STUDY ON NON-TUBERCULOUS AEROBIC BACTERIAL PROFILE OF LOWER RESPIRATORY TRACT INFECTIONS IN HOSPITALISED GERIATRIC POPULATION IN A TERTIARY CARE HOSPITAL

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ABSTRACT

BACKGROUND

The increasing number of persons above 65 years of age forms a special population at risk for infections. The vulnerability of this age group is related to impaired host defences such as diminished cell-mediated immunity. LRTIs place a considerable strain on the health budget and are more serious than upper respiratory infections. Accurate diagnosis of respiratory tract infections in older people is problematic because of the lack of clear symptoms and signs. In addition, the increasing prevalence of bacterial resistance to antibiotic therapy has complicated the antimicrobial selection process and highlights the importance of appropriate treatment.

MATERIALS AND METHODS

The study was observational and prospective, undertaken over a period of one year from October 2015 to September 2016 conducted in the Institute of Microbiology in association with the Department of Geriatric Medicine, Madurai Medical College, Madurai. A total of 110 patients of 65 years of age and above admitted with lower respiratory tract infections [LRTIs] were studied. Microbiology methods included direct microscopic examination for potassium hydroxide wet mount and Gram staining. Culture was done by semi-quantitative method.

RESULTS

A total of 110 patients of 65 years of age and above admitted with lower respiratory tract infections [LRTI] were studied. Majority of the patients were in the age group of 65 – 74 years (82.7%). From the 110 patients, sputum was collected from 93 (84.5%). Other respiratory specimens included bronchial wash- 11 (10.0%), bronchoalveolar lavage- 4 (3.6%), pleural fluid- 2 (1.8%). Among the 93 sputum samples, 65 (59.1%) were positive for any one pathogen. The different spectrum of organisms grown were *Klebsiella pneumonia* 28 (36.8%), *Klebsiella oxytoca* 3 (3.9%), *Pseudomonas aeruginosa* 17 (22.3%), *Acinetobacter baumannii* 9 (11.8%), *Staphylococcus aureus* 8 (10.5%), *Proteus mirabilis* 4 (5.3%), *Escherichia coli* 3 (3.9%), *Streptococcus pneumonia* 2 (2.6%), *Citrobacter koseri* 2 (2.6%).

CONCLUSION

From this prospective study, it is quite clear that the elderly patients are at high risk for contracting lower respiratory tract infections due to Gram-negative bacteria. The emergence of resistant strains in the patients of the present study emphasises the need of prudent use of antibiotics.

KEYWORDS

ESBL, Respiratory Tract Infection, Geriatric, DDST, PCDDT, MBL.

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BACKGROUND

The increasing number of persons above 65 years of age forms a special population at risk for infections. The vulnerability of this age group is related to impaired host defences such as diminished cell-mediated immunity.¹ Factors which influence immunocompetence are immune senescence, changes in non-adaptive immunity, chronic diseases, medications, malnutrition and functional impairments. Respiratory infections are common at all ages

but are particularly sinister among the elderly, because of the fragility and chronic comorbidity associated with this age group. Lower respiratory tract infections (LRTIs) cause disease in the alveolar sacs.²

LRTIs place a considerable strain on the health budget and are more serious than upper respiratory infections. Chronic obstructive pulmonary disease and pneumonia is the most common respiratory tract infections observed in older people and are the leading causes of morbidity and mortality associated with infection. Accurate diagnosis of respiratory tract infections in older people is problematic because of the lack of clear symptoms and signs. In addition, the increasing prevalence of bacterial resistance to antibiotic therapy has complicated the antimicrobial selection process and highlights the importance of appropriate treatment.³

The presence of pathophysiological changes and organ involvement, together with fever, raised white blood cell count or elevated ESR are strongly supportive of a diagnosis of infection.⁴ This study was conducted to determine the aetiological agents causing lower respiratory tract infections

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in geriatric population attending tertiary care hospital. Antibiotic susceptibility patterns of the pathogens and the predisposing factors for LRTI were also evaluated, which would provide information to optimise accurate timely diagnosis, treatment and care of the elderly. The aim of this study was to isolate and identify the aerobic bacterial pathogens, to identify and correlate the risk factors, to study the antibiotic susceptibility patterns of the isolated pathogens and to determine the extended spectrum beta-lactamase producing organisms commonly causing lower respiratory tract infections in geriatric patients.

MATERIALS AND METHODS

The study was observational and prospective, undertaken over a period of one year from October 2015 to September 2016 conducted in the Institute of Microbiology in association with the Department of Geriatric Medicine, Madurai Medical College, Madurai. The study was approved by the Institutional Ethical Committee, Madurai Medical College. Informed consent was obtained from the study population. All patients satisfying the inclusion criteria were documented. Patients were interviewed by structured questionnaire.

Patients of 65 years of age and above, admitted for lower respiratory tract infections in geriatric medical ward were included in the study. Patients below 65 years of age and those who were being started on antibiotic therapy for lower respiratory tract infections after admission were excluded. Patients admitted for diseases other than lower respiratory tract infections were also excluded from the study.

Under strict aseptic precautions, samples were collected from the patients and transported immediately to the laboratory in appropriate method and sample processing was done as per CLSI standard. Samples collected were sputum, bronchial wash, bronchoalveolar lavage pleural fluid, and blood. Early morning sputum samples after deep expectoration was collected.

Microbiology methods included direct microscopic examination for potassium hydroxide wet mount and Gram staining. Culture was done by semi-quantitative method. The liquefied samples were re-suspended in sterile saline. Three serial dilutions (1/10, 1/1000, 1/100000) were made, and a calibrated loop was used to plate out 0.01 mL of the fluid. Diagnostic thresholds were $>10^5$ CFU/mL for sputum, $>10^4$ CFU/mL for bronchoalveolar lavage. The specimens were plated out on MacConkey agar, blood agar, chocolate agar and incubated at 37°C for 24 hours. All plates were checked for growth overnight and then after 24 and 48 hours up to 5 days.

Antibacterial susceptibility tests were done by disc diffusion method (Kirby Bauer's). The technique was performed as per CLSI guidelines. The antibiotic discs used for Gram-positive cocci were: Penicillin 10 units, Cefotaxime 30 µg, Amikacin 30 µg, Ciprofloxacin 5 µg, cotrimoxazole 1.25/23.75 µg, Erythromycin 15 µg, Chloramphenicol 30 µg, Tetracycline 30 µg. When testing for Streptococcus pneumoniae, the diameter of zone of inhibition varies for these drugs. Oxacillin (1 µg) disc or Cefoxitin (30 µg) disc was used to detect Methicillin-resistant Staphylococcus aureus. Broth macrodilution method was done to obtain MIC for Vancomycin. Staphylococcus aureus with MIC ≤ 2 µg/mL for Vancomycin was reported as sensitive.⁵ The antibiotics disc

used for Gram-negative bacilli were: Amikacin 30 µg, Amoxicillin/clavulanic acid 20/10 µg, Ceftazidime 30 µg, Cefotaxime 3 µg, Ciprofloxacin 5 µg, cotrimoxazole 1.25/23.75 µg, Ofloxacin 5 µg, gentamicin 10 µg, Imipenem 10 µg, Piperacillin/Tazobactam 100/10 µg. The quality and efficacy of the antibiotic discs checked with ATCC strains (E. coli ATCC 25922, Klebsiella pneumoniae ATCC 25923, Pseudomonas aeruginosa ATCC 27853).

Detection of beta-lactamases production in Gram-negative bacteria done with Screening of Strains with diameter of Zone of inhibition ≤ 27 mm for Cefotaxime (30 µg), ≤ 22 mm for Ceftazidime (30 µg) were considered as possible ESBL producers and selected and proceeded with confirmatory test as per CLSI guidelines such as Double disk diffusion synergy test (DDST), Phenotypic confirmatory disc diffusion test (PCDDT).⁶

Detection methods for production of Metallo β -Lactamases (MBL) in Gram-negative bacilli by Imipenem-EDTA combined disk method⁷ as per CLSI guidelines.

Detection of Methicillin-resistant Staphylococcus aureus (MRSA) was done by Disc diffusion method: Oxacillin 1 µg or Cefoxitin 30 µg disc and using Oxacillin agar.

Statistical analysis was carried out using Statistical package for Social Sciences (SPSS) by a statistician. The proportional data of this cross-sectional study were tested using Pearson's Chi Square Analysis test and Fisher exact probability test.

RESULTS

Sex	No. of Cases	Percentage
Male	69	62.7%
Female	41	37%
n=110		

Table 1. Sex Distribution of Cases

Males (62.7%) were predominantly affected when compared to females (37%) with a p-value of >0.05 , which is statistically insignificant [Table 1].

Clinical Presentation	No.	Percentage
Cough	91	82.7%
Dyspnoea	79	71.8%
Myalgia	75	68.02%
Productive Sputum	74	67.02%
Pleuritic chest pain	39	35.5%
n=110		

Table 2. Clinical Presentation of Patients to OPD

Majority of the patients with lower respiratory tract infections presented with cough (82.7%), dyspnoea (71.8%), myalgia (68.2%), sputum production (67.2%) and pleuritic chest pain (35.5%) [Table 2].

n=110		
Sample	No.	Percentage
Sputum	93	84.5%
BAL*	4	3.6%
Pleural fluid	2	1.8%
* BAL - Bronchoalveolar Lavage		

Table 3. Distribution of Sample Collected for Processing

From the 110 patients, sputum was collected from 93 (84.5%). Other respiratory specimens included bronchial wash -11 (10.0%), bronchoalveolar lavage -4 (3.6%), pleural fluid-2 (1.8%). Among the 93 sputum samples, 65 (59.1%) were positive for any one pathogen [Table 3].

Antibiotic	Klebsiella pneumoniae (n=28)	Klebsiella oxytoca (n=3)	Pseudomonas aeruginosa (n=17)	Acinetobacter baumannii (n=9)	Proteus mirabilis (n=4)	Escherichia coli (n=3)	Citrobacter koseri (n=2)
AK	25 (89.2%)	2 (66.6%)	13 (76.5%)	4 (44.4%)	4 (100%)	3 (100%)	2 (100%)
CIP	20 (71.4%)	3 (100%)	14 (82.3%)	5 (55.5%)	2 (50.0%)	2 (66.6%)	1 (50%)
GENTA	16 (57.1%)	3 (100%)	11 (64.7%)	5 (55.5%)	3 (75.0%)	3 (100%)	1 (50%)
CEF	10 (35.7%)	2 (66.6%)	8 (47.1%)	5 (55.5%)	2 (50%)	1 (33.3%)	0
CZ	13 (46.4%)	2 (66.6%)	10 (58.8%)	7 (77.7%)	3 (75.0%)	1 (33.3%)	0
OF	21 (75.0%)	2 (66.6%)	13 (76.5%)	6 (66.6%)	0	1 (33.3%)	0
CS	19 (67.8%)	3 (100%)	11 (64.7%)	8 (88.8%)	4 (100%)	2 (66.6%)	0
IMI	28 (100%)	3 (100%)	17 (100%)	9 (100%)	4 (100%)	3 (100%)	2 (100%)

Table 4. Antibacterial Sensitivity Pattern of Gram-negative Bacilli

AK-Amikacin, CIP-Ciprofloxacin, CEF-Cefotaxime, GENTA-Gentamicin, CZ-Ceftazidime, IMI-Imipenem, CS-Cefoperazone/sulbactam, OF-Ofloxacin.

All the isolated pathogens were sensitive to Imipenem among the clinical samples. Acinetobacter and Pseudomonas showed resistance to Amikacin (AK) and Cefotaxime (CEF) [Table 4].

Organism	ESBL	Amp C Beta-Lactamase
Klebsiella pneumoniae n=28	12 (42.8%)	4 (14.3%)
Klebsiella oxytoca n=3	1 (33.3%)	-
Pseudomonas aeruginosa n=17	4 (23.5%)	1 (5.8%)
Acinetobacter baumannii n=9	3 (33.3%)	-
Proteus mirabilis n=4	2 (50.0%)	-
Escherichia coli n=3	2 (66.6%)	-
Citrobacter koseri n=2	2 (100%)	-

Table 5. Detection of ESBL and Amp-C beta-lactamases Production among Gram-negative Bacilli by Screening Test

Among the clinical isolates Klebsiella pneumoniae and Pseudomonas aeruginosa producing ESBL and Amp-C Beta-lactamases. Others were ESBL producers [Table 5].

Organism	Screening Test	DDST	PCDDT
Klebsiella pneumoniae n=28	12 (42.8%)	12 (42.8%)	12 (42.8%)
Klebsiella oxytoca	1 (33.3%)	1 (33.3%)	1 (33.3%)

n=3			
Pseudomonas aeruginosa n=17	4 (57.1%)	4 (57.1%)	4 (57.1%)
Acinetobacter baumannii n=9	3 (33.3%)	3 (33.3%)	3 (33.3%)
Proteus mirabilis n=4	2 (50.0%)	2 (50.0%)	2 (50.0%)
Escherichia coli n=3	2 (66.6%)	2 (66.6%)	2 (66.6%)
Citrobacter koseri n=2	2 (100%)	2 (100%)	2 (100%)

Table 6. Detection of ESBL producers among Gram-negative Bacteria

Among the clinical isolates, Citrobacter koseri and Escherichia coli were more prevalent in ESBL production followed by Pseudomonas aeruginosa and Proteus mirabilis [Table 6].

Chart 1. Sex Distribution of Cases

Chart 2. Clinical Presentation of Patients to OPD

Chart 3. Distribution of Sample Collected for Processing

Chart 4. Antibacterial Sensitivity Pattern of Gram-negative Bacilli

Chart 5. Detection of ESBL and Amp-C Beta-lactamases Production among Gram-negative Bacilli by Screening Test

Chart 6. Detection of ESBL Producers among Gram-negative Bacteria

DISCUSSION

Lower respiratory tract infections are among the most common diseases encountered in elderly people. In most studies carried out, age is a major risk factor for both occurrence and severity of lower respiratory tract infections. The expansion of the classification of those age 65 and older has been expanded to three sub-populations commonly referred to as the young old [65 to 74 years], the old [75 to 84 years] and the old-old [≥ 85 years] groups, due to the dramatic increase in the number of people reaching age 65, coupled with their increased life expectancy.⁸

This study was conducted at Geriatric Medical ward to detect the common non-tuberculous aerobic bacterial pathogens causing lower respiratory tract infections in hospitalised geriatric patients and to determine their antimicrobial susceptibility pattern. This study also focused on the risk factors which contribute substantially to the development of lower respiratory tract infections in elderly. During the study period of one year, respiratory samples were collected from 110 hospitalised geriatric patients with lower respiratory tract infections. The respiratory specimens included 93 (84.5%) sputum samples, 11 (10.0%) bronchial washings, 4 (3.6%) bronchoalveolar lavages and 2 (1.8%) pleural fluid samples. Among the 110 samples, 65 (59.1%) exhibited significant bacterial growth. This result is in consistence with the study of Tamang MD et al, in which respiratory pathogens were recovered from 50.41% of sputum samples.⁹

In this study, males (62.7%) were found to be predominantly affected than females (37.3%). In the retrospective study of 455 elderly patients, conducted by E Hak et al from Netherlands, a higher prevalence of males (63%) with lower respiratory tract infections was found.¹⁰ Similar finding was also observed in the study by Adel Khattab et al from Egypt, in which 61.4% of patients with LRTI were males.¹¹

In this study, an acute exacerbation of chronic obstructive pulmonary disease (55.5%) was the most common presentation of LRTI. This is also statistically significant, with a p-value of 0.04. Pneumonia and acute bronchitis accounted for 36.4% and 8.2% respectively. Bont J et al in their study also showed a higher incidence of acute exacerbations of chronic obstructive pulmonary disease (48.1%), when compared to acute bronchitis (35.3%) and pneumonia (16.5%).¹²

History of smoking was presented by 56 male (50.9%) patients. The p-value obtained for smoking was < 0.01 . This shows significant association of smoking with LRTI. The

prevalence of the predisposing factors like hypertension (53.6%), diabetes mellitus (49.1%), alcohol intake (44.5%) and cardiac disease (17.3%) was determined. In the Leiden 85-plus study of A. Sliedrecht et al, smoking was mentioned as the greatest contributor of LRTI with a population attributable risk of 32%.¹³ Irma Koivula et al in their study in Finland populations, observed hypertension in 36.4% of the total 184 cases. They also reported the prevalence of diabetes mellitus (13.1%), alcoholism (2.2%) and cardiac disease (17.3%) among the elderly people.

In the present study, atypical features like myalgia (68.2%), confusion (18.2%), vomiting (10%) and diarrhoea (1.8%) were observed in the elderly people with LRTI, along with respiratory symptoms like cough (82.7%), sputum production (67.3%) and pleuritic chest pain (35.5%). Cough was the most common symptom observed in our study. The statistical analysis depicts a strong association of cough with lower respiratory tract infections, with a p-value of 0.0029.

The susceptibilities of the bacterial isolates towards the various antibiotics were determined by disc diffusion method and broth macrodilution method. The most prevalent organism, *Klebsiella pneumoniae* reported 100% sensitivity to Imipenem, followed by Amikacin (89.2%), Ofloxacin (75.0%), Ciprofloxacin (71.4%), Cefoperazone/Sulbactam (67.8%), Gentamicin (57.1%), Ceftazidime (46.4%) and Cefotaxime (35.7%). Christopher Aye Egbe et al in their study had reported the sensitivity pattern of *Klebsiella pneumoniae*, obtained from samples of patients with LRTI.¹⁴ They showed sensitivity to Ofloxacin (95.6%), Ciprofloxacin (93.5%), Ceftazidime (88.0%) and Gentamicin (85.9%).

Among the 76 pathogens isolated, 26 (34.2%) were Extended Spectrum Beta-Lactamase (ESBL) producers. *Klebsiella pneumoniae* was the predominant ESBL producing organism. 12 (42.8%) strains of the 28 isolates of *Klebsiella pneumoniae* were confirmed to be ESBL producers by double disk diffusion synergy test and CLSI phenotypic confirmatory disc diffusion test. Broth Macrodilution Method was also done for the ESBL producing *Klebsiella pneumoniae* isolates. A decrease in more than three two-fold dilutions of MIC for Cefotaxime/Sulbactam versus the MIC for Cefotaxime was noted.

In the present study, among the 17 isolates of *Pseudomonas aeruginosa* 4 (57.1%) strains were ESBL producers. Five (6.6%) Amp-C Beta-Lactamase producing Gram-negative bacteria were determined in this study by screening test. *Klebsiella pneumoniae* accounted the majority of Amp-C Beta-Lactamase producing organisms. Out of 12 strains of *Klebsiella pneumoniae*, 4 (33.3%) strains were screened as Amp-C beta-lactamase producers. In this study, all the isolates of Gram-negative bacteria were sensitive to Imipenem.¹⁵

In the study, 7.9% of the total isolates were found to be Methicillin-Resistant *Staphylococcus aureus* (MRSA). Out of the 8 isolates of *Staphylococcus aureus*, 6 (75%) isolates were MRSA. All the strains of *Staphylococcus aureus* were sensitive to Vancomycin.

Blood cultures from the 110 patients yielded 3 pathogens (2.7%) including two strains of *Klebsiella pneumoniae* and one isolate of *Streptococcus pneumoniae*. The sputum from the patients showing positive blood culture also reported the same pathogens with similar antibiogram. This finding was

supported by the Nima Afshar et al in their systematic review, in which they reported 0% to 14% of cases of community-acquired pneumonia showing true blood culture results.¹⁶

CONCLUSION

From this prospective study, it is quite clear that the elderly patients are at high risk for contracting lower respiratory tract infections due to Gram-negative bacteria. The emergence of resistant strains in the patients of the present study emphasises the need of prudent use of antibiotics.

REFERENCES

- [1] Strausbaugh LJ. Emerging health care-associated infections in the geriatric population. *Emerging Infectious Diseases* 2001;7(2):268-71.
- [2] Conlon CP, David R. Mosby's color atlas and text of infectious diseases. 7th edn. Chapter 4, 2010:67-76.
- [3] Bonomo RA. Resistant pathogens in respiratory tract infections in older people. *J Am geriatr Soc* 2002;50(7 Suppl):S236-41.
- [4] Fox RA. Atypical presentation of geriatric infections. *Geriatrics* 1988;43(5):58-9, 63-4, 68.
- [5] Clinical and laboratory standards for antimicrobial susceptibility testing; twenty-first informational supplement M 100-S21, 2011.
- [6] Ruben FL, Dearwater SR, Norden CW, et al. Clinical infections in the noninstitutionalized geriatric age group: methods utilized and incidence of infections. The Pittsburgh good health study. *AM J Epidemiol* 1995;141(2):145-57.
- [7] Singhal S, Mathur T, Khan S, et al. Evaluation of methods for AmpC beta-lactamase in gram negative clinical isolates from tertiary care hospitals. *Indian J Med Microbial* 2005;23(2):120-4.
- [8] The demographic of aging. Transgenerational design. Pediatrics use and geriatric use of drug and biological products. CQ'S WEB blog on the issues in biostatistics and clinical trials 2009.
- [9] Tamang MD, Deys S, Makaju RK, et al. Prevalence of *Moraxella catarrhalis* infections of the lower respiratory tract in elderly patients. *Kathmandu University medical Journal* 2005;3(9):39-44.
- [10] Cook NB. Textbook of Fundamentals of Diagnostic Mycology. 14th edn. W.B. Saunders 1998:P 352.
- [11] Khattab A, Said A, Raafat H, et al. Study of the pattern of respiratory infections in elderly patients at ain shams university hospitals. *Egyptian Journal of Bronchology* 2010;4(2).
- [12] Bont J, Hake E, Hoes AW, et al. A prediction rule for elderly primary care patients with lower respiratory tract infections. *Eur Respir J* 2007;29(5):969-75.
- [13] Slidrecht A, Verheij TJ, Westendorp RG, et al. Incidence and predictive factors of lower respiratory tract infections among the very elderly in the general population. The Leiden 85-plus study. *Thorax* 2008;63(9):817-22.
- [14] Egbe CA, Ndiokwere C, Omoregie R. Microbiology of lower respiratory tract infections in Benin city, Nigeria. *Malaysian J Med Sci* 2011;18(2):27-31.

- [15] Aggarwal R, Chaudry U, Bala K. Detection of extended-spectrum β -lactamase in *pseudomonas aeruginosa*. Indian Journal of Pathology and Microbiology 2008;51(2):222-4.
- [16] Afshar N, Tabas J, Afshar K, et al. Blood cultures for community-acquired pneumonia: are they worthy of two quality measures? A systematic review. Journal of Hospital Medicine 2009;4(2):112-23.