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Multidrug resistant Gram-negative bacilli in lower respiratory tract infections

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ABSTRACT

Background: Lower respiratory tract infections are among important causes of morbidity and mortality for all age groups. The emergence of multidrug resistant Gram-negative bacilli is an issue of increasing concern.

Materials and Methods: A retrospective study including respiratory specimens (sputum and BAL) was conducted in our tertiary care centre. Samples were processed for microscopy, culture and susceptibility testing following standard methods. Multidrug resistant Gram-negative bacilli causing lower respiratory tract infections were studied for their causation of disease. The effect of appropriate treatment on clinical outcome was observed.

Results: A total of 472 Gram-negative pathogens were isolated from sputum and broncho-alveolar lavage fluid specimens during the study period. Among these Gram-negative pathogens 175 (37%) were found to be multidrug resistant. *Klebsiella pneumoniae* 85 (48.6%) and *Acinetobacter spp.* 59 (33.7%) were the predominant multidrug resistant Gram-negative bacilli isolated. Based on clinico-microbiological correlation, 138 (78.9%) multidrug resistant isolates were found to be pathogenic and the rest 37 (21.1%) were considered as colonizers. After initiating appropriate antibiotic therapy, clinical improvement was seen in 110 (79.7%) patients. In the patients who showed improvement, amikacin (34.3%) and cefoperazone-sulbactum (21.8%) were found to be the most effective drugs.

Conclusion: A large majority of the isolated multidrug resistant Gram-negative bacilli were found to be pathogenic. Regular surveillance which directs appropriate empirical therapy; and good clinic-microbiological workup of each case of lower respiratory tract infection can reduce the morbidity and mortality associated with multidrug resistant organisms.

Keywords: *Acinetobacter spp.*, Gram-negative bacilli, *Klebsiella pneumoniae*, Lower respiratory infection, Multidrug resistance

INTRODUCTION

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 (1). LRTIs are among the most common infectious diseases affecting humans' worldwide (2) and are important causes of morbidity and mortality for all age groups (3).

The etiological diagnosis of LRTI is frequently confounded by the presence of commensal flora, as well as that of potentially pathogenic organisms in the oropharynx. Today, the increasing trend of antibiotic resistance in respiratory bacterial pathogens poses a challenge to their empiric treatment with conventional agents. Infections with multidrug resistant (MDR) Gram-negative bacilli lead to worse outcomes, including longer hospital stays, increased mortality, and greater costs of hospitalization (4).

Inappropriate initial antibiotic therapy is a potentially modifiable factor that has been associated with increased mortality in patients with serious infections (5). For the clinician to select empirical antibiotics based on Gram stain findings of specimens

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collected from lower respiratory tract, studies are critical to identify the microorganisms causing LRTI in the local context and to determine their susceptibility to various antimicrobials The initial empirical broad-spectrum therapy can then be narrowed based on the culture results.

Multidrug resistant organisms might be associated with either symptomatic illness (i.e., clinical disease or infection) or asymptomatic carriage (i.e., colonization). Differentiating colonization from infection can be difficult and requires clinical correlation. Good communication between the treating clinician and the clinical microbiologist will aid in clinical decision making.

Information on the role of MDR Gram-negative bacilli in LRTI is limited. Hence, this retrospective study was planned to document MDR Gram-negative bacilli causing LRTIs, to correlate their isolation with the causation of disease and to study the effect of appropriate treatment on clinical outcome.

MATERIALS AND METHODS

Lower respiratory specimens, including sputum and broncho-alveolar lavage fluid (BAL) were collected from patients with LRTIs. The specimens received in the microbiology laboratory in our tertiary care teaching hospital during a period of six months (January to June 2012) were studied. 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 oro-pharyngeal flora. Those containing greater than 25 leucocytes and fewer than 10 squamous epithelial cells per field were optimal specimens and further processed (6). Broncho-alveolar lavage fluid was processed by quantitative culture with positive threshold of 10^4 CFU/ml (7).

The specimens were plated on Blood agar, MacConkey agar and Chocolate agar and incubated at 37°C for 18-24 hours. Identification of bacterial isolates was done following standard bacteriological techniques (8). Antibiotic susceptibility was determined by the Kirby Bauer's disc diffusion method on Mueller-Hinton agar (BD) following the clinical laboratory standard institute (CLSI) guidelines (9). Duplicate isolates from the same patient were excluded from analysis. These strains were then checked for extended spectrum beta-lactamase production (ESBL) using double disc approximation method (10). 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 *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella spp, Enterobacter spp, Acinetobacter spp. and Citrobacter spp.* isolates showing resistance to at least 3 different antimicrobial groups (4). These MDR isolates were considered as pathogenic if microbiologically same isolate was repeatedly isolated from multiple samples or in case of BAL sample growth of $\geq 10^4$CFU/ml was observed. Clinically isolates were considered pathogenic if there was presence of fever (temperature >38°C), raised leucocyte count (>12.0 × 10^9 cells/L), presence of purulent sputum, positive chest auscultatory findings and radiological findings of chest infection. Response to appropriate treatment (resolution of fever, improvement of respiratory function, decrease in leucocyte count, and stable or no further worsening of chest x-ray) was also analyzed.

RESULTS

Sputum (2264) and broncho-alveolar lavage fluid (315) specimens received in microbiology laboratory during the study period were studied. From these specimens, a total of 472 Gram-negative pathogens were isolated. *Klebsiella pneumoniae* 175 (37%) was the most common Gram-negative bacilli followed by *Pseudomonas aeruginosa* 135 (28.6%), *Acinetobacter spp.* 107 (22.7%) and others. Among these Gram-negative pathogens 175 (37%) were found to be multidrug resistant. Of these MDR Gram-negative bacilli, 146 (83.4%) were from sputum specimens and 29 (16.6%) from broncho-alveolar lavage fluid. Majority of the patients 91 (52%) from whom MDR Gram-negative bacilli were isolated had underlying pneumonia (Table 1).

Klebsiella pneumoniae 85 (48.6%) and *Acinetobacter spp.* 59 (33.7%) were the predominant MDR Gram-negative bacilli isolated (Table 2).

Table 1. Percentage of the relative frequencies of LRTI s in which MDR Gram-negative bacilli were isolated.

Type of LRTI	No.	Percentage
Pneumonia	91	52
Chronic obstructive pulmonary disease	55	31.4
Pleural effusion	11	6.3
Empyema	9	5.1
Pulmonary tuberculosis	8	4.6
Pyopneumothorax	1	0.6
Total	175	100

A large number of these MDR Gram-negative bacilli were ESBL producers (n=70, 40%). Based on clinico-microbiological correlation, 138 (78.9%) MDR isolates were found to be pathogenic and the rest (37, 21.1%) were considered as colonizers. After 3 days of initiating appropriate antibiotic therapy, clinical improvement (resolution of fever, improvement of respiratory function, decrease in leucocyte count, and stable or no further worsening of chest x-ray) was seen in 110 (79.7%) patients. Mortality was observed in 10 (7.2%) patients infected with MDR Gram-negative bacilli. (Table. 3)

The susceptibility profile of MDR isolates is given in Table.4.

FR ceftriaxone (30 µg), CZ ceftazidime (30 µg), PC piperacillin (100 µg), FEP cefepime (30 µg) CI ciprofloxacin (5 µg), CT trimethoprim-sulfamethoxazole (23.75/1.25 µg), AK amikacin (30 µg), GM gentamicin (10 µg), NET netilmicin (30 µg), CS cefoperazone-sulbactum (75/30 µg), PT piperacillin-tazobactum (100/10 µg), MR meropenem (10 µg), NT not tested.

DISCUSSION

Drug resistant infections and their related morbidity and mortality are on the rise around the world. The World Health Organization has identified antimicrobial resistance as 1 of the 3 greatest threats to human health (11). Initial empirical therapy with broad-spectrum antimicrobials is a treatment strategy for severe antimicrobial infections. With an awareness of local pathogen prevalence and resistance profiles, as well as a consideration of patient clinical characteristics, the physician can implement an initial empirical antimicrobial regimen that is likely to be active against the probable causative pathogen, thereby decreasing the risk of death and the potential for complications (12).

In the present study, *K. pneumoniae* followed by *P. aeruginosa* were the most common Gram-negative bacilli isolated in cultures from LRTIs. Lin *et al.* (13) have reported similar data in hospitalized patients with acute exacerbation of chronic obstructive pulmonary disease, whereas Goel *et al.* (14) found *P.*

Table 2. Percentage of the relative frequencies of various multidrug resistant Gram-negative bacilli.

Organism No. (%)	MDR GNB No. (%)	ESBL Producers among MDR GNB No. (%)
Klebsiella pneumoniae 175 (37)	85 (48.6)	56 (65.9)
Acinetobacter spp. 107 (22.7)	59 (33.7)	0
Escherichia coli 30 (6.4)	17 (9.7)	12 (70.6)
Pseudomonas aeruginosa 135 (28.6)	10 (5.7)	0
Enterobacter spp. 15 (3.2)	2 (1.1)	2 (100)
Citrobacter spp. 10 (2.1)	2 (1.1)	0
Total	17, (100)	73 (41.7)

Table 3. Prognosis of patients (n = 138) infected with multidrug resistant Gram-negative bacilli after appropriate treatment.

Outcome	Number of patients (%)
Improved	110 (79.7)
Discharge against medical advice	18 (13)
Death	10 (7.2)
Total	138

aeruginosa to be most common isolate (35%) from patients with LRTIs admitted to intensive care units. Gonlugur *et al.* (15) found *Klebsiella spp* (35%), *A. baumanii* (27%), and *E. coli* (15%) to be the most common organisms causing LRTIs. In the present study, a significant number (37%) of Gram-negative bacilli were found to be MDR with *K. pneumoniae* 85(48.6%) and *Acinetobacter spp.* 59(33.7%) being the most common MDR Gram-negative bacilli isolated from LRTIs. Higher rates of multidrug resistance (83%) among Gram-negative pathogens have been reported by Gagneja *et al.* (16) Goel *et al.* (14) found very high rate of resistance (60-100%) among *A. baumannii* and *K. pneumoniae* isolates. In contrast to the present study, Goel *et al.* (14) 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. Another important finding in the present study is presence of ESBL production in 65% and 70% of MDR strains of *K. pneumoniae* and *E. coli* respectively. The importance of ESBL producing strains lies in the fact that they are difficult to treat because they carry plasmids that confer resistance to many other antibiotics. Betalactam-betalactamase inhibitors and carbapenems are the only options to treat these infections. Gonlugur *et al.* (15) have reported lower rates of ESBL production among the respiratory isolates of *K. pneumoniae* (12%) and *E. coli* (21%).

Over the years we are witnessing the emergence of multidrug resistant strains in our laboratory that can be explained partially by inappropriate use of antibiotics. The increasing trend of antimicrobial resistance is most worrisome for Gram-negative bacteria because there has been little successful development of new antibiotic agents targeting this class of pathogen (5). In the present study, maximum resistance was observed towards quinolones and cephalosporins. Similar pattern of antimicrobial resistance was seen in previous studies conducted on respiratory specimens. In contrast, Gonlugur *et al.* (15) and Okesola and Ige (17) have found very low rates of resistance to various antibiotics in patients with LRTIs. Cefoperazone-sulbactum (79.4%), imipenem (72%) and amikacin (69.7%) were the most effective drugs. All MDR enterobacteriaceae, *Acinetobacter spp.* and *P. aeruginosa* were uniformly susceptible (100%) to colistin.

In the present study 78.9% of isolated MDR Gram-negative bacilli were suggested to be pathogenic strains. This highlights the importance of MDR Gram-negative bacilli in causing lower respiratory tract infection. MDR Gram-negative bacilli should not be considered as mere hospital flora and this finding highlights the importance of correlation of microbiological reports with clinical findings of patients as it aids the clinician in therapeutic decisions. This also avoids unnecessary therapy with higher or reserved antibiotics if the isolate turns out to be a mere colonizer. Strict enforcement of hospital

Table 4. Susceptibility profile of multidrug resistant Gram-negative bacilli isolated from Lower respiratory tract infections.

Organism (No.)	FR	CZ	PC	FEP	CI	CT	AK	GM	NET	CS	PT	MR
Klebsiella spp. (85)	1.1%	NT	NT	8.2%	29.4%	35.2%	90.5%	49.4%	67.2%	81.1%	52.9%	94.1%
Acinetobacter spp. (59)	1.5%	NT	NT	13.5%	25.4%	33.8%	30.5%	23.7%	28.8%	77.9%	28.8%	30.5%
Escherichia coli (17)	0	NT	NT	5.8%	0	17.6%	100%	64.7%	94.1%	94.1%	76.4%	100%
P. aeruginosa (10)	NT	60%	40%	50%	70%	NT	60%	NT	20%	40%	60%	70%
Citrobacter spp. (2)	0	NT	NT	50%	0	50%	100%	50%	100%	100%	100%	100%
Enterobacter spp. (2)	0	NT	NT	0	50%	0	100%	100%	100%	100%	100%	100%
Total (175)	6%	60%	40%	12.6%	27.4%	32.7%	69.7%	38.9%	54.9%	79.4%	48.6%	72%

infection control measures are of absolute necessity. The majority of patients (79.7%) infected with MDR Gram-negative bacilli responded to appropriate therapy. Regular surveillance which directs appropriate empirical therapy and good microbiological workup of each case of LRTI and treatment with antibiotics according to the susceptibility reports can reduce the morbidity and mortality associated with multidrug resistant organisms.

To conclude, the presence of MDR isolates in respiratory samples must be correlated with clinical findings of patients to see their pathogenic role and not merely considered as colonizers. Initiation of appropriate treatment well in time, according to their susceptibility pattern, can result in favourable clinical cure of respiratory infections.

REFERENCES

1. Woodhead M, Blasi F, Ewig S, Garau J, Huchon G, Leven M. Guidelines for the management of adult lower respiratory tract infections - Full version. *Clin Microbiol Infect* 2011; 17 Suppl 6: E1-59.
2. Carroll KC. Laboratory Diagnosis of Lower Respiratory Tract Infections: Controversy and Conundrums. *J Clin Microbiol* 2002; 40: 3115-3120.
3. Egbe CA, Ndiokwere C, Omoregie R. Microbiology of lower respiratory tract infections in Benin City, Nigeria. *Malays J Med Sci* 2011; 18: 27-31.
4. Pop-Vicas AE, D'Agata EMC. The rising influx of multidrug-resistant Gram-negative bacilli into a tertiary care Hospital. *Clin Infect Dis* 2005; 40: 1792-1798.
5. Kollef MH, Golan Y, Micek ST, Shorr AF, Restrepo MI. Appraising contemporary strategies to combat multidrug resistant Gram-negative bacterial infections–proceedings and data from the Gram-negative resistance summit. *Clin Infect Dis* 2011; 53(S2): S33-55.
6. Lentino JR, Lucks DA. Nonvalue of sputum culture in the management of lower respiratory tract infections. *J Clin Microbiol* 1987; 25: 758-762.
7. Chastre J, Combes A, Luyt CE. The invasive (Quantitative) diagnosis of ventilator-associated pneumonia. *Respir Care* 2005; 50: 797-807.
8. Collee JG, Miles RS, Watt B (1996). Tests for identification of bacteria. In: *Mackie and McCartney Practical Medical Microbiology*. Ed, Collee JG, Fraser AG, Marmion BP, Simmons A. Churchill Livingstone, 14th ed. New York, pp. 131-149.
9. Clinical and Laboratory Standards Institute (2011). performance standards for antimicrobial susceptibility testing. twenty-first informational supplement. CLSI document M100-S21. Clinical and Laboratory Standards Institute. Wayne, Pennsylvania, USA.
10. Tofteland S, Haldorsen B, Dahl KH, Simonsen GS, Steinbakk M, Walsh TR et al. Effects of phenotype and genotype on methods for detection of extended-spectrum-beta-lactamase-producing clinical isolates of Escherichia coli and Klebsiella pneumoniae in Norway. *J Clin Microbiol* 2007; 45: 199-205.
11. Gilbert DN, Guidos RJ, Boucher HW, Talbot GH, Spellberg B, Edwards Jr JE et al. The 10 × ′20 initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020. *Clin Infect Dis* 2010; 50:1081-1083.
12. Kollef MH. Broad-Spectrum antimicrobials and the treatment of serious bacterial infections: Getting tt right up front. *Clin Infect Dis* 2008; 47: S3-13.
13. Lin SH, Kuo PH, Hsueh PR, Yang PC, Kuo SH. Sputum bacteriology in hospitalized patients with acute exacerbation of chronic obstructive pulmonary disease in Taiwan with an emphasis on *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. *Respirology* 2007; 12: 81-87.
14. Goel N, Chaudhary U, Aggarwal R, Bala K. Antibiotic sensitivity pattern of Gram negative bacilli isolated from the lower respiratory tract of ventilated patients in the Intensive care unit. *Indian J Crit Care Med* 2009; 13: 148-151.
15. Gonlugur U, Bakici MZ, Akkurt I, Efeoglu T. Antibiotic susceptibility patterns among respiratory isolates of Gram-negative bacilli in a Turkish university hospital. *BMC Microbiol* 2004; 4: 32.
16. Gagneja D, Goel N, Aggarwal R, Chaudhary U. Changing trend of antimicrobial resistance among Gram-negative bacilli isolated from lower respiratory tract of ICU patients: A 5-year study. *Indian J Crit Care Med* 2011; 15: 164-167.
17. Okesola AO, Ige OM. Trends in bacterial pathogens of lower respiratory tract infections. *Ind J Chest Dis All Sc* 2008; 50: 269-272.