Prevalence of Antibiotics Resistance in the Isolated Bacteria from Bronchial Washing Fluids in Ramadi Teaching Hospital, Iraq

Irak Ramadi Eğitim Hastanesinde Bronş Yıkama Sıvılarından Izole Edilen Bakterilerde Antibiyotik Direnci Prevalansı

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ABSTRACT

The study proved defining the resistance of bacterial pathogens taken from the inferior respirational region from patients who were admitted to Ramadi Teaching Hospital. 76 samples of tracheal discharges were extracted. The highest percentage of bacterial isolate of males was *Streptococcus pneumonia* which about 26.2% for males. Generally, the lowest percentage is 4.8% for *Staphylococcus* spp. and *Bordetella* spp. The lowest percentage of females was 2% in the previous two genera. The outcome of the culture was determined by *p* value, the outcomes revealed that the growth of a pathogen with patients was equal to 0.782 for the *Klebsiella pneumonia* and the low *p* value appeared in the results of the *Acinetobacter* sp. strain level to 0. The confrontation of the antimicrobial is determined by the rate of MICs. The resistance appeared MIC ≥ 64 for *Pseudomonas aeruginosa* against antimicrobial Ciftazidim and resistance of *Bordetella* spp. showed MIC ≤ 0.5 against antimicrobial Colistin.

Keywords: Pneumonia, Bronchitis, Resistant bacteria, VITEK 2, Ramadi city.

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ÖZET

Çalışma, Ramadi Eğitim Hastanesine başvuran hastalardan alt solunum bölgesinden alınan bakteriyel patojenlerin direncini tanımladığını kanıtladı. 76 trakeal deşarj örneği çıkarıldı. Erkeklerde en yüksek bakteriyel izolat yüzdesi, erkeklerde yaklaşık %26.2 olan *Streptococcus pneumonia* olmuştur. Genel olarak, en düşük yüzde *Staphylococcus spp* ve *Bordetella spp*. için %4,8'dir Dişilerin en düşük yüzdesi önceki iki cinste %2 idi. Kültürün sonucu p değeri ile belirlendi, sonuçlar *Klebsiella* pnömonisi için hastalarda bir patojenin büyümesinin 0.782'ye eşit olduğunu ve *Acinetobacter sp*'nin sonuçlarında düşük p değerinin ortaya çıktığını ortaya koydu. suş seviyesi 0'a. Antimikrobiyalin karşılaşması MİK'lerin oranı ile belirlenir. *Pseudomonas aeruginosa* için antimikrobiyal Ciftazidim'e ve *Bordetella spp*. direncine karşı direnç MIC ≥ 64 olarak ortaya çıktı. antimikrobiyal Colistin'e karşı MIC ≤ 0,5 gösterdi.

Anahtar Sözcükler: Pnomoni, Bronşit, Dirençli bakteri, VITEK 2, Ramadi

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INTRODUCTION

Many pulmonologists analyze bronchial washing fluids to evaluate the etiology of hemoptysis by microbiological and cytological examinations (1). The bronchial washing samples are obtained for isolation bacteria by clinicians (2). Some studies exhibited rate of high drug resistant because of inadequate or non-completion antibiotic treatment and infection control practices. These negative statuses may make recurrence the bacterial pulmonary infections which lead to developing the drug resistance (3).

The counts of the collected bacteria from lower respiratory tract infections in most of patients reached ≥ 10^3 cfu/ml in Alabama, USA in the period 1978-1981 (4). Patients aged 51-60 years old had 19.7% of *Streptococcus pneumoniae* isolates compared as other age groups that related with Lower respiratory tract infections. This bacteria was resistant 1100% to Erythromycin (5).

Bronchial washing is sometimes achieved to get samples for cytological and/or microbiological examinations during bronchoscopy in patients with bronchiectasis that have developed hemoptysis (2). *Pseudomonas aeruginosa* was isolated from bronchial washing of a patient in King Khalid hospital, Saudi Arabia during 2013 (3). Anaerobic species associated with mixed bacteria pneumonia were identified including *Bacteroides melaninogenicus*, *Bacteroides ruminicola*, *Bacteroides oralis*, *Fusobacterium* spp., *Peptostreptococcus* spp. *Peptococcus* spp., *Veillonella* spp., [4). However, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterobacter aerogenes, Klebsiella pneumoniae, Ochrobactrum anthropi, Mycobacterium tuberculosis, Mycobacterium lentiflavum, Mycobacterium avium, Mycobacterium intracellulare, Mycobacterium abscessus* and *Mycobacterium fortuitum* were isolated from bronchial washing and sputum specimens in Seoul (2).

However, bronchial washing is a useful method to identify multi-resistant microorganisms when patients with bronchiectasis (2). Vitek 2 is a widely used commercial antimicrobial susceptibility testing system (6). The identification of the microbes using VITEK 2 present in the transtracheal washes is the best way to make the diagnosis (7). The VITEK 2 is an accuracy automated system to identify microbes and to test the susceptibility of antibiotics (7,8). Antimicrobial resistance may be caused by some random mutations in chromosomal DNA, or by transferring genes from resistant bacteria (9) or caused by transferring ribosomal DNA (10). Some recent studies have investigated emergence of antimicrobial resistance (AMR) in Iraq (11–13).

Therefore, a comprehensive investigation is required to uncover the scope of multi-drug resistance (MDR) problem among local population in Iraq. The current study aims to evaluate types of bacterial pathogens associated with Bronchitis patients, and to determine their antimicrobial susceptibility patterns using VITEK 2.

MATERIAL and METHODS

Collection and Isolation of Bronchial washing samples bacteria

This Laboratory-based reviewing investigation of Bronchial washing samples cultures and susceptibility tests were achieved at the Bacteriology Laboratory, in Department of Microbiology, Ramadi Teaching Hospital (TRH) within a period of one year between July 2017 and June 2018. One isolate per patient was randomly collected from different Bronchial clinical specimens of patients. About 76 isolates of human pathogenic microbes has isolated from 42 males and 34 females. The samples have been collected as a part of the routine clinical management of patients entered in various divisions of TRH. The collected samples were achieved in sterile containers by physicians and transferred to the laboratory within 30 min after the collection.

The bacterial samples were processed following the standard microbiological procedures by centrifugation, inoculating the precipitate on MacConkey Agar, Chocolate Agar, and Blood Agar and incubated at 37 °C aerobically. For creating micro-aerophilic conditions for the fastidious bacterium, the agar plates were put in in the candle jar, which provided 5%-10% CO_2 concentration. The plates were examined for the finding bacterial colonies after 18-20 hr of incubation. Plates which did not exhibit any growth were further incubated for an additional 24 hr.

Identification of bacteria and Sensitivity test using VITEK 2 System

Organisms were identified by standard microbiological methods including morphology of colonies, staining, serological and biochemical tests. They were declared using Vitek 2. Antibiotic sensitivity test was conducted on pure culture isolates employing the VITEK system-2 Susceptibility tests with the Vitek 2 (bioMérieux, Inc., Durham, NC) system were performed using software version 5.01 and cards of AST (antimicrobial susceptibility testing) according to the manufacturer's instructions. Each isolate was tested concurrently with both methods using isolated colonies from a single 18 to 24 hr blood agar plate. Purity plates were prepared following inoculation of each test by sub-culturing an aliquot of inoculum suspension onto blood agar plates and incubating for 18-24 hr (6).

Statistical analysis

The statistical analysis was performed by using the Pearson Chi-square test and the analysis of variance (ANOVA). Data were analyzed using SPSS 17.0 (USA). Furthermore, the differences were significant at $p< 0.01$.

The study was approved by Local Ethics committee in University of Anbar. Informed consent/assent was obtained from the patients (42 males and 34 females) in Teaching Ramadi Hospital in Ramadi.

RESULTS

As in Table 1, about 76 isolates of human pathogenic microbes has isolated from 42 males and 34 females in Teaching Ramadi Hospital in Ramadi, Iraq from July 2017 and June 2018. About 8 genera have identified using the basic general methods. They were declared using Vitek 2. They are *Streptococcus pneumonia*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus* spp., *Bordetella* spp., *Acinetobacter* sp. and *Oligella ureolytica*. As in Table 1, the higher percent was 26.2% *Streptococcus pneumonia* in males, followed by 21.4%, 14.3% and 11.9% for *Pseudomonas aeruginosa*, *Klebsiella pneumonia* and *Proteus mirabilis* respectively. *Staphylococcus* spp. and *Bordetella* spp. had the lower percent 4.8%. In females, *Streptococcus pneumonia, Klebsiella pneumonia* and *Bordetella* spp. recorded the higher percent of pathogenic bacteria were 20.6% for each. However, the lower percent was recorded reached 2.9% for *Oligella ureolytica*. *Acinetobacter* sp. did not record any existence in the bronchial samples of female patients.

Streptococcus pneumonia had higher percent reached 26.2% in males compared with 20.6% in females significantly (p<0.01). *Oligella ureolytica* record the lower percent 2.9% in females compared as 9.5% in males. About 7.1% of bacteria were *Acinetobacter* sp. appeared only in males. In females, *Klebsiella pneumonia*, *Bordetella* and *Staphylococcus* spp. recorded higher percentages 20.6%, 20.6% and 11.8% compared with 14.3%, 4.8% and 4.8% in males respectively. However, *Proteus mirabilis* and *Pseudomonas aeruginosa* recorded higher percentages 11.9% and 21.4% in males in comparison with 5.9% and 17.6% in females respectively. Generally, *Streptococcus pneumonia* has the highest percent 23.7% than other bacteria in males and females. However, *Acinetobacter* sp. has the lowest percent 3.9%.

Table 1 Number of the isolated bacteria from bronchial samples in Ramadi

Name of isolate	Male %	Female %	Total	LSD (p<0.01)
Streptococcus pneumonia	26.2	20.6	18	0.346
Klebsiella pneumonia	14.3	20.6	13	0.782
Proteus mirabilis	11.9	5.9	7	0.257
Pseudomonas aeruginosa	21.4	17.6	15	0.439
Staphylococcus spp.	4.8	11.8	6	0.414
Bordetella spp.	4.8	20.6	9	0.096
Acinetobacter sp.	7.1	0.0	3	-
Oligella ureolytica	9.5	2.9	5	0.18
Total			76	0.359

Effect of ages on the pathogenicity of bacteria caused Bronchitis appeared in table 2. The significant infection (p<0.05) was 43.2% in males with ages from 21-40 years, followed by 27.3% and 20.5% with males 40-60 years and >60 years respectively. Males with 10-20 years recorded the lower infection 9.1%. Females has higher infection with 10-20 years, followed by 25% for reach 21-40 years and >60 years.

Table 2 Ages and sexes distribution of the culture positive samples

Age groups (Years)	Male		Female		Total	LSD (p<0.05)
	No.	%	No.	%		
10-20	4	9.1	11	34.4	15	0.071
21-40	19	43.2	8	25.0	27	0.034*
41-60	9	20.5	5	15.6	14	0.258
> 60	12	27.3	8	25.0	20	0.371

*: The difference is significant at the level of 1%

Table 3 showed the antimicrobial susceptibility test using 15 antibiotics are including Amikacin, Gentamicine, Ciproflaxacin, Imipenem, Ceftriaxone, Ciftazidim, Tetracycline, Meropenem, Cefepim, Azthreonam, Colistin, Augmentin, Cefotaxim, Cephalothin and Clindamycin. In general, the resistance (100%) to Cephalothin, Gentamicine, Ciftazidim, Colistin, Ciproflaxacin, Augmentin, Cefotaxim and Tetracycline was observed with some pathogenic bacteria. All isolates (No=8) of *Streptococcus pneumonia* were resistant to Cephalothin, and susceptible to Ciproflaxacin which did not effect on its growth. Of 18 *S. pneumonia* isolates, it found that 16 isolates (88.9 %), 14 isolates (77.8 %), 12 isolates (66.7 %) were resistant to Cefepim, Augmentin and Cefotaxim respectively. Results of *S. pneumonia* isolates found that only 2 isolates (11.1 %) were resistant to Gentamicine (Table 3).

Isolates (No=13, 100%) of *Klebsiella pneumonia* were resistant to Gentamicine and Ciftazidim. Of 13 isolates, 10 (76.9%) and 11 (84.6%) isolates were resistant to Cefepim and Azthreonam respectively. However, only one isolate (7.7%) was found to be resistant to Amikacin, Meropenem and Colistin, while 3 isolates (23.1%) was resistant to Ciproflaxacin. All isolates (No=7) of *Proteus mirabilis* were resistant to Colistin. Of 7 isolates, 6 (85.7%) isolates were resistant to Gentamicine. However, Of 7 isolates, 2 (28.6%) isolates was resistant to Meropenem, while only one isolate (14.3%) was found to be resistant to Ciproflaxacin and Azthreonam. The 7 (100%) isolates of *Proteus mirabilis* were susceptible to Amikacin, Ciftazidim and Cefepim.

All isolates (No=15) of *Pseudomonas aeruginosa* were resistant to Ciftazidim. Of 15 isolates, 14 (93.3%) isolates were resistant to Imipenem and Meropenem. However, of 15 isolates, 6 (40%) and 3 (20%) isolates were resistant to Amikacin and Ciproflaxacin, while only 2 (13.3%) isolates were found to be resistant to Cefepim. All isolates (No=6) of *Staphylococcus* spp. were resistant to Ciproflaxacin, Augmentin and Cephalothin. Of 6 isolates, 5 (83.3%) isolates was resistant to Cefotaxim, while only one (16.7%) isolate was found to be resistant to Ceftriaxone and Clindamycin. The 6 (100%) isolates of *Staphylococcus* spp. exhibited susceptibility to Gentamicine.

Of 9 isolates of *Bordetella* spp., 4 (44.4%) and 3 (33.3%) isolates showed resistance to Azthreonam and Gentamicine respectively. However, of 9 isolates, 2 (22.2%) isolates were resistant to Amikacin, Ciftazidim and Colistin, while only one isolate 1 (11.1%) was resistant to Ciproflaxacin and Meropenem. The 9 (100%) isolates of *Bordetella* spp. exhibited susceptibility to Cefepim. All isolates (No=3) of *Acinetobacter* sp. exhibited resistance to Augmentin and Cefotaxim. Of 3 isolates, only one (33.3%) isolate was resistant to Ceftriaxone. The 3 (100%) isolates of *Acinetobacter* sp. showed susceptibility to Amikacin, Gentamicine, Ciproflaxacin and Imipenem. All isolates (No=5) of *Oligella ureolytica* were resistant to Tetracycline. Of 5 isolates, 4 (80%) and 2 (40%) isolates were resistant to Augmentin and Cephalothin respectively, while only one (20%) isolate was resistant to Ciproflaxacin and Imipenem. The 5 (100%) isolates of *Oligella ureolytica* exhibited susceptibility to Gentamicine and Ceftriaxone.

Table 3 Number and percentages of resistant and sensitive isolates against antibiotics

Antibiotics		*Streptococcus pneumonia*	*Klebsiella pneumonia*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Staphylococcus* spp.	*Bordetella* spp.	*Acinetobacter* sp.	*Oligella ureolytica*
Amikacin	S		12 (92.3%)	7 (100%)	9 (60%)		7 (77.8%)	3 (100%)	
	R		1 (7.7%)	0	6 (40%)		2 (22.2%)	0	
Gentamicine	S	16 (88.9%)	0	1 (14.3%)		6 (100%)	6 (66.7%)	3 (100%)	5 (100%)
	R	2 (11.1%)	13 (100%)	6 (85.7%)		0	3 (33.3%)	0	0
Ciproflaxacin	S	18 (100%)	10 (76.9%)	6 (85.7%)	12 (80%)	0	8 (88.9%)	3 (100%)	4 (80%)
	R	0	3 (23.1%)	1 (14.3%)	3 (20%)	6 (100%)	1 (11.1%)	0	1 (20%)
Imipenem	S				1 (6.7%)			3 (100%)	4 (80%)
	R				14 (93.3%)			0	1 (20%)
Ceftriaxone	S					5 (83.3%)		2 (66.7%)	5 (100%)
	R					1 (16.7%)		1 (33.3%)	0
Ciftazidim	S		0	7 (100%)	0		7 (77.8%)		
	R		13 (100%)	0	15 (100%)		2 (22.2%)		
Tetracycline	S								0
	R								5 (100%)
Meropenem	S		12 (92.3%)	5 (71.4%)	1 (6.7%)		8 (88.9%)		
	R		1 (7.7%)	2 (28.6%)	14 (93.3%)		1 (11.1%)		
Cefepim	S	2 (11.1%)	3 (23.1%)	7 (100%)	13 (86.7%)		9 (100%)		
	R	16 (88.9%)	10 (76.9%)	0	2 (13.3%)		0		
Azthreonam	S		2 (15.4%)	6 (85.7%)			5 (55.6%)		
	R		11 (84.6%)	1 (14.3%)			4 (44.4%)		
Colistin	S		12 (92.3%)	0			7 (77.8%)		
	R		1 (7.7%)	7 (100%)			2 (22.2%)		
Augmentin	S	4 (22.2%)				0		0	1 (20%)
	R	14 (77.8%)				6 (100%)		3 (100%)	4 (80%)
Cefotaxim	S	6 (33.3%)				1 (16.7%)		0	
	R	12 (66.7%)				5 (83.3%)		3 (100%)	
Cephalothin	S	0				0			3 (60%)
	R	18 (100%)				6 (100%)			2 (40%)
Clindamycin	S					5 (83.3%)			
	R					1 (16.7%)			

R: resistant, S: susceptible, blank: Non-Detected.

Minimum inhibitory concentrations (MICs) of nine antibiotics are including Amikacin, Gentamicine, Ciproflaxacin, Imipenem, Ciftazidim, Meropenem, Cefepim, Azthreonam and Colistin against four human pathogenic bacteria (*Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Bordetella* spp.) from some positive Bronchial samples cultures were checked as in table 4. Table 4 reported that *Pseudomonas aeruginosa* has the high-level resistance to Ciftazidim (MIC ≥64 µg⁄ml) followed by Imipenem, Meropenem (MICs ≥16 µg⁄ml) and Amikacin (MICs =8 µg⁄ml) respectively. However, *Pseudomonas aeruginosa* has the low-level resistance to Ciproflaxacin and Cefepim (MICs ≤1 µg⁄ml).

Also, *Klebsiella pneumonia* and *Proteus mirabilis* have a high-level resistance to Gentamicine and Azthreonam and Gentamicine and Colistin (MICs ≥16 µg⁄ml) respectively. *Proteus mirabilis* have resistance to Ciftazidim reached MICs =8 µg⁄ml. *Klebsiella pneumonia* and *Proteus mirabilis* showed the low-level resistance (≤0.25-4 µg⁄ml) with the reset antibiotics. *Bordetella* spp. has a low-level resistance (≤2-0.5 µg⁄ml) to Amikacin, Gentamicine, Ciproflaxacin, Imipenem, Ciftazidim, Meropenem, Cefepim, Azthreonam and Colistin.

Table 4 MICs Breakpoints of some antibiotics (µg⁄ml) against 4 pathogenic bacteria of Bronchial samples cultures

Antibiotics	*Klebsiella pneumonia*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Bordetella* spp.
Amikacin	=4	≤2	=8	≤2
Gentamicine	≥16	≥16	ND	≤1
Ciproflaxacin	=1	=1	=1	=1
Imipenem	ND	ND	≥16	ND
Ciftazidim	=8	≤1	≥64	≤1
Meropenem	≤0.25	=2	≥16	≤0.25
Cefepim	= 4	≤1	≤1	≤1
Azthreonam	=16	≤1	ND	=2
Colistin	=1	≥16	ND	≤0.5

MICs: minimum inhibitory concentrations, ND: Non-Detected.

DISCUSSION

Results of this study agrees with (2) who indicated to finding *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumoniae* in bronchial washing specimens of patients Bronchiectasis with in Seoul. *Streptococcus pneumonia* is the main responsible about Pneumonia and Bronchitis among Bacterial Pulmonary Infections (4), that agree with this study (Table 1). However, 20-40 years recorded the lower percentage reached 15.6%. results of this study for age group 41-60 near to results of (5).

The results of Table 3 agree with results of (11) who referred to resistance of *Pseudomonas aeruginosa* to Imipenem, Meropenem and Ciftazidim and resistance of *Klebsiella pneumonia* to Gentamicine and Cefepim. Also, the results of *Streptococcus pneumonia* are comptible with (5). *Acinetobacter* sp. exhibited resistance to Augmentin and Cefotaxim that may be due to the antibiotic resistant genes (12). Also, resistace of *Staphylococcus* spp. in this current study may be return to some antibiotic resistant genes (9). About 7% of *Acinetobacter* sp. isolated from sputum of patiants in some hospitals in Turkey and it was found resistant to many antibiotics (14)

Pseudomonas aeruginosa isolated from bronchial washing showed resistance to Amikacin, Amox-Clav, Ampicillin, Cefazoline, Cefepime, Cefotaxime, Cefoxitin, Cefuroxime, Ciprofloxacin, Ertapenem, Fosfomycin, Gentamicin, Imipenem, Levofloxacin, Mezlocillin, Pip-Tazo, Tetracycline, Tobramycin, and Trimeth/Sulfa (3). Thus, using of antibiotic to treatment of some patients is changing due to the results of microorganisms in bronchial washing fluid (2).

CONCLUSION

The conclusion from this study is that bacterial infections are the record widespread diseases of the inferior respirational system among patients in hospitals in addition to bacteriological antibiotics cannot be the model resolution for the dealing of bacterial septicity, since it is a recurrent routine indications to the creation of a novel strain resistant to antimicrobial Similarly, the antibiotics are not expected a new effect in the case of various infections.

Conflict of interest

No conflict of interest was declared by the authors.

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