

Haematological Study for Burns, Wound and Urinary Tract Patients Infected with *Klebsiella spp.* and their Antibiotic Sensitivity

Sawsan H. Authman

Khalida J. Ibraheem

Zainab M. Atawei

College of Science . Al-Mustansiriyia Univesity

Summary

The study included (513) clinical samples distributed to 238 burn swabs, 139 wound swabs, 136 urine during two successive years 2008 and 2009 from different ages and both genders. Bacteriological culture, morphological and biochemical tests employed to diagnose *Klebsiella*. The results showed they were 370 isolates. The highest frequency of *Klebsiella* isolated recorded were (63.8)% during 2009, and highest incidence of *Klebsiella spp.* isolated from burn swabs 50% followed by wound and urine 29.1% and 24.6% respectively. And the highest isolated for *Klebsiella spp.* was found in burn female 58.4% compared with male. The most predominant age group were between 20-30 years with percentage of isolate 21.9%.

The haematological values Hb, PCV, ESR, MCHC and WBC counts were determined on the patients. The results showed that the highest isolation percentage of *Klebsiella* was found in patients with Hb value range between 10-15 gm/dL (79.8%) and PCV value 32-50% (79.8%) and ESR ranged between 20-40 mm (40.7%) and MCHC were 30-34 gm% (65.7%). WBC count showed numerical differences but not significant in comparable to the normal value.

The antibiotics susceptibility tested showed that *Klebsiella* isolates were complete resistance to Amoxycillin, Ampicillin, Cefoxitin, Cloxacillin, Penicillin and Tetracyclin (100%). Also the isolation source affected on the bacterial sensitivity.

Introduction

Klebsiella species are ubiquitous in nature and probably have two common habitats, one being the environment, where they are found in surface water, sewage and soil and on plants (1), and the other being the mucosal surfaces of mammals such as humans, horses which they colonize. In humans *K. pneumoniae* is present as a saprophyte in the nasopharynx and in the intestinal tract, carrier rates differ considerably from study to study (2).

Klebsiella is well known to most clinicians as a cause of community – acquired bacterial pneumonia, occurring particularly in chronic alcoholics (3), and due to severe pyogenic infection which was a high fatality rate if untreated (4).

The vast majority of *Klebsiella* infections, however are associated with hospitalization. As opportunistic pathogens, *Klebsiella spp.* Primarily attack immunocompromised individuals who are hospitalized and suffer from severe underlying disease such as diabetes mellitus (5).

Al-Faltawi (6) showed that *K. pneumoniae* isolated from 9% of patients suffering independent diabetes mellitus. Ibraheem and Hindi (7) found a high percentage contamination in the burns patients and ward, wherever *Klebsiella spp.* Was 22.1% and mentioned that *Klebsiella spp.* Survive on the hand of personal longer than other gram negative bacteria. Al-Reda (8) showed that the most common pathogen infecting urinary tract was *E. coli* 41.7% then *K. pneumoniae* 8.3%, other study indicated that the infection caused by *Klebsiella spp.* In children in Mosul suffering diarrhea was 4% (9).

A variety of infections have occasionally been associated with hemolytic anemia. The mechanism involved vary, some organisms among them such common pathogens as *Escherichia coli*, *Klebsiella spp.* Can produce red cell agglutination *In vitro*, whether this phenomenon is important in initiating *In vivo* hemolysis. Bacteria may also produce destruction of red cell indirectly when bacterial polysaccharides are absorbed onto erythrocytes. Also *Klebsiella* bacterium can cause Disseminated Intravascular Coagulation (DIC) accompanied by renal contractions, the mechanism by which DIC is produced may be related to toxin that activates platelets and induces IL-1 secretion by macrophages(10).

Over the past several years, the medical community has become increasingly concerned over the ability of certain bacteria to develop

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resistance to antibiotics (11). Accordingly there is a danger of losing the battle against certain pathogens by using the antibiotics in the treatment(12).

The study aimed to : isolation and identification of *Klebsiella* were present in wound, burns and urine samples obtained from patients attended the hospitals during the years 2008 and 2009. And study the different factors (age, sex) which may effect on the incidence of *Klebsiella* infection and determination of the most effective antimicrobial agent by performing antimicrobial susceptibility tests. Also perform a hematological study on patients to identify any specific hematological changes, in each patient groups suffering from wound, burns and UTI.

Materials & Methods

1 - Collection of samples:

1-1: Burns and Wounds Swabs:

A total numbers of 377 patients both male and females with different age were enrolled in this study during the period from January 2008 to December 2009. The samples included 238 samples of burns swabs and 139 samples of wound swabs collected from patients admitted to the Baghdad Teaching Laboratory which is situated of Baghdad. Sterilized swabs were used and all obtained were directly cultured in two prepared media Blood agar and MacConkey agar. All culture plates were examined after overnight incubation at 37C (8).

1-2: Urine Samples:

Urine samples were obtained from 136 patients both male and females with different age groups during 2008 to 2009, from patients admitted to the Baghdad Teaching Laboratory. The samples were collected aseptically in sterile containers, mid-stream specimens were taken from the patients and the samples were cultivated using culture media including the blood agar and MacConkey agar, Nutrient agar which were prepared according to manufactures recommendations (Liofilchen / Italy) (7).

1-3: Blood Specimens:

Blood specimens of 5mL were collected by venous puncture using disposable syringes from all patients (513). An constricted blood flow was allowed to accumulate to plastic tubes with and without EDTA for hematological tests (9) which included : Haemoglobin estimation (Hb), haematocrit packed cell volume (PCV), the mean cell haemoglobin concentration (MCHC), Erythrocyte sedimentation rate (ESR) and WBC count. These tests were done according to (13).

2 - Identification of Isolates:

After incubation, colonies morphologically suggestive of *Klebsiella* were identified by conventional biochemical reactions (IMVIC, motility, triple sugar iron, oxidase, catalase, urease) by standard methods (14-15).

3 - Sensitivity Test to Antimicrobial Agents:

Antimicrobial susceptibility testing was performed with the standard disk diffusion method according to the Clinical and Laboratory Standard Institute (CLSI) (16-17). A total of 25 antimicrobials were tested.

Results and Discussion

Five hundred and thirteen samples were obtained from burn and wound swabs and urine which were obtained from 191 patients with burn and wound infection and UTI during 2008 and 223 patients during 2009. These patients which were 10<-60> years old attended outpatient clinic and diagnosed by physician as having those infections. And most probably reside in the regions around Al-Medicine City Hospital.

Table (1) shows a total of 191 samples (burn, wound, urine) of which 134 isolates (36.2%) were identified as *Klebsiella spp.* During 2008 and 236 (63.8%) isolates during 2009. The results implicate that the most frequently for *Klebsiella* encountered were during 2009. These seemingly high isolation rates observed of *Klebsiella* could be related to environmentally acquired isolates. It is worthy to notice that *Klebsiella* are opportunistic pathogens and can give rise to sever disease such as septicemia, pneumoniae, UTI and soft tissue infections (14). In this context highest incidence of *Klebsiella spp.* isolated from burn swabs 67(50%) during 2008, and 118 isolates (50%) during 2009 followed by wound swabs 39 isolates (29.1%) and 60 isolates (25.4%) during 2008 and 2009 respectively. Followed by urine samples which were 28 isolates (20.9%) and 58 isolates (24.6%) during 2008 and 2009 respectively (Table-2).

A high percentage of contaminated samples were indicated in the burns ward may be due to patients themselves or particles capable of being air born and low hygiene and sanitation condition in the ward. The result agree with Plowman et al (18) who recorded a high infection rate in burn words was 50%.

From the total 370 isolates of *Klebsiella spp.* 128 (58.4%) isolates were in burn female and 40(18.3%) were female urine (Table-3), the highest isolated for *Klebsiella spp.* was found in burn females. It is worthy to notice that differences were detected between males and females. While the percentage of isolates was found in male urine higher than in female

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urine (30.5%) and (18.3%) respectively. The study agree with (19) who found that 80% of bacterial UTI and prostatitis causes results from infection of *Escherichia coli* and *Klebsiella spp.*

Table (4) shows the distribution of age group in patients infected with *Klebsiella spp.* which ranged from 9 to more than 60 years old, that revealed this organism affected any age groups (children, young and adults) but the most predominant age groups between (20-30) years old (21.9%) of isolates and (30-40) years old, the isolation percentage (18.9%) from the total infected patient with *K. spp.* that's means a high incidence was observed in adults groups without a big differences between male (22.2%) and female (23.6%). As the researches mentioned the *Klebsiella* generally do not cause disease but they become pathogen only when they reach tissue outside of their normal intestinal or other less common normal flora sites, and the organism may reach the blood stream and cause sepsis(14).

The hematological values, Hb, PCV, ESR, MCHC and WBC counts were determined on the patients with burn, wound and urinary tract infection which are shown in tables (5-9).

The average hemoglobin concentration (Hb) was between 5.1-15 gm/dL for all 513 patients (Table-5) and the highest isolation percentage of *Klebsiella* (79.8%) was found in wound patients with hemoglobin values range between 10-15 gm/dL. As know the normal Hb value ranged between 12-16 gm/dL as (13) thus the study patients mostly were correctable by nutritional therapy with in an uncorrectable Hb value (20). The same results was found in PCV value which ranged between (16-50%) (Table-6) also the highest percentage for *Klebsiella* isolates was found in wound patients (79.8%) followed by burn patients (69.2%) and UTI patients (65.1%) with PCV ranged between 32-50%. However the higher total number of isolates was (263) isolates was recorded in patients ranged their PCV value between 32-50%. Again we mentioned that the patients infected with *Klebsiella* get less PCV value compared with normal value which ranged between 37-52% (13).

The infection with *Klebsiella* decrease their PCV value due to the low haemoglobin catabolic rate due to agene defect (21).

Table (7) shows the patients ESR value infected with *Klebsiella*. The higher incidence of *Klebsiella* infection was found 141(38.1%) isolates at ESR ranged between 20-40 mm and the highest percentage of isolates was found (40.7%) in patients with UTI at ESR value ranged

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between 20-40 mm. ESR is the erythrocyte sedimentation rate and the normal value is 0 to 5mm at the end of 1 hr (13). So this study determine the effect of *Klebsiella* infection on the ESR value, when the infection increase the value more than 20mm, in spite of the infection case (wounds, burns, UTI).

The mean cell haemoglobin concentration (MCHC) ranged between 31-35 gm% as normal value (13). In this study the (MCHC) ranged between 26-34 gm% which mean the infection with *Klebsiella* effect on the (MCHC) value, when the highest number of isolates was found (243) (65.7%) in patients with MCHC were 30-34 gm% in comparison with the normal value 31-35 gm%. The mechanism involved vary, some organisms such common pathogens as *Klebsiella* can produce red cell agglutination but it is not know whether this phenomenon is important in initiating *In vivo* haemolysis. Bacteria can also produce destruction of red cells indirectly when bacterial polysaccharide absorbed onto erythrocyte (22).

WBC count showed numerical differences but not significant in comparable to the normal value. In the infected patients with *Klebsiella* there is a little decline in the WBCs count, when it range between 2-6 $10^3/\text{mm}^3$ in UTI patients with highest percentage of *Klebsiella* isolates (46.5%) (Table-9). This may be due to the way which the bacteria affect through impair the gyrase, an enzyme which plays a major role in the replication of DNA (23). And this may cause decrease in immune response by reducing total WBCs count in blood.

In this experiment determined of antibiotics sensitivity was done by agar diffusion method to the 370 isolates of *Klebsiella spp.* isolated from patients suffered from burns, wound and UTI. Table (10) showed that *Klebsiella spp.* isolates were complete resistance to Amoxycillin, Ampicillin, Cefoxitin, Cloxacillin, Penicillin and Tetracyclin (100%). The results obtained were in agreement with (24). And the isolates less resistance to Augmentin (86.6%) Amikacin (86.6%), Cefuldin (85.1%). Yuan (25) isolated *Klebsiella* with highly resistant to third and fourth generation of cephalosporins from urine. Also the results observed the effect of the isolation source on their antibiotic sensitivity, when the *Klebsiella* isolated from burns patient give much resistance to the antibiotics than these isolated from urine.

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Table-1: Percentage of patients infected with *Klebsiella spp.* during the years 2008 and 2009.

%	No. of isolates	%	Total No. of infected patient	Year
36.2	134	37.2	191	2008
63.8	236	62.8	322	2009
100	370	100	513	Total

Table-2: Percentage of *Klebsiella* isolates according the type of samples during 2008 and 2009.

%	No. of isolates	No. of sample	Sample	Year
50	67	84	Burn	2008
29.1	39	58	Wound	
20.9	28	49	Urine	
100	134	191		Total
50	118	154	Burn	2009
25.4	60	81	Wound	
24.6	58	87	Urine	
100	236	322		Total

Table-3: Prevalence of *Klebsiella* isolates according to gender and samples.

Total No. of isolates	No. of isolates				Samples
	%	Female	%	Male	
185	58.4	128	37.7	57	Burn
99	23.3	51	31.8	48	Wound
86	18.3	40	30.5	46	Urine
370	100	219	100	151	Total

Table-4: Distribution *Klebsiella* isolates according to the age and gender.

Gender				%	No. <i>Klebsiella spp.</i> isolates	Age group
%	Female	%	Male			
14.8	30	22.1	37	18.1	67	9 – 19
23.6	48	19.8	33	21.9	81	20 – 30
18.2	37	19.8	33	18.9	70	30 – 40
14.8	30	6.6	11	11.1	41	40 – 50
12.8	26	15.6	26	14.1	52	50 – 60
15.8	32	16.1	27	15.9	59	60 >
100	203	100	167	100	370	Total

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Table-5: The relationship between patients hemoglobin value and *Klebsiella* isolates according to the type subjects.

%	Total	Subjects			Hb gm/dL
		Urine	Wounds	Burns	
		No. of isolates			
30.8	114	30 (34.9%)	20 (20.2%)	64 (34.6%)	5.1 – 10
69.2	256	56 (65.1%)	79 (79.8%)	121 (65.4%)	10 – 15
	370	86	99	185	Total

Table-6: The relationship between patients PCV value and *Klebsiella* isolates according to the type subjects.

Fungal isolates according to the type subjects:					
%	Total	Subjects			PCV %
		Urine	Wounds	Burns	
		No. of isolates			
28.9	107	30 (34.9%)	20 (20.2%)	57 (30.8%)	16 – 32
69.2	256	56 (65.1%)	79 (79.8%)	128 (69.2%)	32 – 50
	370	86	99	185	Total

Table-7: The patients ESR value infected with *Klebsiella* according to the type of subjects.

%	Total	Subjects			ESR mm
		Urine	Wounds	Burns	
		No. of isolates			
31.4	116	35 (40.7%)	30 (30.3%)	51 (27.6%)	20 >
38.1	141	35 (40.7%)	36 (36.4%)	70 (37.8%)	20 – 40
18.4	68	13 (15.1%)	10 (10.1%)	45 (24.3%)	40 – 60
7.0	26	3 (3.5%)	10 (10.1%)	13 (7.1%)	60 – 80
5.1	19	0 (0 %)	13 (13.1%)	6 (3.2%)	80 >
	370	86	99	185	Total

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Table-8: The patients MCHC value infected with *Klebsiella* according to the type of subjects.

%	Total	Subjects			MCHC gm/dL
		Urine	Wounds	Burns	
		No. of isolates			
34.3	127	27 (31.4%)	36 (36.4%)	64 (34.6%)	26 – 30
65.7	243	59 (68.6%)	63 (63.6%)	121 (65.4%)	30 – 34
	370	86	99	185	Total

Table-9: The total count of white blood cell (WBC) in patients infected with *Klebsiella* according to the types of subjects.

%	Total	Subjects			Total count of WBC 10 ³ /mm ³
		Urine	Wounds	Burns	
		No. of isolates			
35.9	133	40 (46.5%)	29 (29.2%)	64 (34.6%)	2 – 6
35.7	132	13 (15.1%)	42 (42.4%)	77 (41.6%)	6 – 10
17.6	65	13 (15.1%)	20 (20.2%)	32 (17.4%)	10 – 14
7.3	27	13 (15.1%)	8 (8.2%)	6 (3.2%)	14 – 18
3.5	13	7 (8.2 %)	0 (0 %)	6 (3.2%)	18 >
100	370	86	99	185	Total

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Table-10: Antibiotic resistance of 370 isolates of *Klebsiella spp.* encountered in study group.

Resistance %			Antibiotics
Urine	Wounds	Burns	
--	56.4	86.6	Amikacin
78.6	100	100	Ampiclox
85.7	100	100	Amocycillin
100	100	100	Ampicillin
100	84.6	86.6	Augmentin
64.3	100	100	Cefotaxime
71.4	69.2	77.6	Cefalexin
28.6	69.2	100	Ceftriaxene
50	100	85.1	Cefuldin
100	100	100	Cefoxitin
100	100	100	Cloxacillin
100	43.6	52.2	Ciprofloxacin
21.4	71.8	100	Chloramphenicol
53.6	100	100	Gentamycin
30	35.9	37.3	Meropenem
100	100	100	Penicillin
50	58.9	67.2	Pefloxacin
100	100	97	Tetracycline
32.1	--	--	Trimethoprim
50	100	100	Streptomycin

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دراسة معالم الدم لمرضى الحروق والجروح والمصابين بالتهاب المجاري البولية والمخمرين ببكتريا *Klebsiella* وحساسيتها للمضادات الحيوية

سوسن حسن عثمان، خالدة جليل ابراهيم، زينب محمد عطوي

كلية العلوم – الجامعة المستنصرية

الخلاصة

تضمنت الدراسة جمع (٥١٣) عينة سريرية . توزعت إلى ٢٣٨ مسحات جروح، ١٣٩ مسحات جروح و ١٣٦ عينة أدرار ولسنتين متتاليتين ٢٠٠٨ و ٢٠٠٩ ومن مختلف الأعمار ولكلا الجنسين. ومن خلال نتائج الزرع البكتريولوجي والتشخيصي المظهري والكيموحيوي تم عزل ٣٧٠ عذلة تعود لبكتريا *Klebsiella* . وكانت أعلى نسبة عزل (٦٣,٨%) خلال سنة ٢٠٠٩ . وبلغت أعلى نسبة عزل للـ *Klebsiella* من مسحات الحروق (٥٠%) يليها الجروح والأدرار (٢٩,١%) و (٢٤,٦%) وعلى التوالي. وجد أن أعلى نسبة لعزل *Klebsiella* كانت من حروق النساء ٥٨,٤% مقارنة بالرجال. فيما كانت الفئة العمرية الأكثر إصابة بالبكتريا هي ما بين ٢٠-٣٠ سنة ونسبة ٢١,٩% . أجريت اختبارات الدم للمرضى وتضمنت قياس Hb ، PCV ، ESR و MCHC وقياس عدد كريات الدم البيضاء WBC . إذ أظهرت النتائج أن أعلى نسبة لعزل الـ *Klebsiella* كانت من المرضى الذين تراوح هيموكلوبين الدم لهم Hb ما بين ١٠-١٥ غرام/ دالتون (٧٩,٨%) وتراوح PCV لهم ما بين ٣٢-٥٠% (٧٩,٨%) وبلغ ESR ما بين ٢٠-٤٠ ملم وكان MCHC لهم ما بين ٣٠-٣٤ غم%. كان عدد كريات الدم لدى المصابين مختلفاً عن الأصحاء وبفارق غير معنوي.

أظهرت نتائج فحص حساسية العزلات لمضادات الحيوية مقاومة تامة من عزلات *Klebsiella* للمضادات الحيوية Amoxycillin ، Ampicillin ، Cefoxitin ، Cloxacillin ، Penicillin و Tetracyclin (١٠٠%) كذلك أثر مصدر العزل على حساسية البكتريا لمضادات الحيوية.