Research Paper

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Prevalence and Antimicrobial Susceptibility of Pyogenic Microorganisms Isolated from Clinical Specimens of Patients of Lower Respiratory Infection

Medical Science

KEYWORDS : Antimicrobial susceptibility, Metallo-β-lactamase, Methicillin resistance

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ABSTRACT

Background :New emerging pathogens and associated antimicrobial resistance mechanisms have been observed in the respiratory tract of patients suffering from pyogenic infection in the last decade. Prevalence rate of metallo-β-lactamase (MBL)-producing Pseudomonas aeruginosa strains, Carbamapenase producing enterobacteriaceae, is growing, apart from prevalent ESBL positive E.coli & Klebsiella pneumoniae. Methods :In this study, 250 respiratory samples (Sputum) of were collected during a 24 months period. Microbiological cultures and antimicrobial susceptibility tests of the most frequently isolated bacteria were performed by standard disk diffusion method as per CLSI guidelines. Results : Out of 250 sputum samples, 142 pathogenic bacteria were isolated and characterized. Among the bacteria, Streptococcus pneumonia, Staphylococcus aureus (both MSSA & MRSA) & P. aeruginosa (Both MBL producing & Non MBL producing) showed highest prevalence. Conclusions : The detection of MBL-producing P. aeruginosa and MRSA in patients confirms that antimicrobial resistance patterns should be always kept under surveillance. Moreover hygiene regulations in pulmonary clinics should prevent a further spread of resistant bacterial strains.

Introduction

The main causes for the high morbidity and mortality in patients with recurrent respiratory infections are predisposing conditions like smoking, viral infection in throat, extreme of age etc. Impairment of the muco-ciliary transport and thus the cleaning function of the upper airway predisposes bacterial colonization which turn into invasion [1]. Colonization is often initiated by respiratory commensal & Later on, becomes more complex when *Pseudomonas aeruginosa* and other gram-negative bacilli invade [2], [3] and [4]. During the course of therapy, these gram negative bacilli develope resistant mutant to ongoing antibiotic therapy, which is more difficult to eradicate[1], [5], [6] and [7]. The rate of methicillin resistance in *S. aureus* is rising [10] and the proportion of ESBL positive Enterobacteriaceae & metallo-β-lactamase-producing *P. aeruginosa* strains is growing [11], [12], [13], [14] and [15]. A continuous microbiological surveillance of the pathogens and a monitoring of the resistance situation is therefore of utmost importance. The aim of this study was to describe the prevalence of bacterial microorganisms in different age groups and to evaluate the antimicrobial susceptibility patterns of the common bacterial pathogens in respiratory illness during a two-year period.

Materials and methods

Patients and samples

Total 250 sputum samples (173 male patients and 77 female patients) were collected at tertiary care hospital affiliated with medical college in western India. The median age of the patients was 18 years, the range of age 6–71 years.

Microbiological cultures

Early morning Sputum was collected from each patient after vigorous gargling with drinking water to wash out oral commensal in sterile universal container. Each patient was instructed to take due possible care that not mix saliva with sputum & expectorate sputum only after deep breathing followed by coughing. Each sample transported immediately to Bacteriology laboratory. In laboratory, each sputum was primarily assessed for it's quality by physical examination. Sputum mixed with saliva or pure salivary samples were rejected. Only mucoid or muco-purulent or purulent sputum were accepted for further investigation, rest of the sputum were rejected & asked patients to collect sputum on next day morning. Each sample were inoculated primarily on Nutrient agar, Blood agar, Chocolate agar & Mac conkey agar & incubated for overnight incubation at 37^0 C. If any growth was observed on next day, identification done by biochemical reaction & Antibiotic susceptibility by disc diffusion method according to the CLSI (formerly NCCLS) criteria were performed. If growth was not observed on next day, further another overnight incubation done at 37^0 C. In order to ruled out mycobacterial infection, each sputum were screened by ZN stain.

Results

A total of 250 sputum samples were collected during a 24-months period. Out of which, 142 strains were isolated and characterized. A sum total of 108 sputum samples showed growth of mix commensal flora of oral cavity. They were considered as a non-pathogenic organism for lower respiratory tract infection. With regard to the prevalence of bacteria in the cohort under study, 36.6% of the isolates were *Strep. pneomoniae*, 27.4% *Staph. aureus*, 20.5 % *P. aeruginosa*, 9.8 % *Klebsiella pneumonia,* 2.9% *H. influenzae*, 2.9% *E.coli*. Out of 29 the *P. aeruginosa,* 9 isolates (6.4%) showed a mucoid phenotype, which are mainly from patients with cystic fibrosis. With regard to the small-colony variant phenotype (SCV), 2 out of 20 non mucoid strain of *P. aeruginosa* (10%) isolates had SCVs., in contrast no SCVs of *Staph. aureus* were detected **(Table 1)**.

Table 1. Prevalence of bacterial species in sputum sample

Bacterial species	No. of isolates (%)
Staphylococcus aureus	39 (27.4%)
Streptococcus pneumonia	52 (36.6 %)
klebsiella pneumonia	14 (9.8 %)
Pseudomonas aeruginosa (Non mucoid isolates)	20 (14.1 %)
Pseudomonas aeruginosa (Mucoid isolates)	9 (6.4 %)
Escherichia coli	4 (2.8 %)
Haemophilus influenza	4 (2.8 %)
Total	142

An analysis of the prevalence of the various bacterial species in different age groups showed a constant increase of rate for mucoid and non mucoid *P. aeruginosa.* No mucoid isolates were detected in patients in the age group of 6–10 years. In contrast, the highest rate for *H. influenza* and *S. aureus* was observed in patients in the age groups of 6–10 years and 11–15 years, respectively. *Strep. Pneumonia* were observed mainly in middle age group (20 – 50 years), considering them as community acquired pathogen. In our study, Mucoid *P. aeruginosa* isolates exhibited a much higher antimicrobial susceptibility than the non mucoid strains. There is no predilection of age for isolated *Klebsiella pneumonia. E.coli* were commonly isolated in age group of 11 – 20 years. **(Table 2)**

Table 2. Bacterial isolates in different age groups

Age (years)	Non mucoid P. aeruginosa	Mucoid P. aeruginosa	S. aureus	H. influenzae	Strep. pneumoniae	E.coli	Klebsiella pneumonia	TOTAL
6–10	0	0	3	4	3	1	3	14
11–20	0	0	20	0	9	3	4	36
21–50	5	3	10	0	27	0	2	47
51–71	15	6	6	0	13	0	5	45
TOTAL	20	9	39	4	52	4	14	142

Table 3. Rate of susceptibility (%) of isolates against different antibacterial agents

Antibotics	Non mucoid P. aeruginosa (n = 20)	Mucoid P. aeruginosa (n = 9)	S. aureus (n = 39)	H. influenza (n = 4)	Strep. Pneumonia (n = 52)	E.coli (n = 4)	Klebsiella pneumonia (n = 14)
Piperacillin	0	3	19	3	21	0	1
Ceftazidime	12	9	4	Not tested	5	0	2
Cefotaxime	2	6	6	4	45	0	2
Meropenem	18	9	32	4	52	4	13
Colistin Sulphate	20	9	Not tested	4	Not tested	4	14
Penicillin G	Not tested	Not tested	4	0	6	Not tested	Not tested
Cefazoline	Not tested	Not tested	31	2	39	0	0
Amikacin	15	8	29	4	26	3	11
Ciprofloxacin	6	3	10	3	16	0	4
Vancomycin	Not tested	Not tested	39	Not tested	52	Not tested	Not tested
Amoxycillin + Clavulanic acid	Not tested	Not tested	30	4	46	4	10
Oxacillin	Not tested	Not tested	29	0	27	Not tested	Not tested

Discussion

Prevalence and antimicrobial susceptibility of microorganisms isolated from respiratory samples of patients of LRTI have been described in several studies [3],[17],[18],[19],[20]. Similar to results reported by others, chronic respiratory tract infection of patients is caused mainly by bacteria such as *Staph. aureus, P. aeruginosa* and *H. influenzae*. In concordance with a previously published report [4], we observed that during the first decade of life, *Staph. aureus* and *H. influenzae* are the most commonly isolated bacteria from sputum, whereas in the second and third decade these species become less frequent, concomitant with a constant increase rate for gram negative bacilli infection.

With regard to the antimicrobial susceptibility, methicillin resistant *Staph. aureus* (MRSA) occurred in 10 patients (25.4%) of our study. In contrast, MRSA prevalence is 19.8% [3]; 25.9%[17],18%[19] and 6%[18] in various parts of the world. Presumably the differences in the MRSA rates in patients correlate with the general nosocomial prevalence of MRSA of various parts of the world.

Nosocomial outbreaks of metallo-β-lactamase (MBL)-producing *P. aeruginosa* have been already described in Japan [11],[14] , in Italy [12], in Brasil [13] and in Canada [15]. However, to the best of our knowledge, we report for the first time the prevalence rate of MBL-producing *P. aeruginosa* in patients. A systematic screening of these multi resistant strains is very important for a correct antimicrobial treatment, since MBL-producing strains are resistant to β-lactam-antibiotics. In our study, only 33% of MBL-producing *P. aeruginosa* were susceptible to ciprofloxacin but the All of them were still susceptible to colistin, a resurrected second-line therapeutic agent. Meropenem was also resistant in 10 % of non mucoid strain of *P. aeruginosa.* One strain of *Klebsiella pneumonia* was also resistant to carbapenem. All strains of *E.coli* (4 out of 4) & 12 out of 14 strain of *Klebsiella pneumonia were* resistant to 3rd generation of Cephalosporin which may be due to ESBL production. We had not observed vancomycin resistance in *Staph. aureus*. Total 35 strain of *staph. aureus* out of 39 strain, were resistant to penicillin G, which is most likely due to β-lactamase enzyme production by *staph. aureus.* Amikacin had shown mild to moderate degree of resistance in both gram positive & gram negative bacteria. So, amikacin is the best choice for institution of empirical antibiotic when clinician is not sure that probable pathogen is gram positive or gram negative.

In our study, mucoid *P. aeruginosa* isolates exhibited a much higher antimicrobial susceptibility than the non mucoid ones. Independently from the antimicrobial susceptibility, mucoid strains in respiratory samples represent a non favourable prognostic factor, since they produce exopolysaccharide/alginate and consequently are resistant to phagocytosis. Furthermore they play a much greater role in lung disease progression than non mucoid *P. aeruginosa* [1], [5], [6] and [7].

Conclusions

The prevalence of different phenotypes of microorganisms in sputum of patients with respiratory illness should be closely monitored. Moreover, antimicrobial resistance patterns should be kept under surveillance, especially the occurrence of methicillin resistance in *S. aureus*, ESBL production in Enterobacteriaceae and MBL production *P. aeruginosa* respectively. Hygiene regulations in pulmonary clinics should prevent a further spread of resistant bacterial strains, as antibacterial treatment options are limited in these patients.

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