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Phytochemical Study with Evaluation the Antimicrobial Activity of *Cressa cretica* Plant Against Some Gram-Positive and Gram-Negative Bacteria

Noor Hamid Hamza
Ministry of Education General Directorate of Educational. Baghdad- Rusafah / first
Noorhamed1983@yahoo.com

Ashwak I. AL- Obaedi
AL- Iraqia University- Research & Islamic Studies Center, Baghdad, Iraq
Ashwak902@gmail.com

Ibrahim S. AIjubory
Department of Pharmacognosy and Medicinal Plants, College of Pharmacy- Mustansiriyah University Baghdad, Iraq.
Ibrahim.a.a@yahoo.com

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Abstract

viruses are responsible for a large proportion of lower respiratory tract infections (LRTIs). Other causes of LRTIs are bacteria: Streptococcus pneumoniae, Haemophilus influenzae, Klebsiella pneumoniae, and Staphylococcus aureus being the most common. Sputum samples are commonly used in the microbiological laboratory for diagnosing lower respiratory infections. **Objective:** The aim of this study to evaluate the causative bacteria and antibiotics sensitivity in culture of sputum samples. **Patients Methods:** A retrospective study performed in the microbiology department of Al Immamin Al Kahdimin Medical laboratory in Baghdad. The results of sputum cultures collected from the files between 2016 and 2019. A total number of 131 included in the study of adults and both sexes. Organisms were identified and tested for the antibiotic susceptibility did for selected cases which ordered by the doctor needed. **Results:** The number of 131 were enrolled. The age of patients was between 17-85 years with mean age 46.69. The higher incidence of patients between ages 51-60 years (21.4 %). The female were 40.5%, the male 59.5%. 65 (49.62%) patients from the medical ward, 50 (38.17) from respiratory care unit (RCU). *Acinetobacter spp* was the most common bacteria isolated, in forty four (33.59%) cases, which was resistant to most antibiotics. followed by *Streptococcus pneumonia* (22.90%), *Pseudomonas aeruginosa* (16.03%),*Escherichia coli* in eleven (8.40) cases, with variable antibiotics sensitivity and resistance. **Conclusion:** sputum culture and sensitivity may help in identifying the organism and choosing the antibiotic, which may be resistant to many drugs as in *Acinetobacter* spp.

Key wards: Bacteriological Profile, Antibiotic Sensitivity, Sputum Culture

1. Introduction

The Infections of upper and lower respiratory tract are a major cause of morbidity and mortality, particularly in patients at extremes of age, those with preexisting lung disease or with immune suppression [1]. Infection can occur because of infectious agents such as bacteria,

virus, fungus, and protozoa [2]. Sputum is most commonly used sample for the diagnosis of lower respiratory tract infections, its easy and non-invasive procedure [3]. The Gram stains and cultures of sputum specimens are performed to detect the potential respiratory pathogens [4].

Role of the sputum culture has been debatable and is limited by a fact that it is difficult to get a deep cough specimen in children and elderly patients and there is a possibility of contamination by the normal upper respiratory tract flora which poses problems in culturing the specimen and has direct and indirect effect on the treatment decisions and clinical outcome of the patients [5, 6]. The yield of the sputum cultures is further diminished if antibiotics received prior to producing the specimen, which occurs in about 25 percent of cases [7].

The Infectious Diseases Society of America/American Thoracic Society guidelines recommend that the sampling in hospitalized adult patients be restricted to those who are able to provide the high quality pretreatment specimen and where the quality performance measures for the sputum collection, transport and processing of samples can be met [8]. The good sputum sample depends on healthcare worker education and patient understanding [9]. The culture of good-quality sputum samples obtained from patients who received antibiotic treatment is probably not cost-effective [10].

During the management of bacterial infections, bacteria might develop resist to the one or more antibiotics [11]. The mechanisms by which organisms exhibit the resistance to antibiotics includes drug inactivation or modification, alteration of target site, alteration in the metabolic pathway, and reduced drug accumulation [12].

The patterns of microorganisms that causing infection and the antibiotic resistance pattern vary from one country to other country, as well as from hospital to hospital [13]. A cross-resistance and multi resistance patterns have been observed, throughout the world [14].

The aim of this study to evaluate the causative bacteria and antibiotics sensitivity in culture of sputum samples that causes lower respiratory tract infections.

2. Patient and Methods

A retrospective study performed in the microbiology department of Al Immamin Al Kahdimin Medical city in Baghdad. The results of sputum cultures collected from January 2016 and March 2019. A total of 131 ncluded in the study of adults and both sexes (female 53 (40.50%) ,and male 78 (59.50%)), their age 17-85, the mean age 46.69±20.80. Information collected include the demographic data of the patient, admission unit \ department. Other data collected included isolated bacteria , sensitivity pattern

All the sputum samples were inoculated on Blood agar, Chocolate agar and Mac Conkey agar at 37°C for 24- 48 hours. The bacterial isolation and identification performed by using standard laboratory methods (15). Culture sensitivity was done by a diffusion method (16). According to the growth on culture, the sensitivity testing has done. Antibiotic used were: Trimethoprim sulfamethoxazole (TS), Rifampin (Rif), Clindamycin (Clin), Imipenem (Imi), Azithromycin (AZ), , Amikacin (Ami), Ciprofloxacin (Cip), Tetracycline (T), Cefotaxime (Cef), Doxycycline (Dox), Netilmicine (Net), Levofloxacin (Lev). Antibiotic susceptibility of isolates was performed by agar disc diffusion method (16). Patients with no growth of sputum culture were excluded from the study. Descriptive statistics made using Statistical Package for Social Sciences (SPSS) 23 and Microsoft Excel 2013. Numerical data were described as mean and standard deviation, while, Categorical data were described as count and percentage.

3. Results

In this study a total number is 131 were enrolled. The age of patients between 17-85 years, divided into ≤20 years, 21-30 years, 31-40 years, 41-50 years, 51-60 years, 61-70 years, and >70 years with mean 46.69. The higher incidence of patients between ages 51-60 years (21.4 %). The female were 40.5% while the male 59.5%, as in **Table 1**.

Table 1. Demographic data of the patients

		value
Sex	Female	53 (40.50)
	Male	78 (59.50)
Age groups	<=20 years	21 (16)
	21-30 years	20 (15.3)
	31-40 years	14 (10.7)
	41-50 years	14 (10.7)
	51-60 years	28 (21.4)
	61-70 years	18 (13.7)
	>70 years	16 (12.2)
Age	Mean± SD	46.69±20.80
	Minimum-maximum	17-85

The distribution of patients according to word / unit, 65 (49.62%) from the medical ward, 50 (38.17) from respiratory care unit (RCU), 13 (9.92%) from out- patients, 3 (2.29%) from Hematology wards, as in **Table 2**.

Table 2. Distribution of patients according to word / unit

Ward unit	No	%
Medical ward	65	49.62%
RCU	50	38.17%
Out-patients	13	9.92%
Hematology wards	3	2.29%
Total	131	100.00%

Acinetobacter spp. was the most common bacteria isolated, in forty four (33.59%) cases, followed by *Streptococcus pneumonia* isolated in thirty (22.90%) cases. *Pseudomonas aeruginosa* was isolated in twenty one (16.03%) cases. Other less common organisms isolate were *Escherichia coli* in eleven (8.40) cases, *Enterobacter* spp eight (6.11%). *Klebsiella pneumonia* seven (5.34%), *Moraxella catarrhalis* four (3.05%), *Staphylococcus aureus* four (3.05%), *Hemophlus influenza* two (1.53%), the lowest are *Proteus* one (0.76%) and *Pseudomonas lenteola* one (0.76%), two cultures showed two bacteria as in **Table 3.**

Table 3. The organism isolated from sputum culture

Organism	No.	%
Acinetobacter spp.	44	33.59%
Streptococcus pneumonia	30	22.90%
Pseudomonas aeruginosa	21	16.03%
E. coli	11	8.40%
Enterobacter	8	6.11%
Klebsiella pneumonia	7	5.34%
Moraxella catarrhalis	4	3.05%
Staphylococcus aureus	4	3.05%
Hemophlus influenzae	2	1.53%
Proteus	1	0.76%
Pseudomonas lenteola	1	0.76%

Regarding the antibiotics resistance for *Acinetobacter* spp, for Amoxicillin\Clavulinic acid (AMC) 87.5%, Amikacin (AK) 56.7%, Cefotaxime (CTX) 100.0%, Ceftriaxone (CRO)

100.0%, Levofloxacin (LEV) 62.5%, Ciprofloxacin (CIP) 88.9%, Gentamycin (GM) 71.1%, Penicillin G (PIZ) 100.0%, Pipracillin (PI) 100.0%, Trimethoprim (TM) 95. 0%, Imipenem (IPM) 83.3%, Ticarcillin (TC) 100.0%, Chloramphenicol (C) 91.7%, as in **Table 4**.

Table 4. Antibiotics sensitivity pattern for *Acinetobacter spp*

	***Acinetobacter* spp.**			
	R	%	S	%
AMC	14	87.5%	2	12.5%
AK	17	56.7%	13	43.3%
CTX	9	100.0%	0	0.0%
FOX	2	100.0%	0	0.0%
CRO	27	100.0%	0	0.0%
LEV	5	62.5%	3	37.5%
CIP	8	88.9%	1	11.1%
CD	3	100.0%	0	0.0%
GM	27	71.1%	11	28.9%
PIZ	5	100.0%	0	0.0%
PI	14	100.0%	0	0.0%
TM	19	95.0%	1	5.0%
IPM	20	83.3%	4	16.7%
TC	13	100.0%	0	0.0%
AMP	1	100.0%	0	0.0%
C	11	91.7%	1	8.3%
ATM	2	100.0%	0	0.0%

Trimethoprim sulfamethoxazole (TS), Rifampin (Rif), Clindamycin (Clin), Imipenem (Imi), Azithromycin (AZ), , Amikacin (Ami), Ciprofloxacin (Cip), Tetracycline (T), Cefotaxime (Cef), Doxycycline (Dox), Netilmicine (Net), Levofloxacin (Lev).Sensative (s) ,Resistence (R)

The antibiotics sensitivity pattern for *Streptococcus pneumonia*: Azithromycin (AZM) 46.2% sensitive and 53.8% resistance, Amoxicillin\Clavulinic acid (AMC) 37.5% sensitive and 62.5%, Amikacin (AK) 75.0% sensitive and 25.0%, Cefoxitine (FOX) 20.0% sensitive and 80.0% resistance, Ceftriaxone (CRO) 80.0% sensitive and 20.0% resistance, Ciprofloxacin (CIP) 75.0% sensitive and 25.0% resistance, Clindamycin (CD) 28.6% sensitive and 71.4% resistance, Gentamycin (GM) 95.0% sensitive and 5.0% resistance, Ipeminem (IPM) 90.9% sensitive and 9.1% resistance, Ampicillin (AMP) 90.0% sensitive and 10.0% resistance, Chloramphenicol (C) 73.7% sensitive and 26.3% resistance, as in **Table 5**

Table 5. Antibiotics sensitivity pattern for *Streptococcus pneumonia*

	Streptococcus pneumonia			
	R	%	S	%
AZM	7	53.8%	6	46.2%
AMC	5	62.5%	3	37.5%
AK	4	25.0%	12	75.0%
CTX	1	50.0%	1	50.0%
FOX	4	80.0%	1	20.0%
CRO	2	20.0%	8	80.0%
CIP	2	25.0%	6	75.0%
CD	15	71.4%	6	28.6%
GM	1	5.0%	19	95.0%
PIZ	1	100.0%	0	0.0%
PI	1	50.0%	1	50.0%
RP	0	0.0%	2	100.0%
TM	3	100.0%	0	0.0%
IPM	1	9.1%	10	90.9%
TC	2	66.7%	1	33.3%

AMP	1	10.0%	9	90.0%
C	5	26.3%	14	73.7%

Trimethoprim sulfamethoxazole (TS), Rifampin (Rif), Clindamycin (Clin), Imipenem (Imi), Azithromycin (AZ), , Amikacin (Ami), Ciprofloxacin (Cip), Tetracycline (T), Cefotaxime (Cef), Doxycycline (Dox), Netilmicine (Net), Levofloxacin (Lev).Sensative (s) ,Resistence (R)

The antibiotics sensitivity pattern for *Pseudomonas aeruginosa*: Amoxicillin\ Clavulinic acid (AMC) 57.1% sensitive and 42.9% resistance, Amikacin (AK) 76.9% sensitive and 23.1% resistance, Cefotaxime (CTX) 20.0% sensitive and 80.0% resistance, Ciprofloxacin (CIP) 50.0% sensitive and 50.0% resistance, Gentamycin (GM) 82.4% sensitive and 17.6% resistance, Pipracillin (PI) 66.7% sensitive and 33.3% resistance, Ipimenem (IPM) 41.7% sensitive and 58.3% resistance, Ticarcillin (TC) 42.9% sensitive and 57.1% resistance, Chloramphenicol (C) sensitive 100.0% as in **Table 6**.

Table 6. Antibiotics sensitivity pattern for *Pseudomonas aeruginosa*

	Pseudomonas aeruginosa			
	R	%	S	%
AZM	0	0.0%	1	100.0%
AMC	3	42.9%	4	57.1%
AK	3	23.1%	10	76.9%
CTX	4	80.0%	1	20.0%
FOX	1	33.3%	2	66.7%
CRO	1	33.3%	2	66.7%
LEV	2	66.7%	1	33.3%
CIP	3	50.0%	3	50.0%
CD	1	100.0%	0	0.0%
GM	3	17.6%	14	82.4%
PI	3	33.3%	6	66.7%
RP	3	100.0%	0	0.0%
TM	1	50.0%	1	50.0%
IPM	7	58.3%	5	41.7%
TC	4	57.1%	3	42.9%
AMP	2	66.7%	1	33.3%
C	0	0.0%	4	100.0%

Trimethoprim sulfamethoxazole (TS), Rifampin (Rif), Clindamycin (Clin), Imipenem (Imi), Azithromycin (AZ), , Amikacin (Ami), Ciprofloxacin (Cip), Tetracycline (T), Cefotaxime (Cef), Doxycycline (Dox), Netilmicine (Net), Levofloxacin (Lev).Sensative (s) ,Resistence (R)

The antibiotics sensitivity pattern for *E. coli*: Amoxicillin\ Clavulinic acid (AMC) 25.0% sensitive and 75.0% resistance, Amikacin (AK) 90.9% sensitive and 9.1% resistance, Cefotaxime (CTX) 20.0% sensitive and 80.0% resistance, Cefoxitime (FOX) 50.0% sensitive and 50.0% resistance, ceftriaxone (CRO) 33.3% sensitive and 66.7% resistance, Gentamycin (GM) 54.5% sensitive and 45.5% resistance, Ipeminem (IPM) 75.0% sensitive and 25.0% resistance, as in **Table 7**.

Table 7. The antibiotics sensitivity pattern for *E. Coli*

	E. coli			
	R	%	S	%
AMC	3	75.0%	1	25.0%
AK	1	9.1%	10	90.9%
CTX	4	80.0%	1	20.0%
FOX	2	50.0%	2	50.0%
CRO	4	66.7%	2	33.3%
LEV	1	100.0%	0	0.0%
CIP	1	50.0%	1	50.0%
CD	2	100.0%	0	0.0%
DXT	1	50.0%	1	50.0%
GM	5	45.5%	6	54.5%
PI	2	100.0%	0	0.0%
TM	1	50.0%	1	50.0%
IPM	1	25.0%	3	75.0%
TC	1	100.0%	0	0.0%
AMP	3	100.0%	0	0.0%
C	0	0.0%	3	100.0%
ATM	0	0.0%	2	100.0%

Trimethoprim sulfamethoxazole (TS), Rifampin (Rif), Clindamycin (Clin), Imipenem (Imi), Azithromycin (AZ), , Amikacin (Ami), Ciprofloxacin (Cip), Tetracycline (T), Cefotaxime (Cef), Doxycycline (Dox), Netilmicine (Net), Levofloxacin (Lev).Sensative (s) ,Resistence (R)

4. Discussion:

Due to increasing usage of antibiotic, the antibiotic resistance became an important medical problem [17]. In this study male is higher than female which may be due to the different in social state. This is in agreement with that of Akansha et al [18] in which male is higher (73.4%) than females (26.6%).

In this study the highest percent in age between 50- 60 years, in Haroon study [19] the highest percent in age between 55- 65 years. Also in this study most of the samples from patients admitted to respiratory care unit and medical word, The Infectious Diseases Society of America/American Thoracic Society consensus (IDSA/ATS) guidelines recognize the limitations of sputum Gram stain and culture [20]. In which the expectorated sputum specimens are recommended for hospitalized patients with any of the following criteria: Intensive care unit admission, Failure of the antibiotic therapy (either hospitalized or outpatients patients), in addition to other criteria.

Regarding the organism isolated from sputum culture in this study, *Acinetobacter spp* is the most common organism isolated (33.59%), this high percent may be due to large number of our patients from ICU, as the risk of infection due to gram-negative organisms, including *Acinetobacter baumannii*, increased with ICU admission, increase the age of patients, use of the catheters or intubation, and prolonged hospital admission [21]. *Streptococcus pneumonia* in this study was 22.90%, in Akansha study [18] *Streptococcus pneumonia* was 20.22%, *Pseudomonas aeruginosa* in this study was16.03%, in Akansha study [18] x9.84%. *E. coli* in this sudy was 8.40%, in [22] study *E. coli* 11.7%. *Enterobacter* in this study was 6.11%, in Jean-Jacques study [23] *Enterobacter* 3%. *Klebsiella pneumonia* in this study 5.34%, in Miriam study [24] study *Klebsiella pneumonia* was 3.6%. *Moraxella catarrhalis* in this study was 3.05%, in Jean-Jacques study [23] was *Moraxella* 3%. *Staphylococcus aureus* in this study was 3.05%, in [23] study study *Staphylococcus aureus* was 3.6%. *Haemophilus influenza* in this study 1.53%, in Jean-Jacques study [23] *Haemophilus influenza* 3%. The Antibiotics sensitivity pattern for common microorganism isolated in this study.

For *Acinetobacter* spp. this study showed very high resistance rate to most of antibiotics. Al-Obeid study (25) for antimicrobial sensitivity of 506, 510 and 936 patients isolates *A. baumannii* during 2006, 2009, 2012, respectively. The resistant of *A. baumannii* to variety of antimicrobial drugs changed as following: 19% in (2006) to 89% in (2012) for meropenem, 36% in (2006) to 91.7% in (2012) for imipenem, 54% in (2006) to 89.2% in (2012) for ciprofloxacin, 71% in (2006) to 83% in (2012) for amikacin, and 75% in (2006) to 83% in (2012) for ceftazidime.

The antibiotics sensitivity pattern for streptococcus pneumonia in this study showed highly sensitive to Amikacin, CRO, Ciprofloxacin, Gentamycin, Imipenem, Amp, while it is highly resistant to AMC, CD, FOX. In Devanath study [25] *Streptococcus pneumoniae* was sensitive to ampicillin, amikacin, gentamicin, co-trimoxazole, penicillin and erythromycin. In Haroon study [19] *Streptococcus pneumoniae* was sensitive to meropenem, gentamicin, linezolid followed by moxifloxacin, ceftriaxone, ciprofloxacin, levofloxacin and azithromycin.

The antibiotics sensitivity pattern for pseudomonas aeruginosa in this study showed highly sensitive to Amikacin, Pi, Gentamycin, and it is highly resistant to CTX, while the resistant rate for Imipenem 58.3 % and for TC 57.1%. In Devanath study (26) Pseudomonas aeruginosa was mainly sensitive to piperacillin, gentamicin and amikacin. In Haroon Study (19) Pseudomonas aeruginosa was mainly sensitive to gentamicin and meropenem. The sensitivity rate was 65% for azithromycin and 28% for amoxicillin–clavulanic acid.

The antibiotics sensitivity pattern for *E coli* pneumonia in this study showed highly sensitive to Amikacin, Imipenem. And it is highly resistant to ACM, CTX, CRO. In Ashis study (27) the sensitivity to Ertapenem 72.91%, Imipenem 85.41%, tigicycline 70.83%, chloramphenicol and amikacin 62.4%.these bacteria resistance to many antibatic because it have many mechanism such as development of enzymes ,target modification ,alternation of membrane permeability and alternation of metabolic pathway(28)

5. Conclusion

This retrospective study which evaluated the bacteriological found that *Acinetobacter* spp. is the most common organism isolated in this study, which has high resistant rate. Other common organism isolated were *Streptococcus pneumonia*, *Pseudomonas aeruginosa*, *E. coli* with variable antibiotics sensitivity and resistant rates. Amikacin and gentamycin are most common sensitive drugs for these common bacteria

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