



Antibiotic Resistance Pattern Among Gram-negative Bacilli involved in Lower Respiratory Tract Infections

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ABSTRACT

The study was aimed at determining the prevalence and antibiotic resistance among Gram-negative Bacilli isolated from the lower respiratory tract of patients attending our tertiary care centre. Lower respiratory tract secretions (tracheal aspirate and sputum) of 500 patients were cultured, identified and antimicrobial susceptibility was performed by standard methods. Out of 500 patients, 216 were culture positive. Out of which 152 were GNB, 45 were *Staphylococcus aureus* and 19 were *Candida* spp. The common GNB isolates were non-fermentative gram-negative bacilli (NFGNB, 34.2%), followed by *Klebsiella* spp.(21.7%) and *Pseudomonas aeruginosa* (21%). In both tracheal and sputum GNB isolates, the highest mean resistance was to ampicillin(87.4%) and cefazolin(75.2%) while lowest to imipenem(12.8%) and amikacin(30.5%). A large majority of the isolated multidrug resistant Gram-negative bacilli were found to be pathogenic. Regular surveillance and good clinic-microbiological workup can reduce morbidity and mortality associated with multidrug resistant organisms.

KEYWORDS : LRTI, Gram-negative bacteria, antibiotic resistance.

Introduction

The most common infectious diseases are those involving the upper and lower respiratory tract. LRTIs are common reason for consultation and hospitalization, imposing an enormous burden on society. Woodhead M., (2011) posited that lower respiratory tract infection is a broad description of a group of disease entities, encompassing acute bronchitis, pneumonia and exacerbations of chronic lung disease. Carroll K.C., (2002) posited that Lower respiratory tract infections are among the most common infectious diseases of human's worldwide and Egbe CA., (2011) stated that LRTI are the important causes of morbidity and mortality for all age groups. Prevalence studies indicate an inevitable tracheal colonisation with GNB in tracheostomies, most of which is asymptomatic. Michael NA., (2001) reported the presence of GNB in tracheal suction samples in all the patients. Inglis TJJ, (1998) posited that in long-term hospitalised patients, bacteria from the ventilator breathing system have been implicated in the pathogenesis of ventilator associated pneumonia. Due to different resistance mechanisms spreading among respiratory pathogens and leading to multi-resistant strains their treatments are frequently difficult. Hence, the present study was undertaken to define the common bacterial profile in LRTI and to study the resistance patterns to common antibiotics and to provide empiric therapy to the clinicians in treating LRTI.

Material and Methods

The present study was carried out in the time span of 6 months from Jan 2015 to June 2015 in the Department of Microbiology NIMS Medical College, Jaipur. A total of 500 lower respiratory tract secretions (361 sputum and 139 tracheal aspirates) were collected from the patients of all age group who had clinically evident Lower Respiratory tract infections were received in diagnostic microbiology for culture and sensitivity. Chronic obstructive pulmonary disease (COPD) and pneumonia were the commonest provisional diagnoses among patients from where samples were obtained. Purulent portion of samples were used for making smears for gram stain and for inoculating blood agar and MacConkey agar. The specimens were cultured on Blood agar, MacConkey agar and Chocolate agar and incubated at 37°C for 18-24 hours. Identification of bacterial isolates was done by their characteristic appearance on the media, Gram's staining, motility testing (by hanging drop method), biochemical tests (Catalase, Coagulase, Indole, Methyl red, voges-proskauer, Citrate, Urease, Triple sugar iron, PPA, Oxidase test), antimicrobial susceptibility tests by Modified Kirby Bauer's disc diffusion method following the clinical laboratory standard institute (CLSI guidelines, 2014). The

panel of antibiotics (discs in mcg) tested was as follows: amikacin(AK) (30), ampicillin(AMP) (10), Cefozolin(Cz) (30), cephotaxime (CX) (30), amoxyclav (AMC) (30), aztreonam(AO) (30), ciprofloxacin(CP) (5), piperacillin/tazobactam(PT) (85) and imipenem(IPM) (10).

Result

During the study period, a total of 500 lower respiratory tract secretions of all ages and both sexes were studied. In the present study, out of 500 samples, 216(43.2%) were culture positive, 221(44.2%) showed normal upper respiratory oral flora and 63(12.6%) showed no growth. Out of the positive culture, 45(20.8%) were GPC, 152(70.3%) were GNB and 19(8.7%) were *Candida* spp. respectively. Among the 500 patients, significant bacterial growth was exhibited by 208(41.6%) cases and polybacterial growth was observed in 8(1.6%) cases. The most common GNB isolates in order of frequency were NFGNB (34.2%), *Klebsiella* spp. (21.7%), *Pseudomonas aeruginosa* (21%), and *Escherichia coli* (7.2%). GNB isolates from tracheal aspirate and sputum were 139(27.8%) and 361(72.2%), respectively (Table 1). A total of 47 (21.7%) and 32(15.8%) isolates were ESBL and AmpC Beta-Lactamases producing organisms, respectively.

Table1: Distribution of gram-negative bacteria (GNB) in LRT secretions

GNB (n=152)	Sputum (n=361)	TA (n=139)	Total (%)
NFGNB	18	34	52(34.2)
<i>Klebsiella</i> spp.	15	18	33(21.7)
<i>Pseudomonas</i> spp.	9	23	32(21.0)
<i>Escherichia coli</i>	4	7	11(7.2)
<i>Enterobacter</i> spp.	4	5	9(5.9)
<i>Citrobacter</i> spp.	3	4	7(4.6)
<i>Proteus mirabilis</i>	1	7	8(5.2)
Total	54(35.5)	98(64.4)	152

TA: Tracheal aspirate; NFGNB: Non-fermenting gram-negative bacilli other than *Pseudomonas* spp; multiple specimens from single patient excluded but multiple isolates from single sample included.

The occurrence of bacterial pathogens varies with age, 41-60years (51.8%) recorded higher isolates while age group 1–20years recorded the least (5.5%). Sex related occurrence of pathogens reveals that, male 133(61.5%) subjects reported higher number of pathogens compared to females 83(38.4%).

The highest mean resistance among GNB of tracheal aspirates (92.4%) and sputum samples (82.5%) was noted to ampicillin. The lowest mean resistance of tracheal aspirate isolates (5.3%) and sputum isolates (20.3%) was to imipenem and to amikacin 36.7%, 24.4% respectively (Tables 2 and 3). NFGNB of tracheal aspirates and sputum showed 32.3% and 33.3% resistance to amikacin respectively (Tables 2 and 3). Tracheal aspirate isolates of *Pseudomonas aeruginosa* showed 39.1% and 69.5% resistance to amikacin and ceftaxime respectively (Table 2) while no resistance was noted to same set of antibiotics in sputum isolates among *Pseudomonas aeruginosa* (Table 3). *Klebsiella spp.* showed no resistance to imipenem. There was no remarkable difference noted in the overall mean resistance in the GNB isolates from tracheal and sputum specimens.

Table 2: Antibiotic resistance (%) among predominant GNB of tracheal aspirates

GNB	AMP	CZ	CX	AO	CP	AK	AMC	PT	IPM
NFGNB	94.1	88.2	61.7	67.6	52.9	32.3	67.6	47	11.7
<i>Klebsiella spp.</i>	83.3	61.1	61.1	72.2	22.2	38.8	27.7	44.4	0
<i>Pseudomonas spp.</i>	100	78.2	69.5	82.6	56.5	39.1	30.4	21.7	4.3
Mean resistance	92.4	75.8	64.1	74.1	43.8	36.7	41.9	37.7	5.3

Column headings are abbreviations for antibiotics, full names given in "Material and Methods"

Table 3: Antibiotic resistance (%) among predominant (GNB) of sputum samples

GNB	AMP	CZ	CX	AO	CP	AK	AMC	PT	IPM
NFGNB	61.1	66.6	55.5	44.4	38.8	33.3	16.6	61.1	27.7
<i>Klebsiella spp.</i>	86.6	80	26.6	53.3	86.6	40	60	40	0
<i>Pseudomonas spp.</i>	100	77.7	0	33.3	44.4	0	44.4	55.5	33.3
Mean resistance	82.5	74.7	27.3	43.6	56.6	24.4	40.3	52.2	20.3

Column headings are abbreviations for antibiotics, full names given in "Material and Methods"

Discussion

This study has demonstrated that the major single pathogens causing LRTI were NFGNB (34.2%), followed by *Klebsiella spp.*(21.7%) and *Pseudomonas aeruginosa* (21%). In the present study 53.7 % of the isolates were GPC and 40.2% of GNB. Quite similar findings were seen in the study by Gorgiana F Brad et al., (2011).The National Nosocomial Infections Study of CDC., (2000) consistently reports that aerobic GNB cause more than 60% of nosocomial pneumonias.

To provide treatment strategies and avoid a false impression of clinically resistant strains, polymicrobial infections are very important to identify. Incidence of mixed bacterial infection in this study was 1.6% and this is consistent with the fact that the incidence of mixed infections does not usually exceed 30% as has been observed in other series (de Roux *et al.*, (2006). Predominance of male over females can be explained by the fact that smoking habits are more pronounced in males that constitute one of the predisposing factors for development of COPD.

The antimicrobial resistance among the respiratory pathogens is a major barrier interfering with an effective treatment. Gilbert DN., (2010) posited that the World Health Organization has identified antimicrobial resistance as 1 of the 3 greatest threats to human health. Carroll KC, (2002) posited that an expanded variety of emerging pathogens likewise provides challenges for the microbiology laboratory. Moreover, in recent years, there has been dramatic rise in antibiotic resistance among respiratory pathogens.

Imipenem among tracheal aspirate and amikacin and ceftaxime among sputum isolates was found to be a better drug for *Pseudomonas aeruginosa* in the present study. However, this needs further evaluation in clinical settings.

Conclusion

We conclude that GNB were the predominant isolates of LRT infections with NFGNB, *Klebsiella spp.* and *Pseudomonas aeruginosa* as the common isolates. Adults, elderly and critically ill patients (tracheal aspirates) are at high risk for contracting GNB LRT infections. Amikacin, imipenem and ceftaxime were found to be effective antibiotics against GNB. Isolation policies have to be adopted to maintain a low level of resistant organisms, while handling colonised or infected patients with drug resistant organisms.

The possibility of reducing resistance by controlling the use of antibiotics is a logical approach, but the implementation of effective policies has proved difficult in most situations. The Present study also highlights the need for periodic surveillance of antimicrobial susceptibility pattern of bacterial isolates, as it would promote the judicious use of antimicrobials given to patients and thus preventing the emergence of drug resistance.

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