Antimicrobial Resistance Pattern Among Aerobic Gram-negative Bacilli of Lower Respiratory Tract Specimens of Intensive Care Unit Patients in a Neurocentre

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ABSTRACT

Background. Analysis of the tracheal and bronchial isolates from the lower respiratory tract specimens of the intensive care unit (ICU)-admitted patients, was carried out for the year 2002 with a perspective of looking at the antibiotic resistance pattern.

Methods. Lower respiratory tract secretions (tracheal or bronchoscopic aspirates) of 370 patients were cultured, identified and antimicrobial susceptibility performed by standard methods.

Results. Out of samples obtained from 370 patients, 274 (74%) were culture positive. A total of 489 bacterial isolates were recovered from 270 patients; 451 were gram-negative bacilli (GNB) and 38 were Staphylococcus aureus. In four of the patients, Candida spp was isolated. The common GNB isolates were non-fermentative gram-negative bacilli (NFGNB, 31.9%), followed by Pseudomonas aeruginosa (21.5%) and Klebsiella spp (19%). Elderly (24.8%) and adults (19.2%) showed increased rate of GNB isolation. In both tracheal and bronchial GNB isolates, the highest mean resistance was to cefazolin (98.8%) and ampicillin (97.6%) while the lowest mean resistance was to amikacin (48.5%). Isolation of two organisms per specimen (41.4%) was commonly seen. Multidrug resistance to the tested antimicrobials was more frequent in NFGNB (6.6%) and Pseudomonas aeruginosa (5%). There were no remarkable differences in the overall mean drug resistance among tracheal and bronchial GNB isolates.

Conclusions. Isolation practices, antibiotic policies, effective surveillance, maintenance of epidemiological trends of infections and, rapid molecular diagnosis are the need of hour in improved and speedy management of lung infections with resistant organisms. [Indian J Chest Dis Allied Sci 2007; 49: 19-22]

Key words: Nosocomial infections, Epidemiology, Antimicrobial resistance, Gram-negative bacteria, Intensive care units, Lower respiratory tract infections.

INTRODUCTION

Infection and antibiotic resistance are important public health issues. The consequences of increased drug resistance are far-reaching since bacterial infection of the lower respiratory tract (LRT) is a major cause of death from infectious disease. One of the major problems worldwide is the increase in antibiotic-resistant strains of bacteria, mainly in hospitals, and also in the community which has proved difficult to control without considerable resources and expenditure. Highly resistant strains of gram-negative bacilli (GNB) continue to spread in hospitals causing therapy problems in many parts of the world, particularly in developing countries and isolation facilities for patients with resistant organisms are often inadequate.

Prevalence studies indicate an inevitable tracheal colonisation with GNB in tracheostomies, most of which is asymptomatic. A study records the presence of GNB in tracheal suction samples in all the patients. In long-term hospitalised patients, bacteria from the ventilator breathing system have been implicated in the pathogenesis of ventilator associated pneumonia.

Nosocomial pneumonia occurs in 0.5-1.5% of all hospitalised patients and 10 to 30 percent of those under artificial ventilation. In developing countries, acute respiratory infection (ARI) is the leading cause of morbidity and mortality in critically ill patients. The main causal agents are Staphylococcus aureus and resistant gram-negative bacteria which form a major problem in intensive respiratory care units. A study discusses that the above organisms are mainly...
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MATERIAL AND METHODS

A retrospective analysis is of the isolates of lower respiratory tract (LRT) specimens constituting trans-tracheal or bronchoscopic aspirates, received in Neuromicrobiology Laboratory of our Institute between January-December 2002, was carried out examining the antimicrobial resistance pattern and eliminating duplicate registrations. The isolates collected from the same specimen source of the same patient were excluded. All consecutive isolates were considered as we did not attempt to distinguish the actual pathology from colonising strains.

The total number of patients (medical and surgical admissions) seen at our Institute during January-December 2002 was 6430; out of whom, 435 patients required ICU care with 382 patients requiring ventilator care.

The diagnostic status was as follows: traumatic injuries (40), cerebro-vascular accidents (46), aneurysms (30) (post-operative), post surgical (94) (tumours, abscesses), Guillain-Barre syndrome (40), Myasthenia Gravis (7) and others (113) (encephalitis, meningitis, brain infarct, extradural haemorrhage etc., all requiring ventilator care).

The specimens were cultured on to thioglycollate broth, chocolate agar and Mac Conkey agar culture plates and incubated at 37 °C for 16-24 hours. Following isolation of organisms, conventional identification was carried out by subjecting to biochemical tests and antimicrobial susceptibility tests by standard Kirby-Bauer disc diffusion method.

The following panel of antimicrobials (discs in mcg) were tested: ampicillin (Am) (10), piperacillin (Pc) (100), cefazolin (Cz) (30), cotrimoxazole (Co) (25), gentamicin (Gm) (10), amikacin (Ak) (30), netilmicin (Nt) (30), cefotaxime (Ce) (30), ceftriaxone (Ci) (30), ceftazidime (Ca) (30) and ciprofloxacin (Cf) (5). Zone diameter was measured in millimeter and interpreted as per CLSI (Clinical and Laboratory Standards Institute) (formerly, NCCLS) guidelines.

RESULTS

During the study period, 370 of the ICU admitted patients were evaluated. Specimens of 274 (74%) patients were culture positive, 39 (10.5%) showed normal upper respiratory tract flora and 57 (15.4%) showed no growth. Out of the culture positives, 270 (98.5%) showed bacterial isolates and four (1.4%) showed Candida spp. The bacterial spectrum was either Staphylococcus aureus (17, (6.2%)) and GNB (232, (85.9%). In a total of 253 specimens, GNB were isolated either alone or along with Staphylococcus aureus.

Four hundred and eighty-nine isolates were obtained from 270 specimens of which 451 (92.2%) were GNB and 38 (7.7%) were Staphylococcus aureus. In 112 (41.4%) there were two isolates per specimen, 106 (39.2%) had single isolate, 49 (18.1%) with three isolates and three (1.1%) samples had four isolates in specimen.

The most common GNB isolates in order of frequency were NFGNB (31.9%), Pseudomonas aeruginosa (21.5%), Klebsiella spp (19%), and Escherichia coli (12.4%). Other organisms are represented in table 1.

Table 1. Number of gram-negative bacilli isolates in lower respiratory tract secretions received from ICU patients

<table>
<thead>
<tr>
<th>GNB (n=451)</th>
<th>Tracheal (n=240)</th>
<th>Bronchial (n=30)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFGNB</td>
<td>136</td>
<td>8</td>
<td>144 (31.9)</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>87</td>
<td>10</td>
<td>97 (21.5)</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>73</td>
<td>13</td>
<td>86 (19)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>43</td>
<td>13</td>
<td>56 (12.4)</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>32</td>
<td>4</td>
<td>36 (8)</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>12</td>
<td>4</td>
<td>16 (3.5)</td>
</tr>
<tr>
<td>Citrobacter spp</td>
<td>6</td>
<td>0</td>
<td>6 (1.3)</td>
</tr>
<tr>
<td>Providencia spp</td>
<td>4</td>
<td>1</td>
<td>5 (1.1)</td>
</tr>
<tr>
<td>Morganella morgani</td>
<td>2</td>
<td>1</td>
<td>3 (0.7)</td>
</tr>
<tr>
<td>Proteus vulgaris</td>
<td>0</td>
<td>2</td>
<td>2 (0.4)</td>
</tr>
<tr>
<td>Total</td>
<td>395 (87.5%)</td>
<td>56 (12.4%)</td>
<td>451</td>
</tr>
</tbody>
</table>

NFGNB=Non-fermentative gram-negative bacilli other than Pseudomonas spp

Rate of multiple drug resistance to all the antibiotics used in the panel as noticed in some common GNB isolates were as follows: NFGNB 30 (6.6%), Pseudomonas aeruginosa 23 (5%), Klebsiella spp eight (1.7%), Escherichia coli five (1.1%), Enterobacter spp four (0.8%) and Citrobacter spp one (0.2%).

The combined highest mean resistance among GNB of both tracheal and bronchial samples was noted to cefazolin (98.8%) and ampicillin (97.6%). The lowest mean resistance was noted to amikacin 53% and 44% (mean 48.5%) in tracheal and bronchial isolates respectively (Tables 2 and 3).

Pseudomonas aeruginosa of tracheal and bronchial specimens showed 59.8% and 40% resistance to amikacin, respectively. In NFGNB, difference in the mean resistance to piperacillin, gentamicin and amikacin in the tracheal versus bronchial isolates in

Susceptibility Testing

To evaluate the susceptibility patterns of the above isolates, we followed the Kirby-Bauer disc diffusion method. Antimicrobials tested included ampicillin (Am) (10 μg), piperacillin (Pc) (100 μg), cefazolin (Cz) (30 μg), cotrimoxazole (Co) (25 μg), gentamicin (Gm) (10 μg), amikacin (Ak) (30 μg), netilmicin (Nt) (30 μg), cefotaxime (Ce) (30 μg), ceftriaxone (Ci) (30 μg), ceftazidime (Ca) (30 μg) and ciprofloxacin (Cf) (5 μg). The zone diameter was measured in millimeter and interpreted as per CLSI (Clinical and Laboratory Standards Institute) (formerly, NCCLS) guidelines.
order was as follows 79.5% vs 87.5%; 76.5% vs 100% and 73.6% vs 87.5% (Table 2 and 3).

Non-fermentative gram-negative bacilli exhibited 71.4% and 75% resistance to amikacin and 38.3% and 62.5% to netilmicin in tracheal and bronchial specimens, respectively. Netilmicin showed highest activity against NFGNB and amikacin against *Klebsiella spp* and *Escherichia coli* of both specimens. There was no remarkable difference noted in the overall mean resistance in the GNB isolates from tracheal and bronchial specimens.

The highest number of GNB isolates was from those above 50 years of age (24.8%) followed by 31-40 years (19.2%) and then those between 21 to 30 years (15.9%), while the lowest number was in patients less than 10 years of age (11.8%).

### DISCUSSION

Ventilator associated respiratory infections continue to be a frequent and often fatal complication in critically ill patients, occurring in 7% to 41% of ventilated patients. Risk of developing infection is directly related to the time spent on the ventilator increasing from 5% at 10 days to 28% at 30 days. Mortality rate ranges from 40 percent to 80 percent.

The National Nosocomial Infections Surveillance (NNIS) of CDC of USA reports 60% of nosocomial pneumonias to be caused by aerobic GNB. We found GNB to be the predominant organism (92.2%) with low isolation of *Staphylococcus aureus*. In 41% specimens, two isolates were obtained while in 39%, there was one isolate.

Fifteen percent of the specimens remained sterile on culture probably due to previous antibiotic therapy or being non representative specimens. Advances in the medical and surgical manipulations and increase in their applications provide suitable environment for nosocomial fungal infection. In the present study, *Candida* species were isolated in four (1.4%) patients. There was an overall preponderance of GNB among the LRT infection isolates with NFGNB, *Pseudomonas aeruginosa* and *Klebsiella spp* as the common isolates.

Multiple drug resistance to the tested antimicrobials was noted more in NFGNB (6.6%) and *Pseudomonas aeruginosa* (4%) among GNB isolates. In this study, overall least mean resistance was noted to amikacin for most of the isolates of both tracheal and bronchial specimens.

The resistance of some GNB to aminoglycosides to a longer extent to gentamicin than to amikacin, has been well recognised in many hospitals. In this study, we noted a combined overall resistance of 88.7% of resistance to gentamicin in both tracheal and bronchial isolates. The causes behind the emergence of such organisms have been a matter for speculation. A limited body of data also suggests that control of the epidemic cross infection by aminoglycoside-resistant organisms may depend on environmental control or barrier type precautions to maintain low level of resistant *Escherichia coli*, *Klebsiella spp* and *Enterobacter spp*. Amikacin, netilmicin, ciprofloxacin and ceftazidime were found to be effective antibiotics against GNB. Because of high rate of progression of resistance to amikacin, a strategy of limited and prudent use of antibiotics is urgently needed. Aminoglycoside resistant strains are more common at sites with poor penetration of the drugs. The serum therapeutic toxic ratio of aminoglycosides is low, hence, penetration into infected respiratory tract may be insufficient to act on the infecting organisms.

Netilmicin had better activity than amikacin on NFGNB isolates of both specimens in the present study. A comparison of mean antibiotic resistance pattern of tracheal and bronchial GNB isolates showed no remarkable difference.

The National Nosocomial Infections Surveillance

| Table 2. Resistance rates (%) to tested antimicrobials for the most common GNB isolates of tracheal specimens |
|---------------------------------------------|---------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| GNB             | Am  | Pc  | Cz  | Co  | Gm  | Ak  | Nt  | Ce  | Cl  | Ca  | Cf  |
| NFGNB           | 97.8| 79.5| 98.6| 79.5| 76.5| 71.4| 38.3| 80.2| 84.6| 78.7| 73.6|
| *Pseudomonas aeruginosa* | 97.8| 52.9| 100| 94.3| 73.6| 59.8| 70.2| 62.1| 80.5| 59.8| 69 |
| *Klebsiella spp* | 98.7| 91.8| 91.8| 63.1| 89| 50.7| 85| 90.5| 89.1| 90.5| 65.8 |
| *Escherichia coli* | 100| 97.7| 100| 69.8| 88.4| 30.3| 83.8| 97.7| 97.7| 95.4| 93.1 |
| **Mean resistance** | 98.5| 80.4| 97.6| 76.6| 81.8| 53| 69.3| 82.6| 87.9| 81.1| 75.3 |

| Column headings are abbreviations for antibiotics, full names given in “Material and Methods” |

| Table 3. Resistance rates (%) to tested antimicrobials for the most common GNB isolates of bronchial specimens |
|---------------------------------------------|---------------|----------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| GNB             | Am  | Pc  | Cz  | Co  | Gm  | Ak  | Nt  | Ce  | Cl  | Ca  | Cf  |
| NFGNB           | 87.5| 87.5| 100| 87.5| 100| 75| 62.5| 75| 87.5| 75| 87.5 |
| *Pseudomonas aeruginosa* | 100| 80| 100| 100| 90| 40| 80| 100| 90| 80| 90 |
| *Klebsiella spp* | 100| 100| 100| 61.6| 100| 23.1| 100| 92.4| 92.4| 100| 77 |
| *Escherichia coli* | 100| 92.4| 100| 77| 92.4| 38.5| 92.4| 92.4| 92.4| 84.7| 92.4 |
| **Mean resistance** | 96.8| 89.9| 100| 81.5| 95.6| 44.1| 83.7| 89.9| 90.5| 84.9| 86.7 |

| Column headings are abbreviations for antibiotics, full names given in “Material and Methods.” |
report indicates that *Staphylococcus aureus* causes approximately 20% of the nosocomial lung infections.\(^2\) In a UK study on gram-positive isolates from respiratory tract of ICU patients, 44% of *Staphylococcus aureus* isolates have been reported.\(^3\) Methicillin-resistant *Staphylococcus aureus* (MRSA) accounts for 52.3% of *Staphylococcus aureus* nosocomial infections\(^4\) and is now endemic in many hospitals and is one of the leading causes of nosocomial pneumonia. In the present study of LRT infections, 14% of the patients had infection with *Staphylococcus aureus*.

Critically ill adults and elderly are at greater risk of contracting GNB LRT infection. Antimicrobial resistance monitoring helps in optimisation of antimicrobial therapy and is more important in the ICUs as infection and antimicrobial consumption are significantly higher.\(^5\)

In an Italian study on control of nosocomial infections, pneumonias were common in ICU patients and were related to tracheal intubation and other additive factors such as poor immune status and numerous invasive diagnostic and therapeutic procedures carried out as well as the duration of hospitalisation.\(^6\) In our study, the patients had more than three days of ICU hospitalisation.

To maintain a low level of resistant organisms, isolation policies have to be adopted while handling colonised or infected patients with drug resistant organisms and also barrier-type precautions need to be taken while cultures of such patients are awaited. Patients transferred from local nursing homes and community hospitals should be screened as a source of resistant bacilli, observing special precautions during patient care while the appropriate culture are being processed in the laboratory.\(^7\)

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. However, a combined approach of antibiotic restriction, effective surveillance and good infection control practices is essential if antibiotic resistance is to be overcome.\(^8\)

There is also a need to encourage research in rapid molecular diagnosis aiding in rapid and improved management of severe infections with resistant organisms. There is an obvious necessity to study not only the trends in epidemiology of nosocomial infections but also the local situations for which multicentre studies need to be carried out in our country to coordinate and arrive at protocols based on local patterns of antibiotic resistance. Alteration and rotation in antibiotic prescribing patterns might decline the antibiotic resistance.

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**REFERENCES**

Surgical Treatment of Pulmonary Aspergilloma: A Series of 72 Cases

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ABSTRACT

Background. The objective of this study was to evaluate the immediate and long term result of resectional surgery in pulmonary aspergilloma.

Methods. Seventy-two patients who underwent pulmonary resectional surgery for symptomatic aspergilloma between 1990 to 2002 were studied. Seventy-nine definitive operations were carried out, including one bilateral lobectomy for recurrent lesions and six thoracoplasties to deal with post-operative complications, besides 21 pneumonectomies and 51 lobectomies. There were 10 bilobectomies as well, included in the lobectomy group.

Results. At a mean follow-up of 3.5 years, there were two post-operative deaths and a few complications occurred in 20 cases translating into a morbidity of 28.57% and a mortality of 2.77 percent. Major complications included were persistent air leak, persistent pleural space, empyema, bronchopleural fistula and massive haemorrhage. All events were seen in cases of complex aspergilloma; cases of simple aspergillomas had an uneventful course.

Conclusions. Surgery offers definitive and long term symptom-free survival in cases of pulmonary aspergilloma at a negligible risk; though almost one-third of those undergoing surgery develop some complications, these are largely manageable. [Indian J Chest Dis Allied Sci 2007; 49: 23-27]

Key words: Aspergilloma, Pulmonary, Tuberculosis, Surgery.

INTRODUCTION

Pulmonary aspergilloma, the so-called fungus ball or mycetoma, is a clinical syndrome of worldwide presence and represents one of the many manifestations of human disease due to the fungus *Aspergillus*. These include asymptomatic inconsequential presence in healthy hosts, chronic necrotising aspergillosis in patients with chronic lung disease, invasive aspergillosis in immunocompromised hosts and allergic bronchopulmonary aspergillosis in asthmatics.¹ The term aspergilloma refers to colonisation of pre-existing lung cavities with the *Aspergillus* fungus, most commonly the fumigatus species, and the lesion itself consists of a tangled mass of fungal hyphae, fibrin, epithelial cells, mucus, debris and blood cells.² Tubercular lesions are the most common cause of such cavities although aspergillomas may occur within cavities of diverse aetiologies including sarcoidosis, bronchiectasis, cysts and bullae, neoplasms, ankylosing spondylitis, Wegener’s granulomatosis, and pulmonary infraction.¹ Though the natural history of such a lesion is not completely understood, it has the propensity to cause recurrent and severe haemoptysis; besides, when super infected, the illness runs a distressing and protracted course.³

Pulmonary aspergilloma is the only surgically relevant manifestation of *Aspergillus* infection and though known for a century- and-a-half now, controversy still surrounds its optimal management.¹⁻³ The controversy surrounds the surgical management stems from the perceived threat of morbidity and mortality consequent to surgery and it has led to recommendations of surgery only as a last resort,⁴ or restricted to symptomatic cases only⁶⁻⁷ or an option for all cases to preempt massive haemoptysis.⁸⁻¹⁰

In this paper we are presenting a series of 72 cases of pulmonary aspergilloma treated surgically at our centre over the last 12 years, to evaluate our indications and results.

MATERIAL AND METHODS

During a 12-year period from 1990 to 2002, 72 patients underwent surgery for pulmonary aspergilloma at LRS Institute of Tuberculosis and Respiratory Diseases in the...