Assiut Univ. J. of Botany and Microbiology ***Printed* ISSN:** *1687-4927*
49(2), pp.60-76 (2020)

ISOLATION, CHARACTERIZATION AND IDENTIFICATION OF *KLEBSIELLA PNEUMONIAE* FROM ASSIUT UNIVERSITY HOSPITAL AND SEWAGE WATER IN ASSIUT GOVERNORATE, EGYPT

Dalia Kamal Rawy[1,5], Mohamed Ahmed El-Mokhtar[2],Sameeh Kamal Hemida[3]*, Ahmed Askora[4], Naiema Yousef[3]
[1]*BloodBank Unit, Hospital of Assiut University, Assuit 71515, Egypt.*
[2]*Department of Microbiology & Immunology, Faculty of Medicine, Assiut University, Assuit 71515, Egypt.*
[3]*Department of Botany & Microbiology, Faculty of Science, Assiut University, Assuit 71511, Egypt.*
[4]*Department of Botany & Microbiology, Faculty of Science, Zagazig University, Zagazig 44519, Egypt.*
[5]*Ph. D student, Department of Botany and Microbiology, Faculty of Science, Assiut University, Assiut71511, Egypt.*

*Corresponding author email address: samehhemida53@yahoo.com

Received:25/7/2019 **Accepted:**2/9/2020 **Available Online:**1/12/2020

***Klebsiella* is an important human pathogen causing opportunistic nosocomial and community acquired infections. A major threat to public health concern regarding *Klebsiella pneumoniae* is the increasing incidence of multidrug-resistant strains. *K. pneumoniae* and *K. oxytoca*, are two clinically important *Klebsiella* species. The two clinically relevant species, are differentiated initially/preliminary by the ability to produce indole from tryptophan, *K. oxytoca* being indole positive. We report here the detailed biochemical and molecular analysis of *Klebsiella* isolates collected from sputum, urine, blood and environmental specimens. Where 75 clinical and environmental specimens' isolates were identified by biochemical tests. To differentiate *K. pneumoniae* from *K. oxytoca*, a multiplex polymerase chain reaction (PCR) was developed using species specific primers (*rpo B* gene for *K. pneumoniae* and of *peh X* gene for *K. oxytoca*). The position of bands on agarose gel, were at 108 bp for *K. pneumoniae* and 343 bp for *K. oxytoca*, confirmed strain identity. *K. pneumoniae rpo B* gene and *K. oxytoca peh X* gene of these isolates were determined by using Multiplex-PCR where, *rpo B* gene regions were determined in 74 (99%) of 75 *Klebsiella* strains while *peh X* gene region was determined in 1 (1%) of 75 *Klebsiella* strains. Results suggested that this newly developed PCR can be used as a diagnostic tool for correct identification and differentiation of *K. pneumoniae* and *K. oxytoca*, the two clinically important *Klebsiella* species. Antimicrobial test for these isolates indicated that all isolates are resistant to Penicillin and Clindamycin, 97.33% of isolates where resistance to Piperacillin and most of the isolates showed resistance to Cefoperazone and Ceftriaxone with a percentage of 94.67%. This study is important for determination the prevalence of *Klebsiella* infections in patients in Assiut University Hospital and in sewage water from different locations in Assiut Governorate and also, in this**

study the majority of isolates were multidrug- resistance to 16 antibiotics which confirms the serious (threat) of *Klebsiella* on human health.

Keywords: Bacteria, *Klebsiella pneumoniae*, Multidrug-resistance, PCR

INTRODUCTION

Klebsiella is one of the most important members of *Klebsiella* genus in Enterobacteriaceae family, which is responsible for pneumonia **[1].** The main *Klebsiella* species caused pneumonia disease is *K. pneumoniae* followed but to lesser degree by *K. oxytoca*. *Klebsiella* spp are facultative, anaerobic, non-motile, lactose-fermenting, and Gram-negative rods (0.3-1 μm in width and 0.6-6 μm in length) arranged singly, in pairs or in short chains that possess a prominent polysaccharide capsule (CPS) **[2]** which gives the colonies their appearance on agar plates. *Klebsiella* colonies appear large, mucoid, and red with diffusing red pigment on MacConkey agar indicating fermentation of glucose and acid production **[3].**

Klebsiella species exist as normal flora in the gastrointestinal tract of animals and humans **[4].** In addition, *Klebsiella* spp can cause severe infections such as meningitis, bronchitis, bacteremia, pneumonia and urinary tract infections in humans and animals **[5-11].** These infections are common particularly in human patients who are suffered from low immune systems and chronic lung diseases. *Klebsiella* spp. are also, normally inhabitants of soil, water and botanical environment **[3, 12].**

K. pneumoniae is an increasingly challenging human bacterial pathogen, causing hospital or community-acquired infections such as pneumonia, urinary tract infection (UTI) and pyogenic liver abscess (PLA) **[13],** that are associated with high rates of antibiotic resistance **[14, 15].**

K. oxytoca can be cultured from intestines of healthy humans and animals, oropharynx, mucous membrane and skin **[3].** It is an opportunistic pathogen that causes primarily hospital-acquired infections, most often involving immunocompromised patients or those requiring intensive care **[16-18].**

Sewage water commonly includes the bacteria belonging to *Coliforms, Clostridia, Enterococcus, Lactobacilli, Micrococci, Proteus, Pseudomonas, Klebsiella, Streptococcus, Staphylococcus.* These bacteria may produce certain toxins which can be responsible for causing different types of illness or it is also possible that they may produce some industrially important bioactive molecules **[19, 20].**

The objective of the present study is aimed to isolate, characterize, and then check for antibiotic sensitivity of *Klebsiella pneumoniae* strains from patients in Assiut University Hospital and in sewage water obtained from different locations in Assiut Governorate, Egypt.

MATERIALS AND METHODS

1-Collection of samples

This study was conducted over a year period, from April (2018) to April (2019) in Assiut governorate, Egypt. One hundred fifty clinical specimens were collected from different patients (males and females) who were suffering from infections of urinary tract, blood stream and respiratory tract, admitted to Assiut University Hospital. The specimens were transported under aseptic conditions quickly to the Microbiology Laboratory and subjected to bacterial culturing. Twenty-five environmental samples were collected aseptically from ten different sewage water locations in Assiut **(Amshoul, Dairout, Qoysia, Manflout, Mankabad, Arab El Madabq, NazletAbdella, El Rajhi hospital, Woman health hospital, and Assiut University Hospital)**. Samples were collected in a sterile bottles and brought to Microbiology lab and stored in clean place.

2-Isolation and purification of *Klebsiella* spp

Spread plate method was used for isolation of *Klebsiella* from the collected human samples. The samples were streaked on MacConkey agar plates **[21]** and incubated at 37°C for 24 h. The *Klebsiella* growth was distinguished by its mucoid growth, appear as pink color. The obtained *Klebsiella* isolates were purified on MacConkey agar as well and sub-cultured on Eosin Methylene Blue (EMB) agar plates **[22]** using the streaking method. A pure single colony then transferred to each slant.

Each collected sewage water sample was diluted by sterilized distilled water as following: one mL of sewage water sample was used for isolating the bacteria through serial dilutions up to 10^{-9} and agar plate culture technique, 500 µL was collected from each dilution and were spread on MacConkey agar plate. The plates were incubated at 37^{o}C for 24 h. After successful growth of microorganisms, the pure colonies of *Klebsiella* spp were sub-cultured in Eosin Methylene Blue (EMB) agar plates and nutrient agar slants, incubated at 37^{o}C to growth. **[23].**

3- Characterization and identification of bacterial culture

3.1-Morphological characteristics

Gram staining was performed **[24]** for all bacterial cultures to differentiate them whether are Gram positive or Gram-negative bacteria.

Morphological characteristics such as shape, size and color were described by microscopic observations. The shape of the colony was studied by observing the margin and elevation. The size of the bacteria was measured by scale and calculated in millimicrons. The color of the bacteria will be identified by observing the colony by microscope **[23].**

For more characterization the following biochemical tests were done: Oxidase test **[25],** Triple Sugar Iron test **[26],** Simmon's Citrate Agar test **[27],** Indole test **[28],** Urease tests **[29]** and Motility test **[30].**

3.2-Molecular identification of *Klebsiella* isolates by PCR

Multiplex PCR was performed for the amplification of *rpo B* gene for *K. pneumoniae* and of *peh X* gene for *K. oxytoca* for detection *Klebsiella* strains**.**

3.2.1- Extraction of genomic DNA

It was done by heating lysis method **[31].** Template DNA was prepared by inoculating *Klebsiella* spp colonies from culture on MacConkey agar plates then were suspended in PCR tubes containing 200 µl of sterilized distilled water, subjected to 98°C for 20 minutes by thermal cycler (Biometra, Germany) then centrifuged at 14.000 rpm for 5 min to pellet the cellular debris. The supernatant was transferred into sterile labeled Eppendorf tubes and stored at -20°C for further steps.

3.2.2- Primers and PCR amplification

Specific primers were used for the amplification of *K. pneumoniae* and *K. oxytoca* in a single reaction mixture (Table 1). PCR was performed in a DNA thermal cycler (Biometra, Germany) with a final volume of 20 µl in 0.2 ml thin-walled tubes.The components of each amplification reaction mixture consisted of: 10 µL of MyTaq HS Red Mix, 2xmaster mix (Bioline, UK), 0.5 µL of each primer (forward and reverse), 4 µl of DNA template, and nuclease-free waterto make final volume of 20 µL. Reaction conditions for PCR were: initial denaturation at 95°C for 10 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min, and final extension at 72°C for 10 min. after the last cycle, the PCR products were stored at -20°C until used for further analysis.

Table (1): List of primers used in this study.

Primer	Sequence (5'-3')	Target	Product	Reference
KP(F)	CAA CGG TGT GGT TAC TGA CG	*rpo B*	108	**[32]**
(R)	TCT ACG AAG TGG CCG TTT TC			
KO(F)	GAT ACG GAG TAT GCC TTT ACG GTG	*peh X*	343	**[33]**
(R)	TAG CCT TTA TCA AGC GGA TAC TGG			

3.2.3- Electrophoresis of PCR Products

The PCR products were visualized by electrophoresis on 2% (w/v) agarose gel (Biometra-Agarose gel mini, Germany) in Tris-acetate-EDTA buffer with ethidium bromide staining. A 50 bp DNA ladder was used as molecular weight marker.

4- Antibiotic sensitivity assay

The assay for antibiotic sensitivity was done by the Kirby- Bauer disc diffusion method based on guidelines of Clinical and Laboratory Standard Institute (CSLI) **[34].** The sterilized Muller Hinton Agar (MHA) **[35]** medium was prepared and poured in the plates. The bacterial suspension was spreaded on MHA plates. Sixteen antimicrobial discs [Ampicillin (10 µg), Penicillin (10 µg), Piperacillin (100 µg), Imipenem (10 µg), Meropenem (10 µg), Cefoperazone (75 µg), Ceftriaxone (30 µg), Cefotaxime (30 µg), Norfloxacin(10 µg),Aztreonam (30 µg),Amikacin(30 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Clindamycin (2 µg), Amoxicillin-Clavulanate (20/10 µg), Trimethoprim Sulfamethoxazole (1.25/3.75 µg)] from Oxoid (England) were placed on the surface of the agar, the plates were incubated overnight at 37^{o}C. The inhibition zone around each disc was measured against each antibiotic. Multidrug resistance (MDR) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories **[36].**

RESULTS

1-Collection and isolation of *Klebsiella* spp from clinical and sewage samples

Examination of clinical samples (sputum, urine and blood) that collected from patients of Hospital and those collected from the environmental sewage water location samples revealed that 75 out of 175 samples were positive for *Klebsiella* (Table, 2). The highest occurrence of *Klebsiella* spp was with respiratory tract infections (37.33%) followed by urinary tract infections (28.00%) while the lowest incidence of these bacteria was observed with a percentage of 20 and 14.67% for isolates from sewage and blood, respectively (Table, 3)

Table (2): *Klebsiella* isolates collected from clinical and environmental samples recovered from MacConkey agar medium.

Source of isolation	Sample	Isolate code:
Assiut university hospital	Blood	KP16, KP87, KP54, KP79, KP80, KP82, KP88, KP94, KP95, KP170, KP174
	Urine	KP171, KP142, KP169, KP43, KP72, KP86, KP98, KP113, KP120, KP145, KP146, KP41, KP81, KP92, KP99, KP100, KP111, KP114, KP140, KP144, KP147
	Sputum	KO37, KP42, KP52, KP53, KP55, KP77, KP91, KP96, KP115, KP124, KP127, KP128, KP141, KP143, KP36, KP56, KP70, KP78, KP83, KP84, KP85, KP97, KP116, KP123, KP125, KP126, KP112, KP38
Arab El Madabq sewage station	Sewage	KP59, KP60, KP61
Amshoul sewer canal		KP66, KP64
Dairout sewage station		KP75, KP76
Qoysia sewage station		KP129, KP130
Manflout sewage station		KP109
Mankabad sewage station		KP155
Assiut University Hospital sewage drain		KP159, KP160
El Rajhi Hospital sewage drain		KP172
Woman health hospital sewage drain		KP173

Table (3): Showed the number of *Klebsiella* isolates and their percentage.

Source of isolation	Number of positive isolates (n)	Percentage
Blood	11	14.67%
Urine	21	28.00%
Sputum	28	37.33%
Sewage	15	20.00%
Total	75	100%

2-*Klebsiella* isolation from clinical and sewage samples

The collected clinical samples were streaking on MacConkey agar and 60 *Klebsiella* isolates were obtained. For sewage samples, the serial dilution method was followed in order to reduce the number of colonies and also to isolate the colonies separately. A total of 15 *Klebsiella* isolates from 25 sewage samples were recovered. All isolates were marked as illustrated in Table (2). *Klebsiella* growth was distinguished by its mucoid growth,appear in pink color. All 75isolates were streaking on Eosin Methylene Blue (EMB) agar plates where gave pink or purple dark-centered, mucoid colonies.

3- Characterization of *Klebsiella* isolates

Isolates tested by Gram's staining to check the morphological characteristics shown that *Klebsiella* spp are a Gram-negative, non-motile, encapsulated, rod shaped bacteria (Figure 1). Different biochemical tests were performed for all 75 isolates to know their biochemical characteristics and results are presented in Table (4).

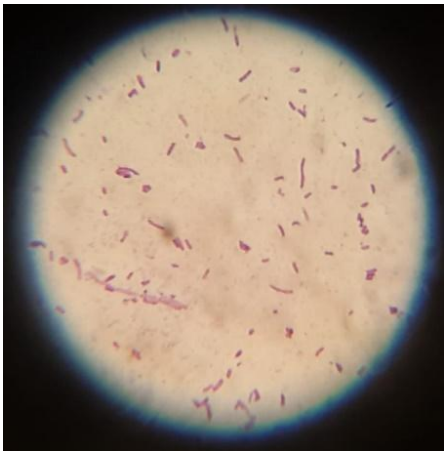

Figure (1): Showing the rod shape of *K. pneumoniae* by light microscopic examination using lens 1000X

Table (4): Biochemical reactions for differentiation and identification of 75 *Klebsiella* isolates

Biochemical tests	*Klebsiella pneumoniae* isolates	*Klebsiella oxytoca* isolate
Oxidase test	Negative (-ve)	Negative (-ve)
Indole test	Negative (-ve)	Positive (+ve)
Motility test	Negative (-ve)	Negative (-ve)
Urease test	Positive (+ve)	Positive (+ve)
Triple sugar Iron test	yellow slant, yellow butt	yellow slant, yellow butt
Simmon's Citrate Agar test	Positive (+ve)	Positive (+ve)

4- Molecular identification of *Klebsiella* isolates

Specific PCR was used to determine the identities of *Klebsiella* species through amplification of the *rpo B* and *peh X* gene fragments for *K. pneumoniae* and *K. oxytoca* respectively. Figure (2) showed agarose (2% w/v) gel indicating *rpo B* and *peh X* gene fragments generated by PCR using genomic DNA extracted from *Klebsiella* species isolated from different samples. Gel electrophoresis of PCR products revealed the desired 108 bp and 343 bp fragments for the *rpo B* and *peh X* gene fragments, respectively. A total of 75 isolates were screened and 74 isolates were positively identified as *Klebsiella pneumoniae* while 1 isolate was identified as *Klebsiella oxytoca*.

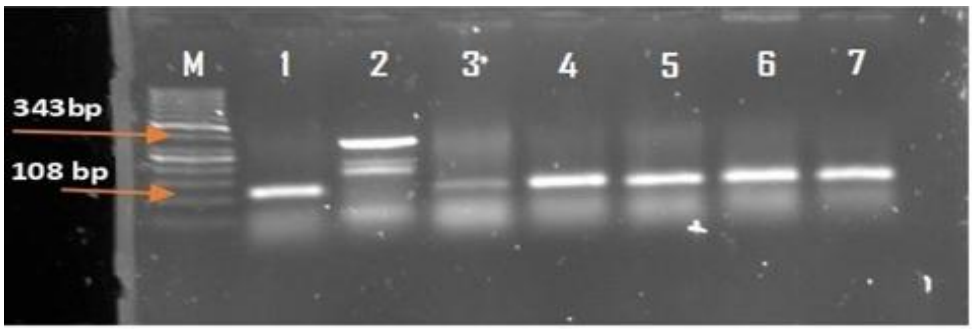

Figure (2): Agarose gel electrophoresis of *K. pneumoniae* and *K. oxytoca* gene.

- Lane M: DNA markers (50-1000) ladder
- Lanes (1 ,3,4,5,6,7) show DNA bands of *Klebsiella pneumoniae* at 108 bp
- Lane (2) show DNA bands of *Klebsiella oxytoca* at 343 bp

5- Antibiotic sensitivity

The seventy-five *Klebsiella* isolates were screened for their sensitivity toward 16 antibiotics and the results are presented in Table 5and revealed that, most of the isolates showed good resistant to antibiotics. All isolates showed resistance to Penicillin and Clindamycin with percentage of 100% and to Piperacillin with 97.33%while most of the isolates showed resistance to Cefoperazone and Ceftriaxone with a percentage of 94.67%**.** Also, the isolates showed high resistance to Ampicillin, Amoxicillin-Clavulanate and Cefotaxime with 93.33%. On the other hand, 37 isolates showed sensitivity to Tetracycline with a percentage of 49.33% followed by 34 isolates showed sensitivity percentage of 45.33% to Imipenem.

Table (5): Showing sensitivity, intermediate and resistant percentage of 75 *Klebsiella* isolates to various antimicrobial agents.

Antibiotic	*Klebsiella* isolates n=75					
	Sensitivity (rate(S)		Intermediate rate(I)		Resistant rate(R)	
	NO	(%)	NO	(%)	NO	(%)
Amikacin (AK)	26	34.67%	8	10.67%	41	54.67%
Meropenem (MEM)	21	28.00%	6	8.00%	48	64.00%
Tetracycline (TE)	37	49.33%	6	8.00%	32	42.67%
Trimethoprim Sulfamethoxazole (SXT)	6	8.00%	1	1.33%	68	90.67%
Ampicillin (AM)	3	4.00%	2	2.67%	70	93.33%
Amoxicillin-Clavulanate (AMC)	3	4.00%	2	2.67%	70	93.33%
Norfloxacin (NOR)	31	41.33%	6	8.00%	38	50.67%
Piperacillin (PRL)	2	2.67%	0	0.00%	73	97.33%
Penicillin (P10)	0	0.00%	0	0.00%	75	100.00%
Cefotaxime (CTX)	5	6.67%	0	0.00%	70	93.33%
Clindamycin (DA2)	0	0.00%	0	0.00%	75	100.00%
Chloramphenicol (C30)	32	42.67%	4	5.33%	39	52.00%
Cefoperazone (CEP)	3	4.00%	1	1.33%	71	94.67%
Aztreonam (ATM)	6	8.00%	1	1.33%	68	90.67%
Imipenem (IPM)	34	45.33%	4	5.33%	37	49.33%
Ceftriaxone (CRO)	2	2.67%	2	2.67%	71	94.67%

DISCUSSION

Enterobacteriaceae are the leading causes of nosocomial infections, with *Klebsiella* being the second leading bacteria after *E. coli*. Majority of *Klebsiella* infections are caused by *K. pneumoniae*. There are mainly case reports and small case studies about *K. oxytoca*. Earlier, it was only *Pseudomonas* that showed high resistance to commonly used antibiotics, but now along with *Acinetobacter*, *Klebsiella* species are rapidly developing multidrug resistance. *Klebsiella* spp are opportunistic pathogens that cause severe diseases in hospital setting. This organism causes pneumonia, urinary tract infection, soft tissue infection and septicemia, which often leads to septic shock **[37].** Hereby 60 *Klebsiella* strains were isolated from patients in Assiut University Hospital whereas only one patient who had *K. oxytoca* infection.

One of the objectives of this study was to isolate the environmental *Klebsialla* from sewage water in Assiut, Egypt.Isolates from sewage water are just as virulent as clinical isolates from Hospitals and can produce important virulence factors **[4].** Fifteen strains of *Klebsiella pneumoniae* were isolated from sewage water samples.

Several groups of biochemical tests have been proposed to allow differentiation among the most common *Klebsiella* species. A scheme was proposed by **Monnet and Freney [38].** In the Clinical Microbiology Laboratory, the routine of preliminary differentiation of *K pneumoniae* and *K oxytoca* by Indole test is based upon the metabolism of tryptophan toindole; a reaction catalyzed by tryptophanase **[39]** all collected isolates in the present study were differentiated initially by the biochemical tests which gave the same result such the stander biochemical test of *K. pneumoniae* and *K. oxytoca* for the previous studies.

Antimicrobial susceptibility test was performed for clinical and environmental *Klebsiella* isolates by the Kirby Bauer disc diffusion method. Results obtained shown that high level of resistance existed among clinical isolates against different classes of antibiotics, which is probably due to the frequent use of these antibiotics to treat the patients. These results agree with findings of **Shawkey *et al* [40].** On the other hand, the resistance rate was found to be 49.33% for Imipenem which is higher than that reported in previous studies **[41, 42].** The increase resistance of *K. pneumoniae* to Imipenem may be attributed to frequent use of Meropenem in Assiut University Hospital.

Members of the genus *Klebsiella* are opportunistic pathogens which are difficult to identify and are often misclassified in clinical microbiology laboratories **[38, 43, 44].**

Several studies have been conducted to evaluate the efficiency of methods in identifying *Klebsiella* species **[5, 11, 45].** PCR analysis has been reported to be more accurate in the identification of *Klebsiella* species when compared with other preliminary identification tests **[46].** So, confirmation of *Klebsiella* identification using PCR analysis is very useful. Correct identification of *Klebsiella* isolates is important for taxonomic and molecular characterization **[43].** Molecular methods based on amplification and sequencing of *rpoB, gyrA, parC* genes, and 16S rRNA region have recently become available for the identification of *Klebsiella* spp **[47-49]** but there is no PCR based method for the identification/differentiation of *K. pneumoniae* and *K. oxytoca*. This study was designed to fill that gap.

The newly developed multiplex PCR was specific for *K. pneumoniae* and *K. oxytoca* since no amplification was observed with other Gram-negative bacteria tested e.g., *Salmonella typhimurium*, *Escherichia coli*, *Yersinia enterocolitica*, and *Shigella flexneri*. The primers used in this study yielded products of 108 bp for *K. pneumoniae* and 343 bp for *K. oxytoca*, enabling clear differentiation of these two species. With this PCR, 74 of the isolates that were identified as *K. pneumoniae* and one isolate could be confirmed as *K oxytoca*.

REFERENCES

[1] Puspanadan, S.; Afsah-Hejri, L.; Loo, Y. Y; Nillian, E.; Kuan, C. H.; Goh, S. G.; Chang, W. S.; Lye, Y. L.; John, Y. H. T.; Rukayadi, Y.; Yoshitsugu, N.; Nishibuchi, M. and Son, R. (2012): Detection of *Klebsiella pneumoniae* in raw vegetables using Most Probable Number-Polymerase Chain Reaction (MPN-PCR). Int. Food Res. J., 19(4): 1757-1762.

[2] Umeh, O. and Berkowitz, L. B. (2009): *Klebsiella* Infections. eMedicine.com. Wealth Health Organisation (2003). Emmerging Issues in Water and Infectious Disease, World Health Organization, Geneva, Switzerland.

[3] Podschun, R. and Ullmann, U. (1998): *Klebsiella* spp. as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. Clin. Microbiol. Rev., 11(4): 589–603.

[4] Podschun, R.; Pietsch, S.; Höller, C. and Ullmann, U. (2001): Incidence of *Klebsiella* species in surface waters and their expression of virulence factors. J. Appl. Environ. Microbiol., 67(7): 3325-3327.

[5] Lai, Y.; Yang, S.; Peng, H. and Chang, H. (2000): Identification of Genes Present Specifically in a Virulent Strain of *Klebsiella pneumoniae*. Infect. Immun., 68(12): 7149-7151.

[6] Boye, K. and Hansen, D. S. (2003): Sequencing of 16S rDNA of *Klebsiella*: taxonomic relations within the genus and to other Enterobacteriaceae. Inter. J. Microbiol., 292: 495-503.

[7] Jonas, D.; Spitzmüller, B.; Daschner, F. D.; Verhoef, J. and Brisse, S. (2004): Discrimination of *Klebsiella oxytoca* phylogenetic groups and other *Klebsiella* species by use of amplified fragment length polymorphism. Res. Microbiol., 155: 17-23.

[8] Trautmann, M.; Held, T. K. and Cross, A. S. (2004): O antigen seroepidemiology of *Klebsiella* clinical isolates and implication for immunoprophylaxis of *Klebsiella* infections, Vaccine, 22: 818-821.

[9] Casolari, C.; Pecorari, M.; Fabio, G.; Cattani, S.; Venturelli, C.; Piccinini, L.; Tamassia, M. G.; Gennari, W.; Sabbatini, A. M. T.; Leporati, G.; Marchegiano, P.; Rupianesi, W. and Ferrari, F. (2005): A simultaneous outbreak of *Serratia marcescens* and *Klebsiella pneumoniae* in a neonatal intensive care unit. J. Hosp. Infect., 61: 312-320.

[10] Maroncle. N., Rich, C. and Foresters, C. (2006): The role of *Klebsiella pneumoniae* urase in intestinal colonization and resistance to gastrointestinal stress. Res. Microbiol., 1587: 184-193.

[11] Lau, H. Y.; Clegg, S. and Moore, T. A. (2007): Identification of *K. pneumoniae* genes uniquely expressed in a strain virulent using a murine model of bacterial pneumonia. Microb. Pathog., 42: 148-155.

[12] Brisse, S.; Grimont, F. and Grimont, P. A. D. (2006): The Genus *Klebsiella.* Prokaryotes, 6:159–196.

[13] Spagnolo, A. M.; Orlando, P.; Panatto, D.; Perdelli, F. and Cristina, M. L. (2014): An overview of carbapenem-resistant *Klebsiella pneumoniae*: epidemiology and control measures. Rev Med Microbiol., 25: 7–14.

[14] Wyres, K. L. and Holt, K. E. (2016): *Klebsiella pneumoniae* population genomics and antimicrobial-resistant clones. Trends Microbiol., 24: 944–956.

[15] European Centre for Disease Prevention and Control [ECDC] (2017): Surveillance of antimicrobial resistance in Europe 2016, in Proceedings of the Annual report of the European Antimicrobial REsistance Surveillance Network (EARS-Net), (Solna Municipality: European Centre for Disease Prevention and Control).

[16] Jeong, S. H.; Kim, W. M.; Chang, C. L.; Kim, J. M.; Lee, K.; Chong, Y.; Hwang, H. Y.; Baek, Y. W.; Chung, H. K.; Woo, I. G. and Ku, J. Y. (2001): Neonatal intensive care unit outbreak caused by a strain of *Klebsiella oxytoca* resistant to aztreonam due to overproduction of chromosomal β - lactamase. J. Hosp. Infect., 48: 281–288.

[17] Decré, D.; Burghoffer, B.; Gautier, B.; Petit, J. C. and Arlet, G. (2004): Outbreak of multi-resistant *Klebsiella oxytoca* involving strains with extended spectrum beta-lactamases and strains with extended-spectrum activity of the chromosomal beta-lactamase. J. Antimicrob. Chemother., 54: 881–888.

[18] Zárate, M. S., Gales, A. C., Picão, R. C., Pujol, G. S., Lanza, A. and Smayevsky, J. (2008): Outbreak of OXY-2–producing *Klebsiella oxytoca* in a renal transplant unit. J. Clin. Microbiol., 46: 2099–2101.

[19] Zaved, H. K.; Rahman, M. M.; Rahman, A.; Arafat, S. M. Y. and Rahman, M. S. (2008): Isolation and characterization of effective bacteria for solid waste degradation for organic manure. KMITl Sci. Tech. J., 8(2): 844-855.

[20] Moges, F.; Endris, M.; Belyhun, Y. and Worku, W. (2014): Isolation and characterization of multiple drug resistance bacterial pathogens from waste water in hospital and non-hospital environment, Northwest Ethiopia. BMC Res. Notes, 7: 215.

[21] MacConkey, A. (1905): Lactose fermenting bacteria in faeces. J. Hyg., 5(3): 333-379.

[22] Holt-Harris, J. E. and Teague. O. (1916): A new culture medium for the isolation of *Bacillus typhosa* from stools. J. Infect. Dis., 18: 596.

[23] Mahesh, S.; Pinjari, A. B. and Kavitha, B. (2017): Isolation and characterization of bacteria isolated from municipal sewage water of Nandyal, Kurnool, A. P., India. Asian J. of Microbiol. Biotech. Env. Sci., 19 (3): 772-777.

[24] Smith, A. C. and Hussey, M. A. (2005): Gram stain protocols. In American Society for Microbiology-ASM Conference for Undergraduate Educators (Vol.2005).

[25] Shields, P. and Catheart, L. (2013): Oxidase Test Protocol. America Society for Microbiology, peer-reviewed, 1-10.

[26] Acharya, T. (2013): Triple Sugar Iron Agar (TSI) Principle, Procedure and Interpretation, Microbeonline.

[27] Murray, P. R.; Baron, E. J.; Jorgensen, J. H.; Landry, M. L. and Pfaller, M. A. (2007): Manual of Clinical Microbiology, 9th ed. American Society for Microbiology, Washington, D. C.

[28] MacFaddin, J. F. (2000): Biochemical Tests for Identification of Medical Bacteria. 3rd Edition, Lippincott Williams & Wilkins, Philadelphia, PA., 173 – 183.

[29] Winn, W.; Allen, S.; Janda, W.; Koneman, E.; Procop, G.; Schreckenberger, P. and Woods, G. (2006): Koneman's color atlas and textbook of diagnostic microbiology, 6th ed. Lippincott Williams & Wilkins, Philadelphia, PA.

[30] Cheesbrough, M. (2000): Biochemical test to identify bacteria. In Cheesbrough M. (ed). Laboratory practice in Tropical Countries. 2: 63-70.

[31] Ahmed, O. B.; Asghar, A. H. and Elhassan, M. M. (2013): Prevalence of TEM, SHV and CTX-M genes in *Escherichia coli* and *Klebsiella* spp Urinary Isolates from Sudan with confirmed ESBL phenotype. Life Sci. J., 10 (2): 191-195.

[32] Chander, Y.; Ramakrishnan, M. A.; Jindal, N.; Hanson, K. and Goyal, S. M. (2011). Differentiation of *Klebsiella pneumoniae* and *K. oxytoca* by Multiplex Polymerase Chain Reaction. Intern. J. Appl. Res. Vet. Med., 9(2): 138-142.

[33] Kovtunovych, G.; Lytvynenko, T.; Negrutska, V.; Lar, O.; Brisse, S. and Kozyrovska, N. (2003): Identification of *Klebsiella oxytoca* using a specific PCR assay targeting the polygalacturonase pehX gene. Res. Microbiol., 154: 587-592.

[34] Clinical and Laboratory Standards Institute (CLSI) (2019): Performance Standards for Antimicrobial Susceptibility Testing; Twenty-ninth Informational Supplement M100-S29 USA. Wayne, PA, USA: CLSI.

[35] Mueller, J. H. and Hinton, J. (1941): A protein-free medium for primary isolation of the Gonococcus and Meningococcus. Experimental Biology and Medicine, 48: 330-333.

[36] Magiorakos, A. P.; Srinivasan, A.; Carey, R. B.; Carmeli, Y.; Falagas, M. E.; Giske, C. G.; Harbarth, S.; Hindler, J. F.; Kahlmeter, G.; Olsson-Liljequist, B.; Paterson, D. L.; Rice, L.B.; Stelling, J.; Struelens, M. J.; Vatopoulos, A.; Weber, J. T. and Monnet, D. L. (2012): Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect., 18: 268–281.

[37] Hagiwara, S.; Murata, M.; Aoki, M.; Kaneko, M. and Oshima, K. (2013): Septic shock caused by *K. oxytoca*: an autopsy case and a survival case with driving Extracorporeal Membrane Oxygenation. Hippokratia., 17(2):171–173.

[38] Monnet, D. and Freney, J. (1994): Method for differentiating *Klebsiella planticola* and *Klebsiella terrigena* from other *Klebsiella* species. J. Clin. Microbiol., 32: 1121–1122.

[39] Maslow, J. N.; Brecher, S. M.; Adams, K. S.; Durbin, A.; Loring, S. and Arbeit, R. D. (1993): Relationship between Indole Production and Differentiation of *Klebsiella* Species: Indole-Positive and -Negative Isolates of *Klebsiella* Determined to Be Clonal. J. Clin. Microbiol., 31(8): 2000-2003.

[40] Shawky, S. M.; Abdallah, A. and Khouly, M. (2015): Antimicrobial activity of Colistin and Tiegecyline against carbapeneme-resistant *Klebsiella pneumoniae* clinical isolates in Alexandria, Egypt. Int. j. Curr. Microbiol. App. Sci., 4(2), 731-742.

[41] Aitta, A. A.; El Said, M.; Gamal, D.; El-Kholy, A.; Omar, M.; Kamel, M. and Mohamed, H. (2013): Biotyping and Molecular Characterization of *Klebsiella pneumoniae* Producing Extended-Spectrum Beta-Lactamase in Cairo, Egypt: A Multicentre Study. Researcher, 5(9): 1-11.

[42] Eida, M.; Nasser, M.; El-Maraghy, N. and Azab, K. (2015): Pattern of hospital-acquired pneumonia in Intensive Care Unit of Suez Canal University Hospital. Egy. J. Chest Diseases and Tuberculosis, 64(3): 625-631.

[43] Alves, M. S.; Dias, R. C. S.; Castro, A. C. D.; Riley, L. W. and Moreira, B. M. (2006): Identification of clinical isolates of indole-positive and indole-negative *Klebsiella* spp. J. Clin. Microbiol., 44: 3640-3646.

[44] Hansen, D. S.; Aucken, H. M.; Abiola, T. and Podschun, R. (2004): Recommended test panel for differentiation of *Klebsiella* species on the basis of a trilateral interlaboratory evaluation of 18 biochemical tests. J. Clin. Microbiol., 42: 3665-3669.

[45] Haryani, Y.; Noorzaleha, A. S.; Fatimah, A. B.; Noorjahan, B. A.; Patrick, G. B.; Shamsinar, A. T.; Laila, R. A. S. and Son, R. (2007): Incidence of *Klebsiella pneumoniae* in street food sold in Malaysia and their characterization by antibiotic resistance, plasmid profiling, and RAPD-PCR analysis. Food Cont., 18: 847-853.

[46] Diancourt, L.; Passet, V.; Verhoef, J.; Grimnont, P. A. and Brisse, S. (2005): Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. J. Clin. Microbiol., 43(8): 4178-4182.

[47] Lopes, A. C.; Rodrigues, J. F.; Clementino, M. B.; Miranda, C. A.; Nascimento, A. P. and Júnior, M. A. (2007): Application of PCR ribotyping

and tDNA-PCR for *Klebsiella pneumoniae* identification. Mem. Inst. Oswaldo Cruz., 102: 827-832.

[48] Brisse, S. and Verhoef, J. (2001): Phylogenetic diversity of *Klebsiella pneumoniae* and *Klebsiella oxytoca* clinical isolates revealed by randomly amplified polymorphic DNA, gyrA and parC gene sequencing and automated ribotyping. Int. J. Syst. Evol. Microbiol., 51: 915-924.

[49] Drancourt, M.; Bollet, C.; Carta, A. and Rousselier, P. (2001): Phylogenetic analyses of *Klebsiella* species delineate *Klebsiella* and *Raoultella* gen. nov., with description of *Raoultella ornithinolytica* comb. nov., *Raoultella terrigena* comb. nov. and *Raoultella planticola* comb. nov. Int. J. Syst. Evol. Microbiol., 51: 925- 932.

عزل وتوصيف وتعريف الكليبسيلا الرئويه المعزوله من مستشفيات جامعه اسيوط ومياه الصرف الصحى بمحافظه اسيوط

داليا راوى كمال ابراهيم

طالبه دراسات عليا دكتوراه - قسم النبات والميكروبيولوجى – كلية العلوم - جامعة اسيوط

د/ محمد احمد المختار محمود

استاذ مساعد الميكروبيولوجيا الطبيه والمناعة - قسم الميكروبيولوجيا الطبيه والمناعة- كلية الطب - جامعة اسيوط

ا.د/ سميح كمال حميدة

استاذ الميكروبيولوجى -قسم النبات والميكروبيولوجى -كلية العلوم -جامعة اسيوط

د/ احمد عبدالرحمن احمد عسكورة

استاذ مساعد البيولوجيا الجزيئيه - قسم النبات والميكروبيولوجى -كلية العلوم -جامعة الزقازيق

ا.د/ نعيمة محمد همام يوسف

استاذ البكتيريولوجى -قسم النبات والميكروبيولوجى- كلية العلوم -جامعة اسيوط

تناولت هذه الدراسه عزل الكليبسيلا وهى ممرض بشري مهم يسبب عدوى مستشفوية انتهازية اومكتسبة من المجتمع وتعتبرتهديدا رئيسيا على الصحة العامة نتيجه كثرة سلالاتها المقاومة للمضادات الحيويه. ويوجد نوعان مهمان سريريا من الكليبسيلا وهما الكليبسيلا الرئويه وكليبسيلا الاوكسيتوكا .وقد تم

تجميع ٦٠ سلالة كليبسيلا من ١٥٠ عينه مزروعه من البلغم والبول والدم وكذلك ١٥ سلالة من ٢٥ عينة بيئيه.

هذا ولقد تم تحديد عزلات الكليبسيلا عن طريق الاختبارات البيوكيميائيه ولتأكيد التمييز بين كليبسيلا الرئويه وكليبسيلا الاوكسيتوكا، تم عمل اختبار تفاعل تسلسلي متعدد البلمرة (PCR) باستخدام البادئات الخاصة بالأنواع (جين *rpo B* لكليبسيلا الالتهاب الرئوي والجين *peh X* لـكليبسيلا الاوكسيتوكا). حيث تم تحديد ٧٤ سلاله من الكليبسيلا الرئويه بينما تم تحديد سلاله واحده على انها كليبسيلا اوكسيتوكا. أشارت النتائج إلى أن PCR المطور حديثًا يمكن استخدامه كأداة تشخيصية لتحديد والتمييز الصحيح بين السلالات المختلفة من الكليبسيلا . ومن ناحية اخرى اوضح اختبار مضادات الميكروبات أن جميع العزلات مقاومة للبنسلين والكليندامايسين بينما أظهرت العزلات مقاومة للمضاد الحيوى البيبراسيلين بنسبه ٩٧.٣٣% وايضامعظم العزلات كانت مقاومة لـ السيفوبيرازون و السيفترايكسون ولكن بنسبة اقل (٩٤.٦٧٪).

لقد بينت الدراسة اهمية تحديد مدى انتشار الكليبسيلا في مرضى مستشفى جامعة أسيوط وكذلك بمياه الصرف الصحي فى مواقع مختلفة بمحافظه أسيوط. وأيضا، في هذه الدراسة، كانت غالبية العزلات مقاومة لـ ١٦ مضادًا حيويًا مما يؤكد خطورة (تهديد) الكليبسيلا على صحة الإنسان.