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Detection of Anaerobic Bacteria in Bronchoalveolar Lavage of Patients with Chronic Chest Lesion

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ABSTRACT

Anaerobic bacteria have important roles in the microbiota of humans and they are significant infectious agents involved in many pathological processes, both in immunocompetent and immunocompromised individuals. The objectives of this study is to detect anaerobic bacteria in bronchoalveolar lavage samples of patients with chronic chest Lesion in chest department in Beni -Suef university hospital.

Patients & Methods : Each sample taken was placed immediately in previously prepared vacutainer containing thioglycolate media. Specimens from Thioglycollate broth after incubation were cultured on Brucella agar (Thermo Scientific™ Oxoid™) supplemented with 5% lysed sheep blood, hemin and Vitamin K1 . The plates were incubated at 37° c for 48-27 h in anaerobic condition. Commercial Kits RapID™ANA II System (Thermo Scientific™ Remel™) used for the identification of anaerobic isolates. Antimicrobial susceptibility testing for anaerobic isolates were done using MIC Test Strip (Liofilchem, Italy).

Results : The isolated anaerobic organism from BAL specimens were 7 out of 25(28%). The anaerobic isolates were *Prevotella* (3), *Veillonella* (2), *Lactobacillus* (1) and *Propionibacterium* (1).

Conclusion : Anaerobic bacteria are the most overlooked bacterial pathogens of the lower respiratory tract. They are often infrequently recovered from this site probably because of improper specimen collection and lack of appropriate anaerobic identification techniques.

Keywords : Anaerobe, Bronchoalveolar Lavage and Lung Abscess

I. INTRODUCTION

Anaerobic bacteria are relatively frequent pathogens in pulmonary infections that are associated with aspiration pneumonitis, lung abscess, necrotizing pneumonia and empyema [1].

Aspiration of oropharyngeal or gastric content, and severe periodontal or gingival disease predispose for anaerobic pleuropulmonary infection. The infection can progress from pneumonitis to necrotizing pneumonia, pulmonary abscess, and empyema [2].

Anaerobic bacteria are involved in all morbid conditions of respiratory infections, and the frequencies of their isolation are high in pneumonia and at the time of acute exacerbation of chronic lower airway infection. They are detected in high rate in lung abscess and pyothorax cases. Thus, anaerobic bacteria should not be neglected, because the frequency of their detection varies considerably among morbid conditions, and anaerobic bacteria do not merely drift down from the upper airway but also show major involvement in morbid conditions. It should be recognized that anaerobic bacteria are extensively involved not only in lung abscess and

aspiration pneumonia, in which anaerobic infection has conventionally been indicated to participate, but also pneumonia and acute exacerbation of chronic lower airway infection [3].

II. PATIENTS AND METHODS

Subjects and data collection: The study was conducted on 25 patients admitted in the departments of chest in BeniSuef University Hospital .The patients were diagnosed as having chronic chest lesions (10 with lung abscess and 10 with lung tumor).The age ranged from 50 to 73 years old. From each patient a full history was taken as regards name, age and sex. All patients were advised to stop antimicrobial therapy at least 3 days before taken the samples. All procedures were done after taken a written concent from the patients.

Sample processing and bacterial identification : BAL were collected by bronchoscope. Specimens inoculated directly on previously prepared vacutainer containing Thioglycollate broth (Thermo Scientific™ Oxoid™) and incubated anaerobically for 48-72 hours at 37°c.. Specimens from Thioglycollate broth after incubation were cultured on Brucella agar (Thermo Scientific™ Oxoid™) supplemented with 5% lysed sheep blood, hemin and Vitamin K1 .The plates were incubated at 37° c for 48-27 h in anaerobic jar with self-contained gas generating systems (Oxoid AnaeroGen, Thermo Scientific™ Oxoid™). Commercial KitsRapID™ ANAII System (Thermo Scientific™ Remel™) used for the identification of anaerobic isolates Procedure and interpretation of the method were followed according to manufacturer's instructions. (Thermo Scientific™ Remel™).

Antibiotic susceptibility testing: Antimicrobial Susceptibility testing for anaerobic isolates were done using MIC Test Strip (Liofilchem, Italy). The interpretations were done according to CLSI-M100 guidelines [4]. The antibiotic used were: amoxicillin/clavlanic acid (AUG), clindamycin (CD), meropenem (MRP) and metronidazole (MTZ).

Medium used was Brucella agar (Thermo Scientific™ Oxoid™) supplemented with 5% lysed sheep blood, 5 μg/mL hemin and 1 μg/mL Vitamin K1.
Interpretative MIC breakpoints of anaerobic species was done according to CLSI-M100 guidelines [4].

III. RESULTS AND DISCUSSION

The isolated anaerobic organism from BAL specimens were 7 out of 25(28%). From lung abscess 5 cases yielded anaerobic organisms (50%) while in cases of lung tumor only two anaerobic isolates (13.3%) as shown in Table (1).

Table (1) Frequency of anaerobic organism isolated from broncho alveolar lavage (BAL)

	BAL(25)	
	Lung abscess (10)	**lung tumor (15)**
Anaerobic organism	Number (%) 5(50%)	Number (%) 2 (13.3%)
Prevotella	3 (60%)	0(0%)
Veillonella	2(40%)	0(0%)
Probionobacterium	0(0%)	1(50%)
Lactobacillus	0	1 (50%)
No growth	5	13

All the identified anaerobic isolates were sensitive to meropenem and Amoxicillin/clavulanic all isolates

were sensitive to this drug. Clindamycin was effective on *Lactobacillus and Veillonella* (100%). *Prevotella* and *Propionobacterium* isolates showed 66.6% and 100% resistance to clindamycin respectively. As regards the resistance of the isolates to metronidazole, *both Lactobacilli* and *Probionobacterium* isolates were resistance to metronidazole. **Table(2).**

Table (2) : Antimicrobial Susceptibility

Antibiotic	Meropenem		Amoxicillin-clav		Clindamycin		Metronidazole	
	S	R	S	R	S	R	S	R
Prevotella(3)	3 (100%)	0 (0%)	3 (100%)	0 (0%)	1 (33.3%)	2 (66.6%)	3 (100%)	0 (0%)
Veillonella (2)	2 (100%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)	2 (100%)	0 (0%)
Lactobacillus(1)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)
Propionibacterium (1)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)

Discussion

Anaerobic bacteria are relatively frequent pathogens in pulmonary infections including aspiration pneumonitis, lung abscess, necrotizing pneumonia and empyema [1].

The isolated anaerobic organism from BAL specimens were 7 out of 25(28%). From lung abscess 5 cases yielded anaerobic organisms (50%) while in cases of lung tumor only two anaerobic isolates. The anaerobic isolates were *Prevotella*(3), *Veillonella* (2), *Lactobacillus* (1) and *Propionibacterium* (1). *Prevotella* is predominant isolated anaerobes, 3 out of 7 with incidence 42.8%.

Prevotella spp. are members of the oral, vaginal, and gut microbiota and are often recovered from anaerobic infections of the respiratory tract. These infections include aspiration pneumonia, lung abscess, pulmonary empyema, and chronic otitis media and sinusitis [5].

Most of anaerobic lung infections involve multiple bacterial species, and approximately half of the patients have anaerobic bacteria combined with potentially pathogenic aerobic or facultative anaerobes [6].

A somewhat unique feature of anaerobic lung infections is the proclivity for necrosis of tissue, resulting in abscess formation or a bronchopleural fistula associated with empyema [7].

In our study; the frequency of anaerobic organism among lung abscess was 5out of 10 (50%). Isolated

organisms were *Prevotella* and *vellionella.* In contrast; the study done by Wang and colleagues [8] who reported that incidence of anaerobic organism in lung abscess was 39%. Mori and colleagues [9] found that anaerobic incidence in lung abscess was 45%. According to Takayanagi and colleagues [10] it was 26%. On the other hand other studies show incidence which more higher 93% by Bartlett [7] and 100% by De and colleagues [11].

Lung abscess caused by anaerobic or mixed bacterial infection of the lower respiratory tract [12]. *Prevotella* and *Vellionella* and other anaerobic organism isolated from lung abscess according to by De and colleagues [11]. and Mori and colleagues [9]. The differences in the design of these studies such as sample size and method of identification may account for the variation in incidence of isolated organisms.

All the identified anaerobic isolates were susceptible to meropenem and amoxicillin/clavulanic . Anaerobic bacteria susceptible to meropenem, which is used in the treatment of mixed aerobic and anaerobic pulmonary infection [13]. Carbapenems possess excellent activity against aerobic and anaerobic bacteria and are often administered in serious infections[14].

In the present study, 2 out of 3 *Prevotella* isolates were resistant to clindamycin 66.6%. Sherrard and colleagues [15] reported that all *Prevotella* isolates in their study were susceptible to meropenem, 56% were clindamycin resistant, 5% were metronidazole resistant and 20% were resistant to amoxicillin/clavulanic.

Clindamycin was considered the drug of choice for the treatment of anaerobic infections but with the emergence of resistance among *Prevotella* spp. (10–40%), other related anaerobic Gram-negative bacteria (~10%) and *Peptostreptococcus* spp (~10%), this drug lost its significance as a first-line drug [16].

In the present study, both *Lactobacilli* and *Probionobacterium* isolates were resistant to metronidazole. Khassebaf and coleagues [17] reported *Propionobacterium* resistance 100% to metronidazole and 10% to clindamycin. On the other hand Hassan and colleagues [18] reported high *Propionobacterium* resistance to clindamycin (70%).

Metronidazole resistance is common in many Gram-positive anaerobic rods (*Actinomyces spp., Propionibacterium spp., Lactobacillus spp.*), while the prevalence of resistant Gram-positive cocci and Gram-negatives is usually very low (<1%) [19].

IV. CONCLUSION

In conclusion anaerobic bacteria were the most overlooked bacterial pathogens of the lower respiratory tract. They were often infrequently recovered from this site probably because of improper specimen collection and lack of appropriate anaerobic identification techniques. Anaerobic microorganisms are now widely accepted as significant pathogens in human diseases, as such, the proper diagnosis and treatment of these infections are important healthcare priorities. The emergence of antimicrobial resistance in anaerobic bacteria is a discernible phenomenon, which deserved the attention of people working in diagnostic microbiology and infectious disease treatment.

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