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Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens isolation from bronchial washing by VITEK 2 system

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ABSTRACT

This study was examined for the purpose of determining the sensitivity of bacterial pathogens taken from the lower respiratory tract of antimicrobial Susceptibility from patients who were taken to Ramadi teaching hospital where 167 samples of bronchial secretions were withdrawn and the proportion of isolated bacteria was calculated from The samples in table 1 where the highest ratio of isolated bacteria of female males are *strep. Pneumon*ia is about 20.6% for males and 25.0% while the lowest percentage is 0% in *Acinetobacter sp.* the lowest percentage of females was 2% for *Bordetella spp.* and staph. Spp. The growth of the culture was then measured by the value of p, where the results showed that the highest growth of a pathogen with patients was equal to 0.782 for the *Kleb. Pneumonia* and the lowest value p appeared in the results of the *Acinetobacter. SP* strain Equal to 0. The resistance of the antimicrobial is measured through the value of the Mac where between the table that resistance MIC ≥ 64 for the type *Pseudomonas. aerug* For antimicrobial Ceftazidime and resistance *bordetella.spp.* MIC ≤ 0.5 for antimicrobial colistin. The conclusion from this study is that bacterial diseases are the most prevalent diseases of the lower respiratory system among patients in hospitals as well as microbial antibiotics cannot be the ideal solution for the treatment of bacterial infection because it is a frequent use leads to the production of a new strain resistant to antimicrobial Also, the antibiotics are not given a new result in the case of multiple infections (two bacterial pathogens).

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INTRODUCTION

Bacterial pathogens are among the most respiratory diseases that cause deaths of about 30% of human beings as a result of acute infections at different ages as they affect children at an early age as the body and lack of immunity. Respiratory diseases are acquired from community and It can be dangerous and lead to pneumonia and bronchitis as well as sinusitis and the degradation of chronic conditions (Silbiger, 2006; Banister *et al.,* 2006) well as adults who suffer from fatigue, weakness of

confirmed bacteria and fungi transmitted to the respiratory tract by inhalation and then pushed through columnar respiratory epithelium are lined through the airway to the lower respiratory tract to produce mucus. As with other infectious diseases, respiratory diseases require laboratory care to identify pathogens as well as laboratory diagnosis and laboratory diagnosis of pathogens that cause respiratory infections based on several factors, including the collection of samples of bacterial pathogens, laboratory tools, staining and culture methods. The traditional microbiological diagnosis of pathogens depends primarily on the culture of specimens such as sputum, bronchial and

bronchial tubes, which can be performed to quantify the bacterial colony unit of some cultured specimens. After confirming that bacterial colonies are tested for antimicrobial sensitivity to determine their resistance against antibiotics, traditional methods require few days to determine the microbial diagnosis (Torres, 2000). So, use a The VITEK 2 system for the rapid and accurate identification of gram-positive cocci, gram-negative rods, and yeasts in routine clinical microbiology tests (Ligozzi *et al.,* 2002; Renaud *et al.,* 2005; Abele-Horn *et al*., 2006). Using the new VITEK 2 NH system Identify eight strains of bacteria1- strep. Pneumonia, Kleb. Pneumonia Proteus. Mirabilis, Acinetobacter. spp, bordetella. spp, Staph. spp Pseudomonas. aerug and Oligella.urelytica. The aim of this study is to detect the phenotypic differences between the bacterial pathogens and their susceptibility test to the microbial antibiotics.

MATERIALS AND METHODS

167 samples of bacterial pathogens were obtained from patients at Ramadi teaching hospital. Bronchial sputum samples were obtained after local anaesthesia and pull by inserting a tube through the mouth into the lungs and then-conservative samples and brush in methylparaben (Wimberley et al., 1979). After obtaining samples of sputum from the pulmonary section with a brush, a small amount of it is stained on a glass slide for the purpose of staining it with a gram dye. The brush is cut and stored in a tube containing 0.1 ml of Ringers lactate solution. Respiratory samples were collected on the blood Agar and chocolate agar (bioMérieux, France) and in 35°C and 5% CO_2 for insulation and identification organisms. Sensitivity to optochin. Blood agar plates were inoculated with alpha haemolytic colonies obtained in the previous cultures and a 5 µg optochin disk was placed in the centre on the inoculated area. These subcultures were incubated for 24 hours at 35°C in an atmosphere of 5% CO_2. Optochin sensitivity was defined as a zone of inhibition ≥ 14 mm in diameter.

RESULTS

As mentioned in the materials and methods of work, 167 samples of eight strains of bronchial isolates were obtained for diagnostic purposes for both sexes and different ages and were derived from blood agar and agar chocolate.

The results of the analysis in Table (1) showed that 11 males had a *strep. Pneumonia* strain was about 25% and its rate in 7 females was about 20.6% while *Kleb. bacteria*. Pneumonia showed 13.6% in 6 males and 20.6% in 7 females. The results showed that patients with Proteus mirabilis were five males with an average transplant rate of 11.4% and 5.9% *Pseudomonas. aerug* showed 20.5% in 9 males and about 17.6% in 6 females. The rate of transplantation of *Staph. spp* bacteria and *bordetella. spp* in two males, 4.5% while *Staph. spp* in 4 females about 11.8% and *bordetella spp* in 7 females 20.6%. The percentage of *Acinetobacter. Sp* in 3 males was 6.8% and the result was zero in females. The analysis of *Oligella urelytica* bacteria showed 9.1% in 4 males and 2.9%. Figure 1 shows the proportions of the bacterial pathogens of the eight breeds mentioned above for both sexes (males and females) if the colour is red. Female and blue ratios Male ratios.

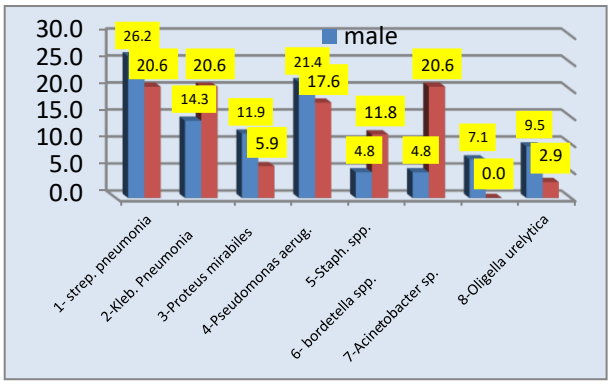

Figure 1: Distribution of the number of patients (males& females) and bacterial isolates rates in the bronchial washing of hospitalized patients

The results of the ANOVA analysis showed that the value of p for 18 samples of all races was 0.346 for the bacterial strain. Pneumonia while p was about 0.782 for *Kleb. Pneumonia* type bacteria. The value of p was 0.277 for *Proteus. Mirabilis* while the value of p in *Pseudomonas. aerug* was 0.439. ANOVA analysis results showed that the value of p is 0.414 for the *Staph. spp* type and *bordetella. Spp.* The value of p is 0.096. The results of the *Acinetobacter. Sp* analysis showed that the value of p was 0 and the value of p was 0.18 in the bacterium *Oligella. urelytica*.

Table (4), the concentration range of the tested antibiotics used in combination experiments depend on the minimum inhibitory concentration (MIC) of single antibiotic against the tested strains. The results indicated that the MICfor the Amikacin was MIC = 4 for samples of *Kleb. Pneumonia* and MIC≤ 2 for both *Proteus. Mirabilis* and *bordetella. spp* while mic = 8 for *Pseudomonas. aerug* and the mic results for antibiotic gentamicin range from MIC≤ 1 for type *bordetella. spp* to MIC≥ 16 for both types *Kleb. Pneumonia*, *Proteus. Mirabilis*. MIC results for Ciprofloxacin have appeared for four strains of aetiology *Proteus. Mirabilis*, *Kleb. Pneumonia*, *Pseudomonas.aerug*. and *bordetella. spp*. that MIC = 1. The results of MIC≥ 16 for antibiotic Imipenem for bacterial *Pseudomonas* and antibiotic ceftazidime are shown to be equal to MIC = 8 for *Kleb. Pneumonia*

Table 1: Key data of the number of patients (males & females) and bacterial isolates rates in the bronchial washing of hospitalized patients

	male		female		
	No.	%	No.	%	
1- strep. pneumonia	11	26.2	7	20.6	18
2-Kleb. Pneumonia	6	14.3	7	20.6	13
3-Proteus mirabiles	5	11.9	2	5.9	7
4-Pseudomonas aerug.	9	21.4	6	17.6	15
5-Staph. spp.	2	4.8	4	11.8	6
6- bordetella spp.	2	4.8	7	20.6	9
7-Acinetobacter sp.	3	7.1	0	0.0	3
8-Oligella urelytica	4	9.5	1	2.9	5
total	42		34		76

Table 2: Distribution of patients with significant bacterial growth

Name of isolate	male	female	total	p value
1- strep. pneumonia	11	7	18	0.346
2-Kleb. Pneumonia	6	7	13	0.782
3-Proteus mirabiles	5	2	7	0.257
4-Pseudomonas aerug.	9	6	15	0.439
5-Staph. spp.	2	4	6	0.414
6- bordetella spp.	2	7	9	0.096
7-Acinetobacter sp.	3	0	3	-
8-Oligella urelytica	4	1	5	0.18
total	42	34	76	0.359

and MIC≤ 1 for *Proteus. Mirabilis* and MIC≥ 64 for *Pseudomonas. aerug* and MIC≤ 1 for *bordetella. spp*

While the results of the antibiotic meropenem that MIC ≤ 0.25 appeared for bacterial *Kleb. Pneumonia* as well as bordetella. SPP was the result of MIC = 2 for Proteus. Mirabilis the same antibiotic direction and reached MIC≥ 16 for *Pseudomonas. aerug* direction meropenem. The *Kleb. Pneumonia* strain appears. MIC= 4 direction antibiotic cefepime and strain *Proteus. Mirabilis* MIC≤ 1, strain *Pseudomonas. aerug* MIC ≤ 1 as well as Bordetella strain. MIC spp ≤ 1. The *Kleb. Pneumonia* strain appears MIC = 16 also strain Proteus. Mirabilis MIC≤ 1 while straining *bordetella. Spp* 5MIC = 2 direction of antibiotic aztreonam, and the analysis results for the *Kleb. Pneumonia* strain MIC = 1, strain Proteus mirabilis MIC ≥ 16 and bordetella strain. MIC spp ≤ 0.5 colistin antibiotic direction.

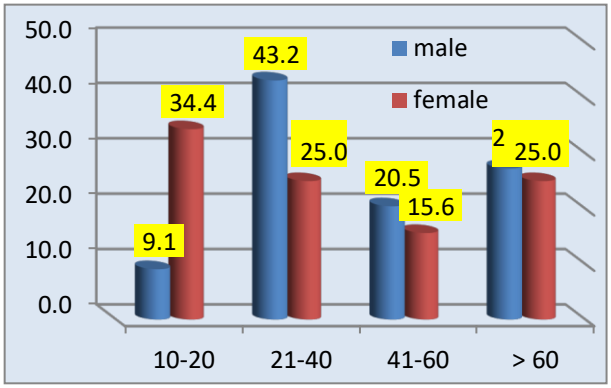

Figure 2: Age and sex distribution of the culture-positive samples

The results (table 4) of the ANOVA analysis of eight strains of bacteria studied showed noticeable differences between the different age groups of both males and females where p-value for the age group 10-20 years was equal to 0.071 and reached the age group 21-40 years about 0.034* While the age range of 41-60 was valuable P is equal to 0.258 and the value of P in the age group > 60 is equal to 0.371. Values of p were represented in fig-2 where red represents females and blue represents males.

DISCUSSION

Bacterial diseases that are the result of a bacterial infection are usually acquired by the interaction between the organism and the environment and the potential host so that patients at the hospital are exposed to pneumonia has developed a lot of methods to take samples of the airways and considered the method of washing the people Aerobic is one of the most commonly used methods where samples from lower respiratory tract secretions are obtained near the site of production while minimizing the contamination of the respiratory tract. Depending on the isolated bacteria, antibiotic resistance is measured as the high resistance of isolated bacteria demonstrates the production of new resistant strains. The results show that 20.6 of the pathogens of three strains. This increase in the results of infection (Seligman, 1978) indicates that the widespread use of antibiotics led to the production of a strain more resistant to antibiotics (Toms *et al*., 1986; Podschun, 1998). The resistance of

Table 3: Susceptibility of isolates according to antibiogram

Bacteria / Antibiotic		*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Amikacin	S	--	12 MIC==4	7 MIC≤2	9 MIC=8	--	7 MIC≤2	3	--
	R	--	1	0	6	--	2	0	--
Gentamicine	S	16	0 MIC≥16	1 MIC≥16	TRM	6	6 MIC≤1	3	5
	R	2	13	6	--	0	3	0	0
Ciproflaxacin	S	18	10 MIC=1	6 MIC=1	12 MIC=1	0	8 MIC=1	3	4
	R	0	3	1	3	6	1	0	1
Imipenem	S	--	--	--	1 MIC≥16	--	--	3	4
	R	--	--	--	14	--	--	0	1
Ceftriaxone	S	--	--	--	--	5	--	2	5
	R	--	--	--	--	1	--	1	0
Ciftazidim	S	--	0	7 MIC≤1	0 MIC≥64	--	7 MIC≤1	--	--
	R	--	13 MIC=8	0	15	--	2	--	--
Tetracycline	S	----------	----------	----------	----------	----------	----------	----------	0
	R	----------	----------	----------	----------	----------	----------	----------	5
Meropenem	S	--	12 MIC ≤0.25	5 MIC=2	1 MIC≥16	--	8 MIC≤0.25	--	--
	R	--	1	2	14	--	1	--	--
Cefepim	S	2	3 MIC= 4	7 MIC≤1	13 MIC≤1	--	9 MIC≤1	--	--
	R	16	10	0	2	--	0	--	--
Aztreonam	S	--	2 MIC=16	6 MIC≤1	TRM	--	5MIC=2	--	--
	R	--	14	1	--	--	4	--	--
Colistin	S	--	12 MIC =1	0 MIC≥16	TRM	--	7 MIC≤0.5	--	--
	R	--	1	7	--	--	2	--	--
Augmentin	S	4	--	--	--	0	--	0	1
	R	14	--	--	--	6	--	3	4
Cefotaxim	S	6	--	--	--	1	--	0	
	R	12	--	--	--	5	--	3	
Cephalothin	S	0	--	--	--	0	----------	----------	3
	R	18	--	--	--	6	----------	----------	2
Clindamycin	S	--	--	--	--	5	--	--	--
	R	--	--	--	--	1	--	--	--

1-Strep. Pneumonia; 2-Kleb. Pneumonia; 3-Proteus mirabiles; 4-Pseudomonas aerug; 5-Staph. sp.; 6-bordetella spp. ; 7-Acinetobacter sp.; 8-Oligella urelytica

Table 4: Age and sex distribution of the culture positive Samples

	male		female		total	
	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 are significant at the 0.05 level

Microbes to the direction of antibiotics may vary due to multiple infections (pathogens), which may increase their resistance. Acharya, (1992) show sensitivity pattern the basis on of the chemotherapeutic agents could be divided into groups. Those appear inclusive sensitivity of less than 25% (their resistance more than 75%) formed the largest group consisting of most of the routinely used antibiotics such as penicillin, ampicillin, amoxicillin, carbenicillin, tetracycline, chloramphenicol and sulphonamides. Gentamycin leads the whole group with 85% sensitivity.

CONCLUSION

It must be noted that, since gentamycin was made freely available over the last 15 years, sensitivity to it had gradually declined from 99% to 95% and show that, quinolones like norfloxacin, ofloxacin, ciprofloxacin and levofloxacin have shown better sensitivity against multiple resistant pathogens than the oldest quinolones (nalidixic acid) and nitrofurantoin which is advent in the middle between the nalidixic acid and new fluoroquinolones.

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