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Microbiological Pattern and Antibiotic Susceptibility of Isolates from Bronchiectasis

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Bronchiectasis is one of the complicated diseases of lower Respiratory Tract. Present study was done to analyze the pattern and antibiotic resistance of bacterial isolates in BE cases. BAL samples were collected from 150 patients, and identified by standard methods. Antibiotic susceptibility of isolates was done by Kirby-Bauer method, from 56 (37.3%) out of 150 BE cases PPMs were isolated. The most frequent isolates were *S.aureus* (21 cases; 37.5%) and *Klebsiella* spp. (16 cases; 28.5%), Three (37.5%) out of 8 Acinetobacter isolates were resistant to imipenem. All of the citrobacter isolates were sensitive to imipenem, Cefepiem, gentamicin, ceftriaxon and ciprofloxacin, and *Klebsiella* isolates were sensitive to all mentioned antibiotics except than ceftriaxone. In gram positive isolates 8(38%) and 3(14%) out of 21 *S.aureus* isolates were resistant against methicillin and vancomycin respectively. The most high sensitivity in *S.aureus* isolates was seen against vancomycin(86%) and then ceftriaxon (71.46%) and tetracycline(71.46%). Results of present study clearly indicates that majority of isolates from BE cases have resistance against commonly used and in some cases choice antibiotics. These findings alarming the health and hospital officials, and emphasizing that screening and surveillance programs should be implemented in necessary conditions.

Key words: Bronchiectasis(BE), Antibiotic Resistance, Lower Respiratory Tract(LTR).

Bronchiectasis(BE) is defined as enlargement and inflammation of bronchial airways of LRT that converts to pockets with destruction of bronchial walls. This process in turn leads to dysfunction of cleaning system in lungs. In addition, losing the functionality of ciliary system causes to accumulation of dust particles, microbes and mucus in bronchioles, which finally predisposes the lungs and other organs to complicated and untreatable infections[1-3].

In fact LRT is sterile in non-smoking humans. But in patients with BE and COPD, lower sections of respiratory system usually colonizing with opportunistic pathogens called PPMs. Scientific evidences indicate that colonization of distal airways with mentioned organisms have the potential of infection in BE patients. Microbial colonization induces inflammatory mediators that may be exaggerates the destruction of lung tissue and airways. The chronic colonization of microbes in lung, secondary inflammatory reactions and progressive destruction of lung makes a vicious

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cycle that necessitates the study of microbes' type and antibiotic susceptibilities, colonizing in the distal air ways for ideal management of disease. For breaking of this closed cycle definite identification of colonized bacteria, and finding proper selective antibiotic of treatment is necessary[1, 3-8].

Some of the intrinsic and extrinsic factors including immunodeficiency, intrinsic abnormalities, childhood respiratory infections (severe pneumonia, pertussis, complicated measles and tuberculosis (TB) entrance of external objects (causing obstruction of airways), reflux of stomach acid, and genetic factors (such as cyctic fibrosis) predisposing humans to BE.[1, 3-5, 9]

Although the European Respiratory Society (ERS) do not recommends microbiological research and routine antibiotic prescription in lower respiratory tract infections[10] , But bacteriologic analysis of BE patients have been studied by many researches all over the world[11-15]. although in special situations such as exacerbations of COPD or BE, medical interfering including suitable antibiotics is necessary[8, 10]. On the other hand it is confirmed in many of studies that colonization of LTR with especially PPMs predisposes patients to dangerous and urgent conditions. In some of the studies it is indicated that BE score is related to the type of colonized bacterium, and also in exacerbations of BE carrier rate of PPMs increases in comparison with clinically stable BE.[3, 12, 15-16] it is also evident that resistance pattern of colonized organisms have been changing in recent decades, and MICs of different antibiotic increasing[17]. So based on the facts mentioned the aim of this study was to identify colonized bacteria and their antibiotic sensitivity pattern in BE patients and comparison of results with similar studies.

MATERIAL AND METHODS

Patients

Ninety five percent (142 cases), and 5 %(8cases) of patients were male and female respectively, that have confirmed by a trained internists using clinical presentation and High-Resolution Computerized Tomography Scan of chest (HRCT-Scan) in Baqiyatallah Hospital, Tehran, Iran. 150 Samples (BAL) were collected by bronchoscopes in sterile conditions and referred to the microbiology laboratory without delay. Then samples were cultured on Blood Agar, Chocolate Agar, Cetrimid Agar and Sabouraud Dextrose Agar for routine aerobic or facultative bacteria, and isolates were identified by standard methods and antimicrobial susceptibility of bacteria determined by Kirby-Bauer method[5].

MAR index

The multiple antibiotic resistance (MAR) index of total isolates was calculated for evaluation of resistance condition in BE patients. MAR index was determined by dividing the number of antibiotics to which the isolate is resistant to the total number of antibiotics tested.

MAR index = Number of antibiotics isolate is resistant to/ Total number of antibiotics tested[18].

RESULTS

From One hundred six (70.66%) out of 150 clinically confirmed cases of BE colonies of bacteria were isolated, 56(37.3%) out of 106 cases PPMs were isolated from patient's samples. Ninety five percent (142 cases), and 5 %(8cases) of patients were male and female respectively, the majority of patients have cylindrical type of BE, and minor percent have varicose type BE. The most frequent isolate were *S.aureus* (21 cases; 37.5%) and Klebsiella spp. (16 cases; 28.5%). Fifty (33%) patients colonized with a mixed group of bacteria that omitted from study. From 44(29.3%) cases no growth of colony was observed. Type and frequency of isolated bacteria have been indicated in Table1.

Table 2 shows resistance pattern of gram negative isolates against selected antibiotics. Three (37.5%) out of 8 Acinetobacter isolates were resistant to imipenem. Acinetobacter spp. was resistant completely against amoxicillin and tetracycline. The highest sensitivity between Acinetobacter spp. was observed to gentamicin(50%). All of the citrobacter isolates were sensitive to imipenem, Cefepiem, gentamicin, ceftriaxon and ciprofloxacin, and klebsiella isolates were sensitive to all mentioned antibiotics except than ceftriaxone. (Table2)

In gram positive isolates 8(38%) and 3(14%) out of 21 *S.aureus* isolates were resistant against methicillin and vancomycin respectively. The most high sensitivity in *S.aureus* isolates was

seen against vancomycin(86%) and then ceftriaxon (71.46%) and tetracycline(71.46%). Streptococci isolates were 85% and 100% sensitive to azitromycin and tetracycline respectively. (Table3)

As indicated in table 4 more than 55% of isolates have MAR index of higher than 0.2, simply implies that majority of isolates have been originated from environments that antibiotic using vastly.

DISCUSSION

Findings of this study indicate that bacterial genus and species such as acinetobacter , citrobacter, klebsiella, *S.aureus* and streptococci have important role in infections of BE patients. In cases of acinetobacter and *S.aureus* resistance to distinguished antibiotics of imipenem, methicillin and vancomycin respectively, implies that high percent of isolates originating from hospital settings and MAR index also confirms that majority of isolates have been cultured from an environment of extensively using antibiotics. Clinical presentation and HRCT have been used in diagnosis of BE cases. Although there was no association between etiology and the results of CT scan in differential diagnosis of BE[9]. But High-resolution computed tomography

Table 1. Frequency of PPMs isolated from total of 150 patients

	Acinetobacter spp.	*Citrobacter* spp.	*Klebsiella* spp.	*S.aureus*	*Streptococci* spp.	Total / percent
Number(percent)	8(5.3%)	6(4%)	16(10.6%)	21(14%)	5(3.3%)	56(37.3%)

Table 2. Gram negative bacilli isolated from BE and pattern of antibiotic resistance

		Acinetobacter spp.	*Citrobacter* spp.	*Klebsiella* spp.
MEM	R	3(37.5%)		
	I			
	S	5(62.5%)	6(100%)	16(100%)
AMX	R	8(100%)	2(33.3%)	13(81.25%)
	I		2(33.3%)	1(6.25%)
	S		2(33.3%)	2(12.5%)
CPM	R	7(87.5%)		
	I	1(12.5%)		
	S		6(100%)	16(100%)
T	R	8(100%)	6(100%)	
	I			
	S			16(100%)
GM	R	4(50%)		
	I			
	S	4(50%)	6(100%)	16(100%)
CRO	R	4(50%)		6(37.5%)
	I	1(12.5%)		1(6.25%)
	S	3(37.5%)	6(100%)	9(56.25%)
KAZ	R	6(75%)	6(100%)	4(25%)
	I	2(25%)		
	S			12(75%)
CP	R	4(50%)		
	I	3(37.5%)		
	S	1(12.5%)	6(100%)	16(100%)

R:Resistant, I: Intermediate, S: Sensitive, MEM : Meropenem 10 µg, AMX:Amoxicillin 20 µg, CPM: Cefepiem 30 µg, T: Tetracycline 30µg, GM : Gentamicin 10 µg, CRO: Ceftriaxon 30 µg , KAZ: Ceftazidime 30 µg, CP:Ciprofloxacin 5 µg

(HRCT) is a reliable and noninvasive method for the diagnosis of BE.[1, 3, 9]

According to different studies in 26% of the BE cases, etiological cause was not found, and in some of the studies in higher percent of patients there is not found definite etiologies. [9, 16] In one study from Northern provinces of Iran for evaluating the microbiological pattern of BAL samples of established BE patients it has been reported that *S. pneumonia* is the most prevalent pathogen, and 85% of patient's lungs have been colonized with potential pathogens. They also reported that rate of colonization in studied area is

Table3. Gram positive cocci isolated from BE and pattern of antibiotic resistance

		S.aureus	*Streptococcus* spp.
OX	R	8 (38%)	5(100%)
	I	3(14%)	
	S	10(48%)	
AMX	R	9(42.8%)	5(100%)
	I	1(4.76%)	
	S	11(50.2%)	
CPM	R	6(28.54%)	5(100%)
	I		
	S	15(71.46%)	
T	R	6(28.54%)	
	I		
	S	15(71.46%)	
TIC	R	7(33.33%)	
	I	1(4.76%)	
	S	13(61.90%)	5(100%)
CRO	R	6(28.54%)	5(100%)
	I	11(50.2%)	
	S	4(19%)	
KAZ	R	15(71.46%)	5(100%)
	I	3(14%)	
	S	3(14%)	
VAN	R	3(14%)	
	I		
	S	18(86%)	
AK	R	6(28.54%)	5(100%)
	I		
	S	15(71.46%)	
AZ	R		1(20%)
	I		
	S		4(80%)

Resistant, I: Intermediate, S: Sensitive, OX:oxacillin 1 µg, AMX:Amoxicillin 20 µg, CPM: Cefepiem 30 µg, T: Tetracycline 30µg, TIC:Ticarcillin 75µg, CRO: Ceftriaxon 30 µg , KAZ: Ceftazidime 30 µg, VAN : Vancomycin 30 µg, AK . Amikacin 30 µg, AZ: Azitromycin 15 µg.

Table 4. Multiple antibiotic resistances (MAR) index and number of total isolates

MAR index	Acinetobacter spp.	Citrobacter spp.	Klebsiella spp.	*Staphylococcus aureus*	Streptococcus spp.	Total number/Percentage
0				3		3(5.35%)
0.1	0		8	9		17(30.35%)
0.2	0	3	2	1	0	6(10.71%)
0.3	1	2	2	1	0	6(10.71%)
0.4	1	0	0	1	0	2(3.57%)
0.5	2	0	4	0	0	6(10.71%)
0.6	0	0	0	0	0	0(0%)
0.7	0	0	0	0	4	4(7.14%)
0.8	1	1	0	6	1	9(16.07%)
.09	3	0	0	0	0	3(5.3%)
1						0(0%)0
Total	8	6	16	21	5	56(100%)

higher than the other countries[19].

Like BAL samples the effects of sputum bacteriology on lung function have been studied in adults. For example in studies by Wilson *et al.,*[11] and Hernandez *et al.,*[14] It has been concluded that the lung infection with *P. aeruginosa* encounters patients with a sever disease in comparison with non-colonized, although no relationships between lung function and microbial infection were indicated in other study[17].

Majority of isolates of this study and specially klebsiella spp., acinetobacter, citrobacter, *S.aureus*, and streptococci strains were resistant to the amoxicillin and tetracycline, although according to the ERS guideline[13] the mentioned drugs are choice drugs of LTR infections .

According to the study of Kwang *et al.,* (on 111 BE cases) a total of 167 (67%) PPMs were isolated from 248 colonies. The most frequent of them was *P. aeruginosa* (23.4%), *K. pneumoniae* (10.5%), and *S. aureus*(8.4%).They also concluded that BE score was higher in patients colonized with *P.aeruginosa* in comparison with other PPMs. [12] the severity of disease in patients colonized with *P.aeruginosa* also confirmed in other studies[16]. In a similar study on a large number of COPD patients it has been indicated that colonization of lower respiratory tract with potentally pathogenic microorganisms (PPMs) especially *P.aeruginosa* is related to exacerbations of disease[13].

In the study of Cabello *et al.,* of BE patients, *S. viridians*, Neisseria spp, Hemophilus spp., *S. pneumoniae*, *H.influenzae*, *M.catarrhalis* were predominant organisms in their study, but pseudomonas spp., rarely isolated from patients[20]. In 2002, Palwatwichai and collogues with evaluation of microbiologic characteristics of BE patients samples in Thailand indicated that *P. aeruginosa* was peredominant(20%) and H. *influenza*(14%) , *K. pneumonia*(14%) and *S. pneumonia*(6%) were isolated with lower percents[21]. Another study with the purpose of bacterial pattern and risk factors of colonization by Angrill etal in BE patients indicated that 64% of patients colonized with potential pathogenic organism. The most frequent of colonized organisms were *H.influenzae*(55%) and Pseudomonas spp.(26%), and 30% of isolated organisms were antibiotic resistant[6]. Shimada *et al*, in a study of antibiotic susceptibility of lower respiratory tract isolated organisms have been found that, 57.7% of *S. aureus* were methicillin resistant. The frequency of penicillin sensitive *S. pneumonia* strains were 37% and all of the *S. pneumonia* isolates were completely sensitive to carbapenems. In this study *P. aeruoginosa* isolates were sensitive to tobramycin and ciprofloxacin, and *K. pneumonia* was susceptible to all of the antibiotics except ampicillin, but *M. catarrhalis* isolates were sensitive to all of the antibiotics[22]. In a study Weinreich UM and Korsgaard J reported that rate of colonization and kind of bacteria in bronchial lavage samples of patients with chronic lung diseases was very different, they reported the *H influenzae* as the most isolated strain. They compared frequency of respiratory infections in different kinds of patients and finally reported that in BE cases infections were higher than other types such as COPD or healthy persons. They were also reported that BE cases harbor high burden of colonized bacteria[23].

King *et al.,* in a long term follow up of 89 BE cases found that *H. influenzae* (47%) and *P. aeruginosa* (12%) were the most prevalent isolates. In their study period (5.7+/-3.6 years) antibiotic resistance in isolates have been increased from 13% to 30%. [16]

So because of the important role in exacerbations and increasing resistance against antibiotics, advanced diagnostic methods such as microbiological analysis of representative samples from affected sites could help to better and definite identification of BE isolated microorganisms, which in turn could help to better management of disease. Based on study design, some limitations were existing in the study. These are including lack of detailed demographic, medical history and predisposing factors. In fact it looks like that the bacteriology of LRT infections is a complicated problem between microbiologists and respiratory tract associated physicians. Although the use of antibiotics in BE is controversial, but because of increasing rate of lungs colonization with resistant strains it is recommended that in LRT infections screening programs for resistant organisms being implemented routinely in hospital settings. It is also necessary to do further studies to elucidate the risk factors, role of different kinds of microbes in lung pathologies and prevention methods of LRT infections.

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