Bacteriological Study of *Klebsiella pneumoniae* Isolated from Burn Patient in Al-Najaf City

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Abstract

A total of (60) clinical specimens were collected from patients suffering from various burns during the period from) November 2018 to January, 2019). These specimens were collected from patients attending to Al-Furat Al-Awsat burn center during the studied period . All specimens were cultured on the MacConkey agar plates and incubated at 37°C under aerobic condition for 18 - 24 hour. In 60 patients, 36 (60%) were female and 24 (40%) were male. Several morphological, physiological and biochemical tests were made to identify bacterial isolates. Results showed that *K.pneumoniae* constitute 15 isolates (25%) of these isolates. A collection of 15 *K. pneumoniae* isolates diagnosed by the morphological ,cultural and biochemical characters, the identification was confirmed by molecular method for the presence of *magA* . The results showed that only 10 (66.6%) isolates was carrying *magA* which are diagnosed as *K.pneumoniae*. The results of antibiotic sensitivity screening test revealed that the isolates of *K. pneumoniae* exhibited highly sensitivity to Azithromycin ,moderate sensitivity for Gentamicin and Imipenem while the findings showed absolute resistant to Erythromycin , highly resistant to Ciprofloxacine , Trimthoprime. According to this result it can considered that Azithromycin as best treatment against *K. pneumoniae*.

Keyword: *Bacteriological, Klebsiella pneumonia, burn patient, clinical specimens and Al-Najaf City*

Introduction

Burn wound infections are one of the most important and potentially serious complications that occur in the acute period following injury[(1)]. These wounds are subsequently colonized with microorganisms, including gram-positive bacteria, gram-negative bacteria and yeasts, which derived from the host's normal flora (gastrointestinal flora, upper respiratory flora) and from the hospital environment [(2, 3)]. The risk of invasive burn wound infection is influenced by the extent and depth of the burn injury, various host factors, and the quantity and virulence of the microbial flora colonizing the wound[(4)]. The common burn wound pathogens are*, Klebsiella spp., Pseudomonas aeruginosa* and *Staphylococcus aureus,* which produce a number of virulence factors that are important in the pathogenesis of invasive infection[(5,6)]. *Klebsiella pneumoniae* is widely distributed in the gastrointestinal, urinary, and respiratory tracts of healthy people. It cause opportunistic infections mainly nosocomial infections, it is a common hospital-acquired pathogen causing severe respiratory infections such as pneumonia. Other infections caused by this organism include urinary tract infection, wound infection, abscesses, sepsis, inflammation and diarrhea, most *K. pneumoniae* are hospital associated with a high fatality rate if incorrectly treated. Treatment of *Klebsiella* infections is complicated[(7)]. *K. pneumoniae* have different virulence factors which give the bacteria the ability to invade the host, such as capsular polysaccharide, lipopolysaccharide, serum resistance, siderophore production, fimbriae and other factors such as the production of urea and enterotoxin[(8)]. However, antibiotic resistance properties are the major factor in its pathogenicity that it resists for wide spectrum of antibiotics and specially β-lactam antibiotics. This is due to the prevalence of infections acquired in hospital which led to the orientation of the research on alternative therapies [(9)]. Due to the common occurrence of *Klebsiella spp*. And high virulence that cause in the absence of accurate and early detection them, severe damage may lead to the death of the patient. The aim of this study was to determine the causative microorganisms of burn wound infection and antibiotic sensitivity.

2-Materials & Methods

-Specimens Collection :

A total of (60) clinical specimens were collected from patients suffering from various burns during the period from) November 2018 to January, 2019). These specimens were collected from patients attending to middle Euphrates burn center during the studied period . All specimens were cultured on the MacConkey agar plates and incubated at 37°C under aerobic condition for 18 - 24 hour [10].

-Isolation and Identification of *Klebsiella pneumoniae*

All specimens were initially cultured on isolation media including MacConkey agar. After incubation of agar media for 24 hr. at 37 ºC, the suspected colonies of pure cultures were investigated. Then the bacteria were confirmed by an additional biochemical test with VITEK-2 compact system and P.C.R technique.

-Antibiotic Susceptibility Test

The detection of the susceptibility of *Klebsiella pneumoniae* to a group of antibiotics Kirby-Bauer discs diffusion method was carried out according to [11]. A pure culture of previously identified bacteria was prepared by adding a growth from isolated colony to 5 ml of sterile normal saline in a cell density equivalent to turbidity of McFarland tube No. (0.5) which approximately equal to bacterial cells density of $1.5x10^8$ cells/ml. A sterile cotton swab was used to obtain inoculums to be streaked on Blood Muller Hinton Agar medium. The antibiotic discs were placed on the surface of the medium at evenly spaced intervals with flaming sterile forceps. Incubate the plate for 18 hr at 37°C. Antibiotic inhibition zone was measured by using ruler. Zone diameter was compared to standard results being recommended by clinical laboratory standards institute documentations.

-Molecular Study of *Klebsiella pneumonia*

-Isolation of Bacterial Chromosomal DNA

Total DNA was extracted from colonies grown on agar plates by boiling method according to [12] with some modifications. One bacterial colony was scraped using sterile toothpick from surface of agar plates and suspended in 40 µl Tris-EDTA buffer. The suspension was heated for 15 min at 100 ºC followed by 5 min on ice rapidly. The suspension containing DNA was stored at -20 ºC until used as template for PCR.

-Primers Selection:

All primers in this study were synthesized by Bioneer company (Korea). The sequences of this primer were *magA*: F5'- CGC CGC AAA TAC GAG AAG TG -3′; R**5′**- GCA ATC GAA GTG AAG AGT GC -3′, the product of this gene 540bp.[13].

-The PCR conditions of the *magA* gene: were the initial denaturation temperature of 94°C for 2min. three thermo-cycler condition (1- denaturation of 94°C for 45sec . 2-an annealing temperature of 52°C for 45sec .3- extension of 72°C for 45sec . The final extension of 5 min at 72°C . This condition used for 35 cycles .

-Agarose Preparation

Agarose was weighted 1g, boiled in100ml (1X) TBE buffer, left to cool at 50 ºC and 5 µl of ethidium bromide is added to agarose and poured on preparing tray. Comb was removed after hardening of agarose leaving wells.

TBE (1X) buffer was added to the electrophoresis tank, tray with agarose was immersed in electrophoresis tank. Each well is loaded with 7µl of DNA sample and standard molecular weight of DNA ladder (marker) is loaded in a first well. Electrophoreses run at 80 volt/cm for 1hr. Gel was visualized with UV transilluminator and photographed by using digital Camera [14].

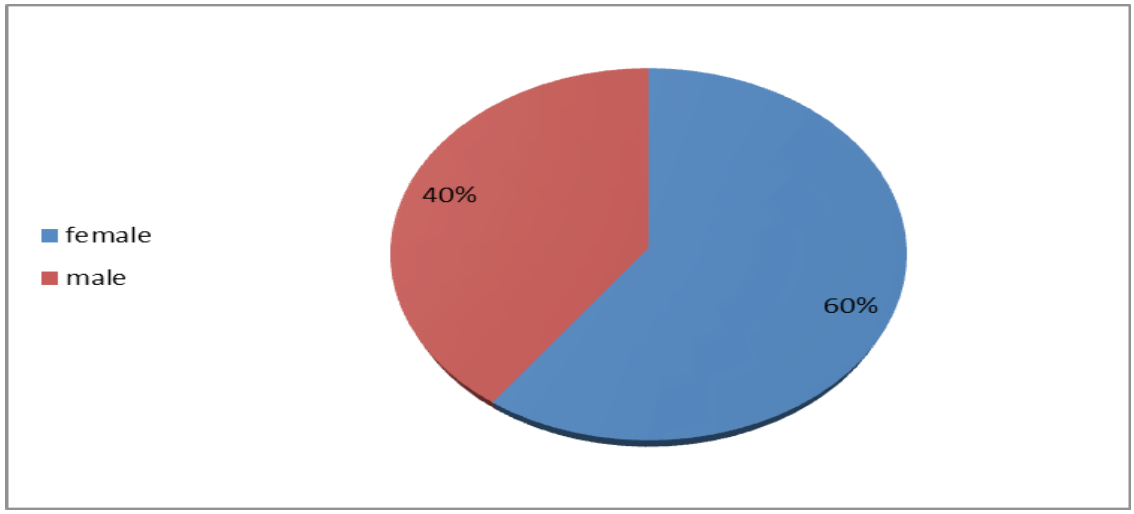
3.The results

-Bacterial Isolation

A total of (60) clinical specimens were collected from patients suffering from various buns during the period from) November 2018 to January, 2019). These specimens were collected from patients attending to middle Euphrates burn center during the studied period . All specimens were cultured on the MacConkey agar plates and incubated at 37°C under aerobic condition for 18 - 24 hour.

In 60 patients, 36 (60%) were female and 24 (40%) were male as in figure(1). However, [15] stated that female infection was higher (54.5 %) than male (45.6 %). In addition, [16] viewed that 209 cases were males and 995 cases females, patients were mainly of the group ≥ 60 years. They referred that male patients above the age of 60 years were subjected to greater frequency

of *K. pneumoniae* infection.



Figure(1): Distribution of infected patients according to sex

-*Klebsiella pneumoniae* Isolation and Identification

Clinical and environmental hospital samples were cultured on to MacConkey agar and incubated for 18-24 h at 37ºC. All lactose-fermenting isolates were tested by morphologic characteristics and standard biochemical tests according to [10]. Confirmation of *K.pneumoniae* was conducted using P.C.R system. Several morphological, physiological and biochemical tests were made to identify bacterial isolates. Results showed that *Klebsiella pneumoniae* constitute 15 isolates (25%) of these isolates, The other bacterial isolates were *Pseudomonas spp.* (6 isolates), *E.coli* (3 isolates) and *Staphylococcus spp.* (1 isolates) . Bacterial isolates were identified according to their cultural, microscopical and biochemical characteristics that were in agreement with [17].

Table (1): The morphological ,cultural and biochemical characters of *Klebsiella pneumoniae*

Test	Result
Indole production	-
Kliglar iron agar (KIA)	(for CO_2) +
Voges –Pproskauer	+
Simmons Citrate	+
Cell shape	Bacilli
MacConkey agar	Lactose ferment
Methyl red	Variable

-Antibiotic Susceptibility Test

Table (2) showed the antibiotic sensitivity screening test to a number of antibiotics on *Klebsiella pneumoniae* isolates by using Kirby-Bauer disk diffusion method .The results were interpreted according to the diameter of inhibition zones and compared with inhibition zones

determined by CLSI, and to decide the susceptibility of bacteria to antimicrobial agent whether being resistant.

The results revealed that the isolates of *Klebsiella pneumoniae* exhibited highly sensitivity to Azithromycin ,moderate sensitivity for Gentamicin and Imipenem while The findings showed absolute resistant to Erythromycin , highly resistant to Ciprofloxacine , Gentamicin and Imipenem.

Erythromycin has been regarded for many years as possessing a good spectrum of activity and safety record for the treatment of respiratory, skin, and soft tissue infections in both adults and children. Azithromycin which differs from erythromycin chemically by a methyl-substituted nitrogen in the macrolide ring This difference produces improvements in spectrum and potency compared with erythromycin[18].

Results showed also that the resistance of *K. pneumoniae* isolates to the aminoglycosids group included gentamicin were 50 %. [19]was rather different from the data. They reported that about 79 % of *Klebsiella* isolates were resistance to gentamicin. In addition, [20] found that all the *Klebsiella* strains in their studies were resistant to gentamicin. In this regard, [21] found that *Klebsiella* isolates producing extended spectrum β-lactamase enzymes were resistant to aminoglycosids.

Resistance of *Klebsiella* isolates to ciprofloxacin which belongs to the quinolones group was 20 %. [22] reported that the resistance to quinolones is releated to change in antibiotic-enzyme (GyrA) binding site.

[23] reported that carbapenem (imipenem) antibiotics have strong activity against ESBLs from *Klebsiella spp*. [23] observed that *K. pneumoniae* isolated from respiratory tract was sensitive against imipenem. In another study *K. pneumoniae* isolated from different clinical specimens was susceptible to imipenem [24]. The result of the susceptibility of *K. pneumoniae* to the imipenem correlated with results of [25] and [26] who revealed that only one isolate was resistant to the imipenem.

While resistance to Sulfonamide group included Trimethoprim + Sulphamethoxazole) was 77.5 %. This blocks two consecutive steps in bacterial biosynthesis of essential nucleic acids and proteins and is usually bactericidal [26]. According to this result it can considered that Azithromycin as best treatment against *K. pneumoniae*.

Table (2): Antibiogram for *Klebsiella pneumoniae* Isolates

Antimicrobial Class	Susceptible	Intermediate	Resistant
Erythromycin	0	0	10(100%)
Azithromycin	10(100%)	0	0
Gentamicin	5(50%)	0	5(50%)
Ciprofloxacine	3(30%)	2(20%)	5(50%)
Imipenem	5(50%)	0	5(50%)
Trimethoprime	1(10%)	0	9(90%)

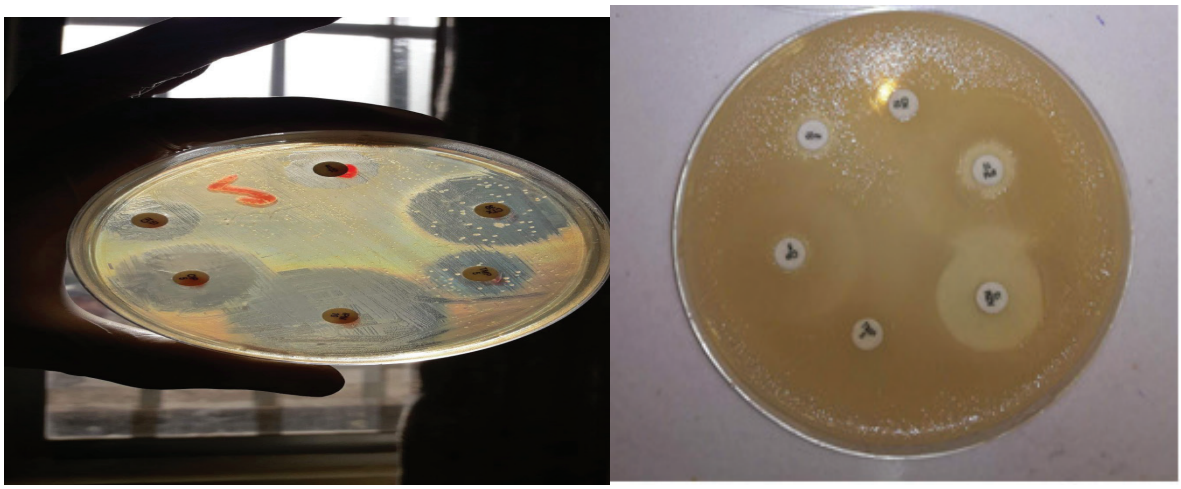


Figure (2): Kirby-Bauer disk diffusion method of *K. pneumoniae* isolate

3. Molecular Study (Genotypic Detection of *K. pneumoniae)*

A collection of 15 *Klebsiella pneumoniae* isolates diagnosed by the morphological ,cultural and biochemical characters, the identification was confirmed by molecular method for the presence of *magA* . the results showed that only 10 (66.6%) isolates was carrying *magA* which are diagnosed as *Klebsiella pneumoniae*, these results was correlate with [27] who found that out of 10^5 *Klebsiella* isolates, 96.2% was identified as *K. pneumoniae* as in the figure (3).

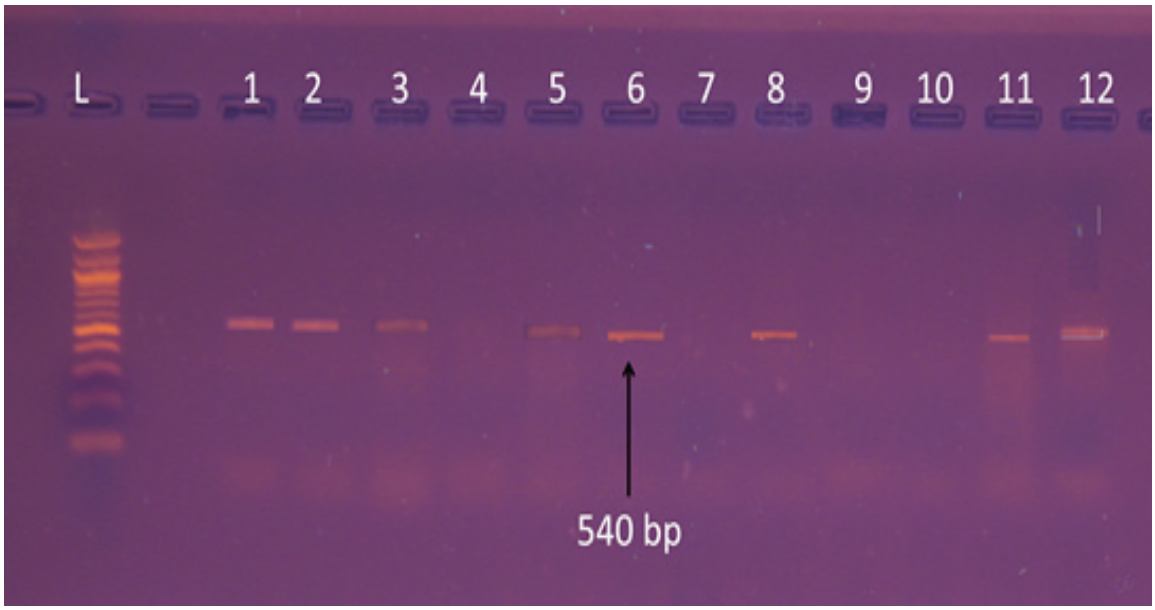


Figure (3): Ethidium bromide-stained agarose gel electrophoresis of PCR amplification products of *Klebsiella pneumoniae* isolates that amplified with *magA* gene primers with product 540 bp for 1 hr. at 80volt/cm.

Conflict of Interest : There was no interest in this study.

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Ethical Clearance : Ethical clearance was obtained from Faculty of Science , University of Kufa ,Iraq.

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