spp. 3 (75%), *Enterobacter* spp. 2 (33.3%) and *Providentia* spp. 1 (100%). [Table 1]

DISCUSSION

The World Health Organization has identified antimicrobial resistance as one of the greatest threats to human health. Two recent reports—one by the Infectious Diseases Society of America (IDSA) and another by the European Centre for Disease Prevention and Control and the European Medicines Agency—demonstrated that there are few candidate drugs in the pipeline that offer benefits over existing drugs and few drugs moving forward that will treat infections due to the so-

called “ESKAPE” pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which currently cause the majority of US hospital infections and effectively “escape” the effects of approved antibacterial drugs.²⁰

Resistance to the current library of antibacterial drugs is a serious problem in all parts of the world including the Asia-Pacific region, Latin America, Europe, and North America.²⁰

In our study, *Klebsiella* spp. followed by *Pseudomonas* spp. were the most common Gram-negative bacilli isolated in cultures from LRTIs. This is in agreement with the study conducted by Lin et al.²¹ and Gonlugur et al.²² Interestingly, in Asian countries *K. pneumoniae* is described as a frequent pathogen causing bacteremia.²¹ In the present study, a significant number (61%) of gram-negative bacilli were found to be MDR with *Escherichia coli* 45/51 (88%), *Acinetobacter* spp. 41/51 (80.4%) and *Klebsiella* spp. 80/119 (67%) being the most common MDR Gram-negative bacilli isolated from LRTIs. This finding is concordant to study conducted by Gagneja et al., they found very high rate of resistance (60-100%) among *A. baumannii* and *K. pneumoniae* isolates.²³ Although *Pseudomonas* spp. was the second most common GNB isolate in our study, the percentage of MDROs 35/108 (32.4%) was relatively less among them. In contrast to the present study, Goel et al.²⁴ found high rates of multidrug resistance among *P. aeruginosa*. This is probably because their study was based on infections in intensive care unit, whereas the present study included both community acquired and nosocomial infections.

The MDR gram-negative bacilli were resistant to most of the so called commonly used antibiotics. Gram negative bacilli showed highest resistance to ampicillin, cephalosporins (cefotaxime, ceftazidime), fluoroquinolones (levofloxacin, ciprofloxacin), amoxicillin-clavulanate and piperacillin-tazobactam. Less resistance was evident to carbapenems (imipenem and meropenem) and tigecyclin. All the GNB isolates were 100% sensitive to colistin. *Pseudomonas* spp. showed a good antibiotic sensitivity pattern among MDR gram-negative bacilli. Most of the MDR *Pseudomonas* spp. were resistant to anti-pseudomonal β -lactam antibiotics.

Further study is required to determine the prevalence and antibiotic susceptibility profile of nosocomial and community acquired MDR GNB pathogens.

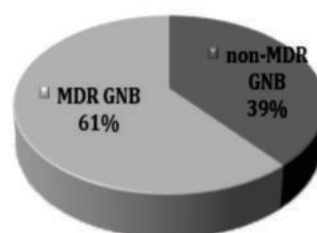
CONCLUSIONS

Correct identification of LRTI patients suspected of being infected with MDR Gram-negative pathogens is crucial. The collaboration of a multidisciplinary team (critical care specialists, pneumologist, infectious disease specialists, microbiologists) is needed to improve the management of the severe cases. The role of the microbiologist, in particular, is of pivotal importance to determine the antimicrobial susceptibility pattern of the pathogen causing LRTI, so that appropriate antibiotic therapy can be initiated as soon as possible. This would avoid excessive use of broad spectrum antimicrobials, which in turn increases the selection of resistant pathogens in-vivo. Antimicrobial stewardship programmes should be instituted in all care settings, based on resistance rates and audit of compliance with guidelines, but should be augmented by improved surveillance of outcome in Gram-negative bacteraemia, and feedback to prescribers.

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Figure 1. Percentage of MDROs among GNB isolates



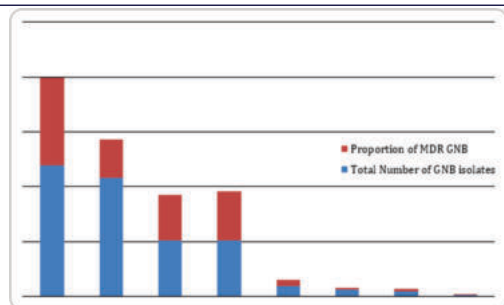


Figure 2. Proportion of MDR among GNB pathogens

Table 1. Percentage of the relative frequencies of various multidrug resistant GNB

Gram-negative bacilli	Total No. (%)	MDR GNB No. (%)
Klebsiella pneumoniae	119 (34%)	80 (67%)
Pseudomonas aeruginosa	108 (31%)	35 (32.4%)
Acinetobacter baumannii	51 (14.6%)	41 (80.4%)
Escherichia coli	51 (14.6%)	45 (88%)
Citrobacter freundii	09 (2.6%)	06 (66.7%)
Enterobacter cloacae	06 (1.7%)	02 (33.3%)
Proteus mirabilis	04 (1.14%)	03 (75%)
Providentia spp.	01 (0.29%)	01 (100%)
TOTAL	349	213

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