Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

Morphological and Molecular Characterization of *Proteus mirabilis* Isolated from Different Clinical Samples in Najaf province

Assist .Lec.Khitam Abdul Kadhim mohsin Al-mashhadi[(1)] and Prof. DrBasaad Abedzaid Al-Fatlawi[(2)]

[1.2.]Faculty of Science ,Dep. of Biology / University of Kufa, Najaf,Iraq.

Email : khitamabdulkadhum93@gmail.com

Abstract

The study included two main parts ,the first was the bacterial diagnosis based on relied diagnostic procedures .Second part were genetic study to virulence factors to common bacteria .A total of 148 samples were collected from different sources of patients that including (urine , vagina swab ,sputum ,tonsil) and from both sexes during a period from August 2020 to November 2020.

These patients ranged from 1-69 years.(85) of them were females and (48)were males .The result indicated ,the most age group were more susceptible to infected (20_29) year from other age group, represented 75 patients with a percentage of (56.3%), among this group females were more susceptible with percentage (63.9%) compared to males (36%).

The result indicated 133 samples (89.86%) gave positive result , while 15 samples (10.13%) was Negative for culture bacteria , primary identification was depended on biochemical tests .Finally identification with Vitek 2 system was done .The results demonstrate 42 isolate *E.coli* ,31 isolate *P.mirabilis* ,25 isolate *K.pneumoniae* , 21 isolate *staphylococcus spp* ,7 isolate *streptococcus spp*,3 isolate *pseudomonas spp* and 4 isolate other bacteria .All isolates (100%) of *E.coli* and *K.pneumoniae* were able to produce capsule and biofilm .

Regarding antibiotic susceptibility testing *P. mirabilis* isolates were resistant to Penicillin and sensitive to Amikacin .

The result indicated all*P .mirabilis* ,contain *motA and motB* genes contain *motA* which play role in the rotary motor of flagella with percentage 100%.

Key word: Morphological ,*Proteus mirabilis*,clinical samples and Najaf province.

Introduction

P. mirabilis is one ofopportunistic pathogen, which can cause severe invasive diseases, Critically, in patients who are immunocompromised[(1)]. This bacterium is one of main source of nosocomial

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

infections[2]. *P. mirabilis* is also the one of causative agents of urinary tract, burns, respiratory, ear and otitis infections[3].

This bacteriumhas wide virulence factors that help to colonize, survive and grow in the host organism such as adhesion, swarming, fimbriae, urease and protease [4]. Furthermore, it is well known that *P. mirabilis* can form biofilms. These biofilms colonize the airways, and provide a highly resistance of antibiotics [5]. These abilities to from biofilm and have a resistance of antibiotics could acquire from different sources such as external environment, transferring gene from different species or strains[6] .

The aim of this study

1.s specimens were collected from (urine ,vaginal swab, tonsillis and sputum,) for bacteriological study.

2.Identification of bacterial isolate by using cultural ,biochemical characters and vitek2 technique.

3.Determine *P.mirabilis* that isolated from (urine ,vaginal swab ,tonsillis and sputum).

4.Detection of some virulence factors of common isolated bacteria and Detection the existence of gene coding for some virulence factors using PCR technique .

Specimens Collection

A total of 148 specimens,133 (89.8 %) specimens revealed positive culture on blood agar and MacConky agar, while 15 (10%) specimens were no growth appear on MacConky agar and blood agar.,were collected from patient suffering from (urine ,sputum , tonsillitis and vaginal) infections during the proid From August 2020 to November 2020, and collected from patients suffering from different clinical specimens from AL-Sader Teaching Hospital , AL-Furat Alawsat Hospital,AL-sajad Hospital and private clinic in Al–Najaf province. Those specimens were collected from patients included both sex (male and female) and age range from 1 to 70 years. Swab was taken swab from infection area and then transport the swab by transport media to the lab.Data about type of infection, sex and age of patients were recorded on the swab. Specimens were collected from patients that not received antibiotic for one week before sample collection. All information, like date, sex, and age were recorded in questionnaire, sheet paper.

Specimens Culture

The collected specimens were inoculated on two types of culture media which included blood agar and MacConkey agar, and spread on the each plates with sterile loop. Plates were incubated at

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

37˚C for 24 hrs. The plates were examined there after for bacterial growth and plates were then a single pure isolated colony was transferred to Brain Heart Infusion agar for the preservation and to submitted the morphological evaluation by Gram staining and carry out other biochemical tests that confirmed the identification of isolates. After incubation period the isolates were identified according to [7].

Vitek – 2 for Identification

GN and GP identification card has been used for identification Gram positive and Gram negative bacteria . The bacterial suspension was adjusted to McFarland standard of 0.5 in 2.5ml of a 0.45% sodium chloride solution with a Vitek -2 instrument(bioMérieux, France). The time between preparation of the inoculum and the card filling was always less than 30 min. The GN identification card is a fully closed system to which no reagents have to be added. The card was put on the cassette designed for use with the Vitek–2 system, placed in the instrument, automatically filled in a vacuum chamber, sealed, incubated at 35.5˚C, and automatically subjected to colorimetric measurement (with a new reading head) every 15 min. for a maximum incubation period of 8 hours. Data were analyzed, using Vitek –2 database, which allows organism identification in a kinetic mode beginning 180 min after the start of incubation [8]

Detection of Biofilm formation

Suspension of tested strain was incubated in the glass tubes containing Brain Heart infusion Broth (BHI broth)aerobically at the temperature of 35 ˚C for the period of 2days .Then the supernatant was discarded ,the glass tube has been stained by 0.1% safranin solution ,washed with D.W. three times and dried .In the case of biofilm formation ,a grainy red structure on the test tube bottom was found.[9]

Antibiotic Sensitivity Test

This test performed by Kirby-Bauer method as the following:

1- From new pure culture plate, 4-5 colonies of bacterial isolate were picked up by sterilized inoculating loop and emulsified in 5ml of sterile normal saline until the turbidity is approximately equivalent to that of the McFarland No. 0.5 turbidity standard which prepared as mentioned .

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

2- A sterile swab was dipped into bacterial suspension.

3- The surface of a Mueller-Hinton agar plate was streaked with the swab, then the plate was rotated through a 45º angle and streaked the whole surface again; finally the plate was rotated another90º and streaked once more. 4- By a sterile forceps the antimicrobial disc was picked up and placed on the surface of the inoculated plate, the disc was pressed gently into full contact with the agar.

5- The step (4) was repeated to all antimicrobial discs under the test, spaced evenly a way from each other.

6- The inoculated plates were incubated at 37ºC for 18-24 hours.

7- After incubation, the plates were examined for the presence of inhibition zone of bacterial growth (clear rings) around the antimicrobial discs, if there was no inhibition zone the organism was reported as resistant to the antimicrobial agent in that disc. If a zone of inhibition surrounded the disc, the diameter of the zone of inhibition was measured (by millimeters) and compared to standard results being recommended by clinical laboratory standards institute documentations [(10)] .

Extraction and Isolation of DNA

After culturing on MacConkey agar, isolates *E.coli*, *Proteus mirabilis,* and *Klebsilla pneumonia,* which are individually inoculated into broth and incubated at 37°C for 24 hours. The Genomic DNA Extraction Kit (Intron Korea) was used to extract DNA according to the manufacturer's instructions.

1- Transfer the appropriate amount of bacterial suspension to 1.5 ml micro centrifuge tube and centrifuge at full speed 13,000 rpm for 1 min, then discard the supernatant.

2- Add 200 µL of buffer CL, 20 µL proteinase K, and 5 µL RNase resuspend the pellet by vortex, incubate lysate at 56°C using water bath for 20 min .

3- Add 200 µL of buffer BL into upper sample tube and mix thoroughly , then incubate the mixture at 70° C for 5 min .

4- Centrifuge the sample tube at 13,000 rpm for 5 min to remove un-lysed tissue particles , then

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

carefully transfer 400 µL of the supernatant into a new 1.5 ml tube.

5-Add 200 µL of absolute ethanol into the lysate.

6-Transfer the mixture to the spin column (in a 2 ml collection tube) and centrifuge at 13,000rpm for 1 min . discard the flow through and place the spin column in a new 2 ml collection tube .

7- Washed the spin column with 700 µL of buffer WA and centrifuge for 1min at 13,000 rpm . discard the flow –through and reuse the collection tube, add 700 µL WB to the spin column and centrifuge for 1min at 13,000 rpm , discard the flow –through and place the column into a new l.5 ml collection tube.

8- To elution ; 100 µL of buffer CE was added directly onto the membrane . Incubate for 1 min at room temperature , then centrifuge for 1min at13,000 rpm to elute.

Estimation of DNA Concentration and purity

The extracted DNA was checked by using Nano drop spectrophotometer, which measured DNA concentration (ng/uL)and check the DNA purity by reading the absorbance at (260/280nm)as following seps:

1.after opening up Nanodrop software,chosen the appropriate application (Nucleic Acid ,DNA).

2.A dry wipe was taken to clean instrument pedestals several time .then carefully pipette 2ul of add H2O on to the surface of the lower measurement pedestals for blank system

3.The sampling arm was lowered and clicked OK to initialized the Nano drop ,then cleaning off the pedestals and 1ul of extracted DNA carefully pipette onto the surface of the lowered measurement pedestals, then concentration and purity of extracted DNA was checked [11].

Polymerase Chain Reaction (PCR) Assay

PCR assay was performed in monoplex pattern in order to amplify different fragments of genes under study for detecting of *K.pneumoniae* *E.coli* and *P.mirabilis* virulence factors of 6 genes from each type were selected to be amplified separately in monoplex PCR technique used in this study table (1)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

Table (1): Determining virulence genes and encoding propertie

type bacteria	Genes	Encoding protein
P. mirabilis	*motA*	swarming motility
	MotB	Outer membrane lipoprotein

Table (2) Primers (Macrogen, Europe) used in the study

Type bacteria	Primer Type	Primer Sequence (5'-3')	amplicon size(bp)	Reference
P. mirabilis	MotA	F:GATGGTGACGGGGAATATGAA R:CCATTTCCCCAGCAGGTCTA	215	(12)
	MotB	F:GCGTTACGTCCACATCTCAA R:ATGTCGCGCATATAGGGTTC	150	(13)

Table (3): PCR program that apply in the thermo-cycler

type of bacteria	Gene	Temperature(°C) /Time					Cycles Number
		Initial Denaturation	Condition of one cycle			Final Extension	
			Denaturation	Annealing	Extension		
Proteus mirabilis	*motA*	94\ 3 min.	94 \ 30 sec.	52\ 30 sec.	72\1min.	72\5min.	30
	motB	94\3 min.	94 \30 sec.	52\30 sec.	72\1 min	72\5min	30

Agarose Gel Preparation and DNA Loading

This procedure was used in accordance as follows:

1. 2g of agarose was dissolved in 100 ml of Tris-borate-EDTA (TBE) (90 ml distilled water + 10 ml TBE) with final concentration 10x
2. The Tris-borate-EDTA solution was boiled, then cooled to 45°C before adding 0.5 mg/ml ethidium bromide..

3.The agarose poured in equilibrated gel tray, and left until cooled and became more hardened.

3. Five microliters of PCR product were loaded to each of agarose gel wells inaddition to one for

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

DNA marker , the gel tray was fixed in electrophoresis chamber and TBE buffer was added to thechamber. The electric current wasperformed at 70 volt for 80 Minutes.

4. Finally, the electrophoresis result was detect using gel documentation system,the positive results were proved when the DNA band base pairs of sample equal to the target product size .

Results and Discussion

Isolation and Identification

During the study period from August 2020 to November 2020,the total of 148 clinical specimens were collected from patients sufferingfrom different infections(urine,vagnial swab,tonsillis and sputum). The results showed that 133 (89.8 %) specimens revealed positive culture on differential media , while 15 (10%) specimens were no growth.infections to each gender : female 85(63.9%) specimens and male 48(36%) specimens.This study agree with [14] they found that *E.coli* prevalence in female was 68% and male 32%.The result of this study agree with [15]was more in female patients compared with male patients probably because the number of patients admitted was more than male patients ,the sexual dimorphism in bacterial infections has been mainly attributed to the differential levels.of sex hormones between males and females as well as to genetic factors.[16][17],with age group from 1-69 years old.In the current study, the prevalence types of infections based on patients age and gender in different groups were shown in table (4).

The highest appeared at the20-29age group that 75(56%) ,these results agree with[18]which was percentage in adult.The cause for such high percentage in young may be due to cross infection because of overcrowded class rooms ,work place ,ventil action in this places and more transfer persens in this age [19] .The lowest incidence was among the (50-59) age . The patient's age ranged from (1-69) years old. The age distribution of the patients can be seen in table (4).

Table (4): Distribution of bacterial isolate according to age and gende

Age	NO	male	female
1_10	7(6.7%)	5(10.4%)	2(2.3%)
10_19	8(7.5%)	1(2%)	7(8.2%)
20_29	75(56.3%)	25(52%)	50(58.8%)
30_39	23(17.2%)	10(20%)	13(15.2%)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

40_49	10(7.5%)	3(6.2%)	7(8.2%)
50_59	2(1.5)	_	2(2.3%)
60_69	8(6%)	4(8.3%)	4(4.7%)
total	133(100%)	48(36%)	85(63.9%)

Biochemical tests of bacterial species that causes of infections :

Many bacterial isolates were diagnosed by using the bacterial cultured, Gram stain ,morphology and biochemical tests ,for the bacterial were isolates and investigated in the laboratory . [(7)]

Table (5) : Biochemical test of bacterial species that causes of infections:

test / bacterial	catalase	coagulase	oxidase	indole	methyl red	voges proskauer	urease	motility
E.coli	+	_	_	+	+	_	_	+
p.mirabilis	+	_	_	+	+	_	+	+
k.pneumonia	+	_	_	_	_	+	+	_
staphylococcus ssp	+	+	_	_	+	+	+	_
streptococcus spp	_	_	_	_	+	_	_	_
pseudomonas spp	+	_	+	_	_	_	_	v

The final identification was performed with the automated VITEK-2 compact system using GP,GN-ID cards which contained 64 biochemical tests and one negative control. Exactly 133 isolates performed identification and confirmed by Vitek-2 system by using Kit(GP-ID cards) to Gram positive bacteria and Kit(GN-ID cards) to Gram negative bacteria.These Isolates grown on blood agar,manitol agar and MacConky agar,and appear positive and negative to Gram stain ,they have been elected bacterial species *E.coli* , *P. mirabilis,K. pneumonia* , *Staphylococcu* spp *(aureus,epidermis and maraxella),* streptococcus spp(*pneumonia,pyogen and agalactie*) , *pseudomonas spp (aeraginosa and fluorescence)* and *enterobacteraerogenes, burkholderiapseudomallei sphingomonas paucimobilis* , which the most frequently than the rest bacteria that neglected recurrence rates,the most common pathogen was *E.coli* 42 (31.5%)The morphological characterization of bacteria in table (6) rareled that the *P.mirabilis* were 31(23.3%) which appeared small in size, Gram negative bacteria and gave negative result for (oxidase ,vp and Hemolysin)while positive result for (catalase, indole and urease).This study similar to study for[(20)]they found *P.mirabilis* 40(19.5%) isolated from different source. Many studies were reported that *P.mirabilis* was the important agent causing different types of infection, in particular or

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

UTI,[21]recorded in their study that *P.mirabilis* was accounted (54%)of bacterial isolates [22] found that (77%)of isolates were identified *P.mirabilis* and the bacteria in the study of [23]were recorded in (65%) of isolates .In contrast, [24]demonstrated that(15%) of isolates were *P.mirabilis* and [25]found (3%) of urine isolates were *P.mirabilis* .[26]found 45(75%) from different result source infection .These different may be due to differentiation in the source of infection ,condition of the collection and identification methods. Twenty five isolates (18.7%) of *K.pneumoniae*.

Table (6) : The positive results of bacterial isolates

type of bacteria	No. of bacteria	percent
E.coli	42	31.5%
p. mirabilis	31	23.3%
k. pneumonia	25	18.7%
staphylococcus spp	21	15.7%
streptococcus spp	7	5.2%
pseudomonas spp	3	2.2%
other bacteria	4	3%
total	133	100%

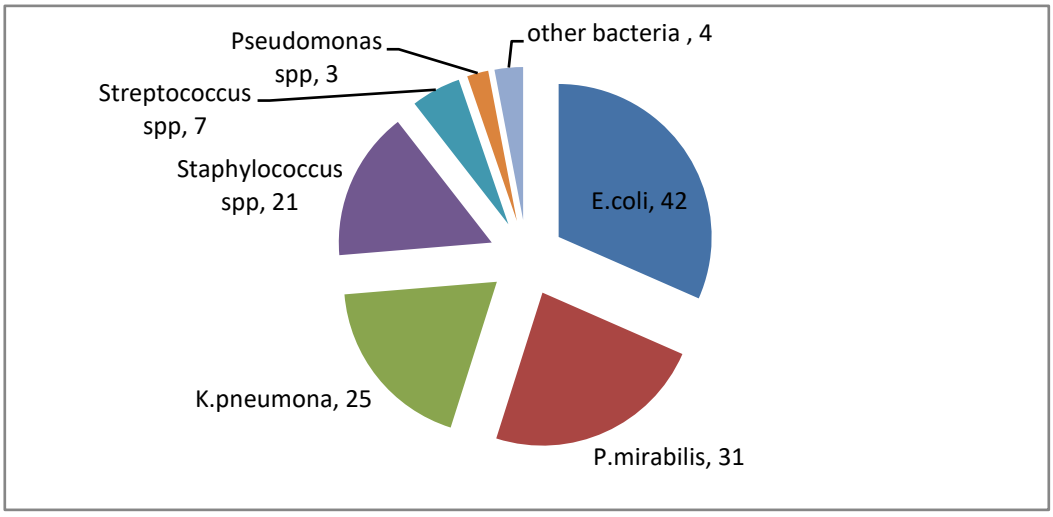


Fig (1): The positive results of bacteria isolates

Disturibution of *E.coli* , *P.mirabilis* and *K. pneumonia* isolates according to the source.

Most of isolate78 (58%) isolates were recovered from urine specimens ,29 (21.8%) isolates

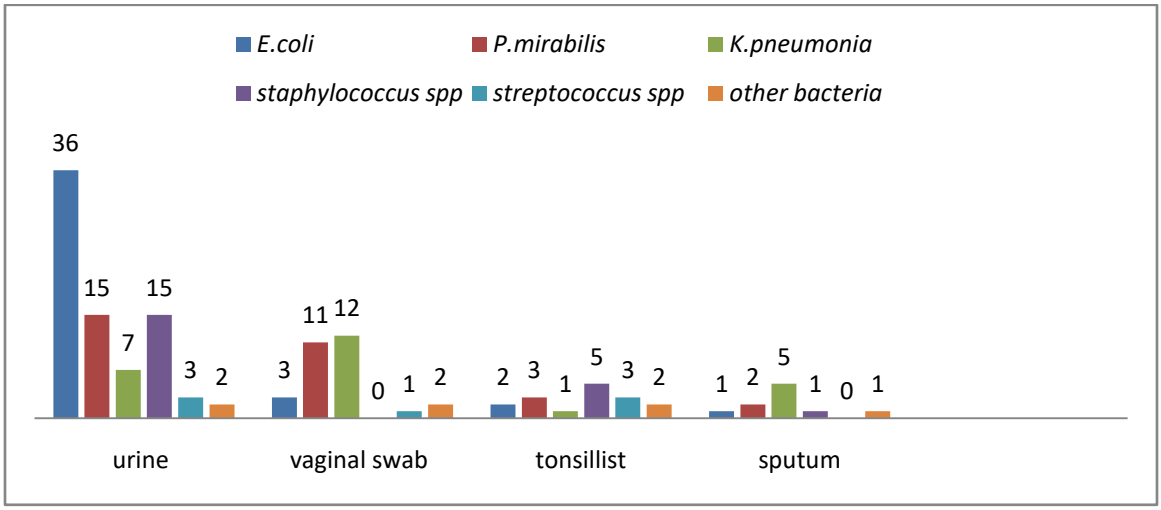
Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

from vaginalswabs ,16(12%) isolates from tonsillist and10 (7.5%) isolates from sputum.table (7) In this study was many isolation bacterial from different sample , the important agent causing different types of infection, in particular UTI ,Genital tract infection,tonsillist and respiratory tract.in urine specimens the high presented bacterial *E.coli* 36 (46%), *P.mirabilis* 15(16%)and *K.pneumonia* 7 (8.9%) while vaginal swab the high commonly bacterial *K. pneumonia* 12(41.3%) , *P.mirabilis*11(37.5%) *and E.coli* 3(10%). Tonsillist *P. mirabilis* 3(18.7%), *E.coli* 2(18.5%)*and k.pneumonia1*(6.2%), *sputum K.pneumonia* 5(50%) *P.mirabilis* 2(20%) and *E.coli* 1(10%).These results were agreed with many previous studies such as that established by[(27)(14)],who found that *E.coli* was the commonest member of Entero bacteriacea that can cause severe infections.many studies were reported that *p.mirabilis* was the important agent causing different types of infection, [(28)(29)]who formed that *p.mirabilis* was couse many infection .This study agree with[(30)(31)] who found that *K.pneumoniae* was the commonest member of *Klebsiellaspp*.that can cause severe infections.Among all patients diagnosed with *K.pneumonia*.[(32).]

Table(7) Disturibution of *E.coli* ,*p.mirabilis* and *k. pneumonia* isolates according to the source.

type of specimens	NO	*E.coli*	*proteus mirabilis*	*Kebsiella pneumonia*	*Staphylococcus sp*p	*Streptococcus spp*	other bacteria
urine	78(58%)	36(46%)	15(19%)	7(8.9%)	15(19.2%)	3(3.8%)	2(2.5%)
vaginal swab	29(21.8%)	3(10%)	11(37.9%)	12(41.3%)	0	1(3.4%)	2(6.8%)
tonsillist	16(12%)	2(12.5%)	3(18.7%)	1(6.2%)	5(31.2%)	3(18.7%)	2(12.5%)
sputum	10(7.5%)	1(10%)	2(20%)	5(50%)	1(10%)	0	1(10%)
total	133(100%)	42(31.5%)	31(23%)	25(18.7%)	21(15.7%)	7(5.2%)	7(5.2%)

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.



Fig(2):Disturibution of type bacteria isolates according to the source.

Detection of some virulence factors for bacteria

caspsule production

The results revealed that capsular phenotype was investigated among E.coli and *K.pnemoniae* isolates ,the results showed that all *E.coli* and *K.pneumoniae* isolates (100%) were positive for capsule production .These results in the present study agreed with[(14)(33)]They found that capsule production is considered as an important virulence factors in bacterial pathogenicity because capsular material forms thick bundles of fibrillous structures covering the bacterial surface in massive layers.

Anew hypervirulent (hyper mucoviscous) variant of some bacteria has emerged.Defining clinical features are the ability to cause serious ,Life threatening community-acquired infection in younger healthy hosts, including liver abscess, pneumonia, meningitis and endophthalmitis and the ability to metastatistically spread ,an unusual feature for enteric Gram-negative bacilli in the non-immunocompromised [(34)] The polysaccharide capsule is amony the most important virulence determinants, providing protection from phagocytosis,resistain to complement-mediated killing and suppression of human beta-defensin expression[(15)].

Biofilm production

The results of this study showed that most isolates of bacteria had ability to biofilm formation, This result were agreed with the result study of [(20)] .they showed that 60% of *P.mirabilis* strain were biofilm producers and agree with[(36) (37)(38)]. In nature bacteria can exist in planktonic and biofilm embedded state[(39)] .Biofilm growth is the most predominant made of growth for bacteria within the environment and is likely a survival mechanism.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

A biofilm is a bacterial population that is adherent to biological or non-biological surface and is enclosed by an extra-polymeric substance .Biofilm development is a sequential process initiated by the attachment of planktonic cells to a surface ,which is followed by formation of micro colonies and biofilm maturation in which individual bacteria , as well as the entire community are embedded in a matrix composed of nucleic acid, protein and polysaccharides [40] .Microorganisms form biofilm , that exist in the environment .whether upon inanimate objects or living animals, the bacteria attach to these surfaces, including soli and aquatic systems, and have been documented in the human literature developing on medical devices ,within the middle ear, external ear ,lunges, heart valves ,surgical implants and tooth enamel [41].

Biofilm formation in Gram negative bacteria occurs when bacteria cells first swing along a surface ,using Flagellar-mediated motility, until attachment occurs at a specific site and their attachment is initially reversible [42].Initial growth of bacteria is the production of Microcolonies(clusters of cells),which form when cells aggregate together. With further movement of the cells using type IV pili-known as twitching motility, the attachment becomes irreversible. This will develop once surface interactions are stable.[43].

Antimicrobial Susceptibility Test

Many factors have contributed to high antibiotics resistance rates in Iraqi hospitals and community , such as excessive antibiotics prescription, antibiotics can be purchased without a prescription and inadequate surveillance also account for the spread of resistance bacteria , The results revealed that showed different degrees of resistance to antibiotics that used in the study. *P. meribilis*the results showed that most of the bacterial isolates were resistance *Penicillins* 31 (100%) this result agree with[44]Similar results were found in the results of[45]. However, the study reveals that *P.mirabilis* was high sensitive to Imipenem (100%), such results were found in a study of[46][47][48]were documented that the sensitivity of *P.mirabilis* is 100% to this antimicrobial. but were different from the results of[48][49]. Some P. mirabilis isolates present an elevation in the resistance level to Imipenem due to many reasons: the loss of outer membrane porins, decreased expression of PBP1a or reduced binding of Imipenem by PBP2 [50]. [51]noted that the development of resistant against imipenem in Proteus mirabilis is due to the absence of 24 kDa OMP.

Amikacin is an amino-glycoside antibiotic used to treat different types of bacterial infection. It is works by binding to the bacterial 30s ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth. In this study *P.mirabilis* isolate show

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

high sensitivity to Amikacin (100%), These result confirmed with result by[52] [47] [48] [53]found the percentage of susceptibility to Amikacin was 87% to98%, which considered an agreement to this result.and other results dis agree with [28].

Tobramycin result showed (100%) of the isolates of *P.mirabilis* were sensitive to this antimicrobial, A study by[54] found the percentage of resistance was (40%) Tobramycin and[55] which considered an disagreement to this result, reported that the Tobramycin was not able to inhibit the growth of proteus species more than 34%.

Table (8): Determination the sensitivity of the bacteria to antibiotics

no	type of antibiotics	*P.mirabilis(31)*	
		S	R
1.	Penicillins (p)	0	31(100%)
2.	Amoxicillin/clavulanic acid(AMC)	6(19.3%)	25(80%)
3.	Imipenem(IMP)	31(100%)	0
4.	Cefotaxime (CTX)	28(90%)	3(9.6%)
5.	Ceftriaxone (CRO)	21(67%)	10(32%)
6.	Ceftazidime (CAZ)	27(87%)	4(12.9%)
7.	Gentamicin (CN)	25(80.6)	6(19.3%)
8.	Amikacin (AK)	31(100%)	0
9.	Tobramycin (TOB)	31(100%)	0

Molecular Detection of *P.mirabilis* Isolates

The verulence genes *motA* and *motB* carried on *P.mirabilis* DNA were investigated these genes have a major role to induce many of *p.mirabilis* infections[12][13].The polymerase chain reaction was used to detect the predominant of those genes in bacterial isolates using specific primer for each one ,the results appear that all isolates (100%) were carrying *motA* and *motB* fig(3) and fig (4).The *matA* and *motB* genes play a role in the rotary motor of flagella[56] .The two *motA* and *motB* genes are required for swimming and twitching motility of *p.mirabilis* the stator complexes are not only in *p.mirabilis* but also in most pathogenic bacteria that need to swarm in over surfaces .[57].These results were similar with the result of, [20] [58] [4]who reported that the genes were encoded in

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

chromosomal DNA of *p.mirabilis* strain.

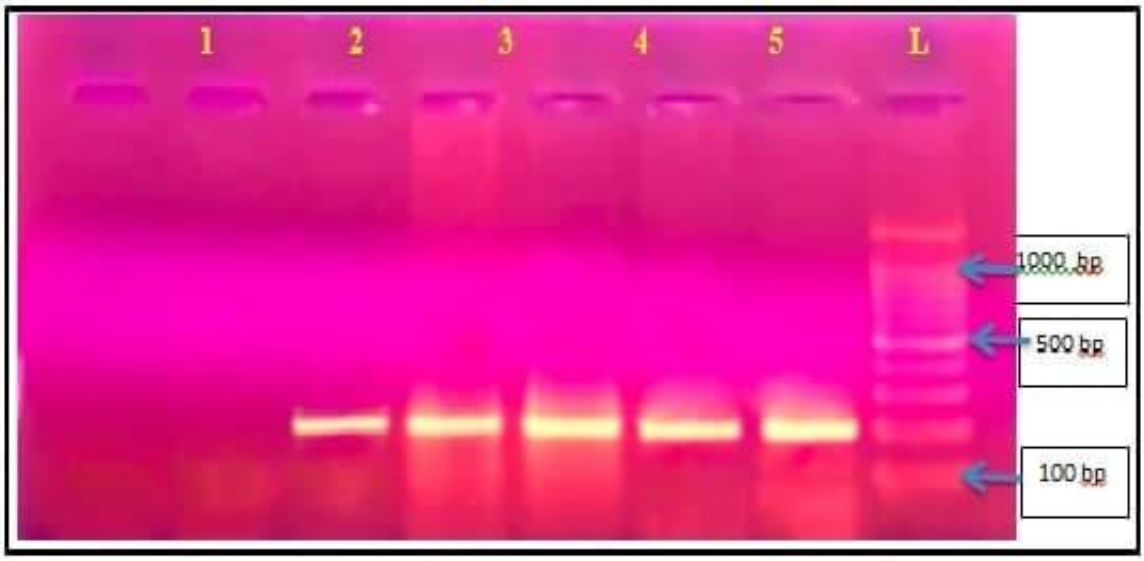


Figure (3): Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *P.mirabilis* using primer *motA* gene with product (215bp). The electrophoresis was performed at 70 volt for 1.5-3hr. lane (L), DNA molecular size marker (100 bp ladder). Lanes (1 to5) show positive results with gene *mot A*.

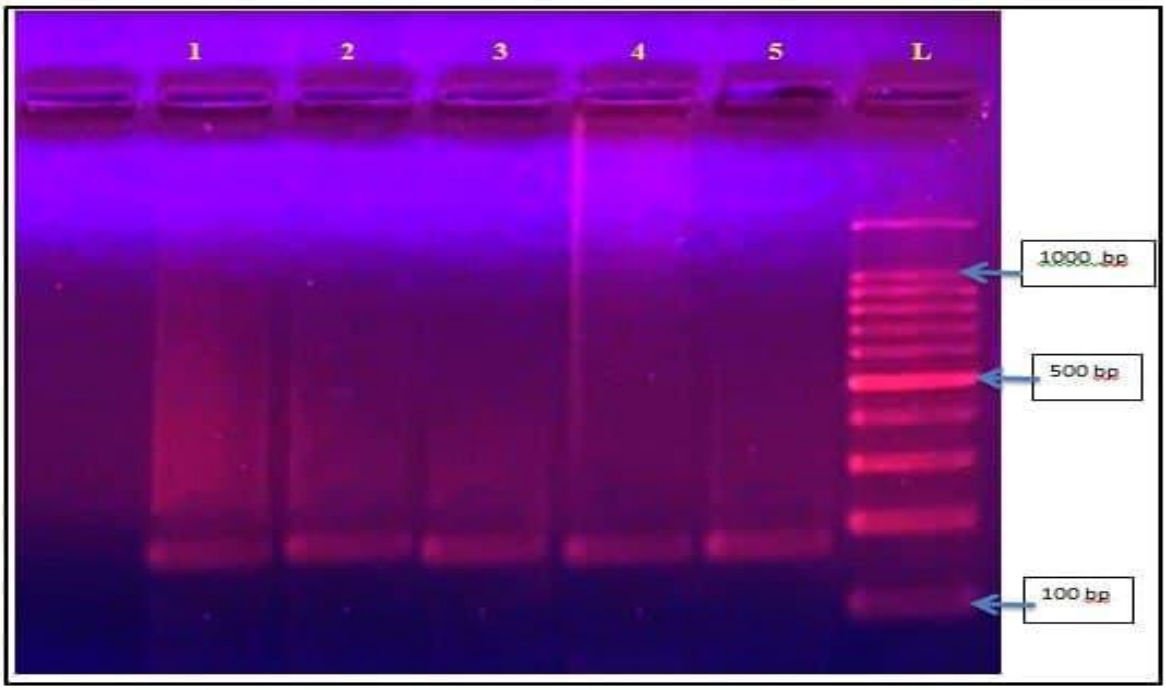


Figure (4): Ethidium bromide-stained agarose gel electrophoresis of PCR products from extracted total DNA of *P.mirabilis* using primer *motB* gene with product 150bp. The

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

electrophoresis was performed at 70 volt for 1.5-3hr. lane (L), DNA molecular size marker (100 bp ladder). Lanes (1 to5) show positive results with gene *motB*.

Reference

1. Abokhalil, R. N.; Elkhatib, W. F. ; Aboulwafa, M. M. and Hassouna, N. A. (2020). Persisters of *Klebsiella pneumoniae* and *Proteus mirabilis*: A Common Phenomenon and Different Behavior Profiles. *C. Microbi.* Vol. 77, 1233–1244.
2. Jamal, M.; Ahmad, W.; Andleeb, S.; Jalil, F.; Imran, M. ; Nawaz, M. A., Hussain, T., Ali, M.; Rafiq, M. and Kamil, M. A.(2018). Bacterial biofilm and associated infections. J. of the Chin. Med. Associa., 81, 7-11.
3. Jamil, R. T.; Foris, L. A. and Snowden, J.(2020) . "Proteus mirabilis infections," in Stat Pearls. (Treasure Island, FL: Stat Pearls Publishing).
4. Wasfi , R.; Abdellatif, G. R.; Elshishtawy H. M. and Ashour, H. M. (2020) First-time characterization of viable but non-culturable Proteus mirabilis : Induction and resuscitation. J.of cellular and molecular medicine. 12(24): 2791-2801
5. AL-Mayahi, F. S. A.(2017). Phenotypic and Molecular detection of Virulence factors in Proteus mirabilis isolated from different clinical sources. Bas. J. Vet. Res, 16.
6. Iribarnegaray,V. ; Navarro, N.; Robino, L. ; Zunino, P. ; Morales, J. and Scavone, P. (2019). Magnesium-doped zinc oxide nanoparticles alter biofilm formation of Proteus mirabilis. Nanomedicine 14(12), 1551–1564.
7. MacFaddin, J.F. (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd edition. Lippincott Williams and Wilkins, USA
8. Guido, F. and Pascale, F. (2005). Performance of the New VITEK 2 GP Card for Identification of Medically Relevant Gram-Positive Cocci in a Routine Clinical Laboratory. J Clin Microbiol, 43(1): 84-88.
9. Aljadoa,H.H.S.; Bunyan , I.A. ;Sahib ,S.(2019).Phenotypic and Genotypic Characterization of Bacterial Throat Infections in Babylon Province .PhD .Thesis submitted of the college of Medicine , University of Babylon,philosophy/in microbiology,In Iraq.
10. CLSI, CLSI M100-ED30:2020 Performance Standards for Antimicrobial Susceptibility Testing, 30th Edition (2020).

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

11. Lee, J. H.;Park, Y.; Choi, J. R.; Lee, E. K .and Kim, H. S. (2010). Comparisons of three automated systems for genomic DNA extraction in a clinical diagnostic laboratory. Yonsei medical journal, 51(1), 104.
12. Cusick, K.; Lee, Y. Y.; Youchak, B. and Belas, R. (2012). Perturbation of FliL interferes with Proteus mirabilis swarmer cell gene expression and differentiation. J. of bacterio., 194, 437-447.
13. Maisuria, V. B.; Hosseinidoust, Z. and Tufenkji, N.(2015). Polyphenolic extract from maple syrup potentiates antibiotic susceptibility and reduces biofilm formation of pathogenic bacteria. Applied and enviro. microbio., 81, 3782-3792
14. Rahman, S. U. and Ahmad, M. (2019). Isolation and identification of Escherichia coli from urine samples and their antibiotic susceptibility pattern.
15. Forma, L.; Rissanen, P.; Aaltonen, M.; Raitanen, J.; and Jylhä, M. (2009). Age and closeness of death as determinants of health and social care utilization: a case-control study. European journal of public health, 19(3), 313-318.
16. Rettew; Inklein, S .L ; Robberts, C .W; editors.(2010). Sex hormones and immunity to infection .Berlin :Springer , pp123-146.
17. Vázquez-Martínez, E. R.; García-Gómez, E.; Camacho-Arroyo, I.; and González-Pedrajo, B. (2018). Sexual dimorphism in bacterial infections. Biology of sex differences, 9(1), 1-20.
18. Folliero, V.; Caputo, P.; Della Rocca, M. T.; Chianese, A.; Galdiero, M.; Iovene, M. R.and Galdiero, M. (2020). Prevalence and antimicrobial susceptibility patterns of bacterial pathogens in urinary tract infections in University Hospital of Campania “Luigi Vanvitelli” between 2017 and 2018. Antibiotics, 9(5), 215.
19. Bengtsson-palme ,J.; Kristiansson ,E.;Larsson,D.G.J.(2018).Environmental factors influencing the development and spread of antibiotic resistance .FEM Microbiol .Reu.,42,fuxo53.
20. Mohsin , R.I .and AL-Muhana, S.J. (2020). Study of Gene Transfer of Some Viruelence Genes of Proteus mirabilis Isolated from Different Infections Msc.Thesis, Submitted to the Council of College of Sciences University of Kufa in Iraq.
21. AL-Oqaili, N. A. D.; AL-Shebli, M. K.; and Almousawi, A. N. (2017). Antimicrobial susceptibility and molecular characterization for some virulence factors of Proteus Mirabilis isolated from patients in Al-Qadisiyah Province/Iraq. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 16(2), 1-7

22. Kadhim, A. K.; Kadhum, S. A. and AL-jbouri, A. M.(2019). Demonstration of the effects of virulence factor and antibiotic resistance on the plasmids of Proteus mirabilis and Proteus vulgaris. J. of Pharmac. Scie. and Res., 11, 1304-1312.
23. Ahmed, S. S.; Shariq, A.; ALSalloom, A. A.; Babikir, I. H. and ALHomoud, B. N. (2019). Uropathogens and their antimicrobial resistance patterns: Relationship with urinary tract infections. Int. J. of health scie., 13, 48.
24. EL-Gamasy, M. A.(2017). Prevalence of infective organisms of infections of urinary tract in a sample of Arab infants and children. J. of Integ. Neph. and Andr., 4, 136.
25. Mohammed, K.; Burhan, F. O. and Nooruldeen, S. Y.(2017). Isolation and Identification of Urinary Tract Infectious Bacteria and Exploring their Anti-drug Potential against Some Common Antibiotics. J Microb Biochem Technol, 9, 6.
26. Alsherees, H. A. A.; Abdzaid, A. J. and Talib, R .(2016). Molecular study of Proteus mirabilis bacteria isolated from urine and wounds in hospitals Al-Najaf province.
27. Zahera, M.; Rastogi, C.; Singh, P.; Iram, S.; Khalid, S. and Kushwaha, A. (2011). Isolation, Identification and Characterization of Escherichia Coli from Urine Samples and their Antibiotic Sensitivity Pattern .Pelagia Research Library. European Journal of Experimental Biology, 1(2), 118-124.
28. Al-Bassam, W. W.; and Al-Kazaz, A. K. (2013). The isolation and characterization of Proteus mirabilis from different clinical samples. Jornal of Biotechnology Research Center, 7(2).
29. Hamilton, A. L., Kamm, M. A., Ng, S. C., and Morrison, M. (2018). Proteus spp. as putative gastrointestinal pathogens. Clinical microbiology reviews, 31(3).
30. Ravichitra, K. N.; Prakash, P. H.; Subbarayudu, S. and Rao, U. S. (2014). Isolation and antibiotic sensitivity of Klebsiella pneumoniae from pus, sputum and urine samples. Int J Curr Microbiol App Sci, 3(3), 115-9.
31. Hussein, E. I., Al-Batayneh, K.; Masadeh, M. M.; Dahadhah, F. W.; Al Zoubi, M. S.; Aljabali, A. A. and Alzoubi, K. H. (2020). Assessment of Pathogenic Potential, Virulent Genes Profile, and Antibiotic Susceptibility of Proteus mirabilis from Urinary Tract Infection. International journal of microbiology, 2020.
32. Lin ,Y. ;Yu-Ping;Wang .; u-DerWang and chang-phone fung .(2015).Community –onset Klebsiella pneumoniae pneumonia in Taiwan :clinical features of the disease and associated microbiological characteristic of isolates from

pneumonia and nasopharynx .Front Microbiol .18(8):122-129.

33. Walker, K. A. and Miller, V. L. (2020). The intersection of capsule gene expression, hypermucoviscosity and hypervirulence in Klebsiella pneumoniae. Current opinion in microbiology, 54, 95-102.
34. Shon, A .S.; Bajwa, R.P.; and Russo, T. A. (2014). Hypervirulent (hypermucoviscous) Klebsiella pneumoniae: A new and dangerous breed. Virulence, 4(2):107–118.
35. Lee, Y. Y. and Belas, R.(2015). Loss of FliL alters Proteus mirabilis surface sensing and temperature-dependent swarming. J. of bacterio., 197, 159-173.
36. Seifi ,K .;Kazemian , H .;Heidari, H .; Rezaghlizadeh , F .;saee, W.;Shirvani ,F .; and Hour ,H.(2016), Evaluation of Biofilm Formation Among Klebsiella pneumoniae Isolates and molecular Characterization by ERIC-PCR.Jundishapur J Microbiol.9(1):30682.
37. AL-Ammar,M. A. M; AL-Khafaji ,J .K. and AL-Shibly , T.K.(2017).molecular characterization of some virulence Determinations of Klebsiella pneumoniae isolated from Different clinicals sampies in Baylon .M.sc Thesis submitted to the council of college of medicine University of Babylon in Iraq.
38. AL-Mashhady ,F.R. and Al-Janabi ,B.A.H.(2020). Antimicrobial Effect Of Gold And Silver Nanoparticles On Resistance Of Antibiotics Bacterial Species That Isolated From UTI Patients In The Province Of Najaf . Msc.Thesis Submitted to the Council of the Faculty of Science / University of Kufa in Iraq.
39. Ghaima, K. K.; Hamid, H. H. and Hasan, S. F. (2017). Biofilm formation, antibiotic resistance and detection of mannose-resistant Proteus-like (MR/P) fimbriae genes in Proteus mirabilis isolated from UTI. International Journal of ChemTech Research, 10(5), 964-971.
40. Branda, S. S.; Vik, Å.; Friedman, L. and Kolter, R. (2005). Biofilms: the matrix revisited. Trends in microbiology, 13(1), 20-26.
41. Singh, A.; Walker, M. and Rousseau, J. (2013). Characterization of the biofilm forming ability of Staphylococcus pseudintermedius from dogs. BMC Vet. Res.;9:93 98.
42. O'Toole, G.; Kaplan, H.B.; and Kolter, R. (2000). Biofilm formation as microbial development. Annu. Rev. Microbiol. 54, 49–79
43. Klausen, M.; Heydorn, A.; Ragas, P.; Lambertsen, L.; Aaes-Jørgensen, A.; Molin, S. and Tolker-Nielsen, T. (2003). Biofilm formation by Pseudomonas aeruginosa wild type, flagella and type IV pili mutants. Molecular microbiology, 48(6), 1511-1524.

44. Filipiak, A.; Chrapek, M.; Literacka, E.; Wawszczak, M..; Głuszek, S.; Majchrzak, M. and Adamus-Białek, W. (2020). Pathogenic Factors Correlate With Antimicrobial Resistance Among Clinical Proteus mirabilis Strains. Frontiers in microbiology, 11, 2896.
45. EL-Gamasy, M. A.(2017). Prevalence of infective organisms of infections of urinary tract in a sample of Arab infants and children. J. of Integ. Neph. and Andr., 4, 136.
46. Yassen, L.T. and I.N. Khelkal (2015). Effect of some fatty acid on virulence factors of Proteus mirabilis. Internal journal of advance biological research, 5(2): 108-117.
47. Al-Jumaily, E. F. and Zgaer, S. H. (2016). Multidrug resistant Proteus mirabilis isolated from urinary tract infection from different hospitals in Baghdad City. Int. J. Curr. Microbiol. App. Sci, 5(9), 390-399.
48. Sokhn, E. S.; Salami, A.; El Roz, A.; Salloum, L.; Bahmad, H. F. and Ghssein, G. (2020). Antimicrobial susceptibilities and laboratory profiles of Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis isolates as agents of urinary tract infection in Lebanon: Paving the way for better diagnostics. Medical Sciences, 8(3), 32.
49. AL-Oqaili, N. A. D.; AL-Shebli, M. K.; and Almousawi, A. N. (2017). Antimicrobial susceptibility and molecular characterization for some virulence factors of Proteus Mirabilis isolated from patients in Al-Qadisiyah Province/Iraq. Al-Qadisiyah Journal of Veterinary Medicine Sciences, 16(2), 1-7.
50. Girlich, D.; Dortet, L.; Poirel, L. and Nordmann, P. (2014). Integration of the blaNDM-1 carbapenemase gene into Salmonella genomic island 1 (SGI1- W) in a Proteus mirabilis clinical isolate. J. Antimicrobial Chemother., dku371
51. Tsai, Y. L.; Chien, H. F.; Huang, K. T.; Lin, W. Y., and Liaw, S. J. (2017). cAMP receptor protein regulates mouse colonization, motility, fimbriamediated adhesion, and stress tolerance in uropathogenic Proteus mirabilis. Scientific reports, 7(1), 1-14.
52. Pebriani, R. ., Jafar, N. ., Wahiduddin, Hidayanti, H. ., Burhanuddin, & Ummu Salamah. (2021). The Effect of Extract of Canarian Nuts on Reduction of Total Cholesterol Levels of Hyperglicemic Rat. Journal of Scientific Research in Medical and Biological Sciences, 2(1), 19-29. https://doi.org/10.47631/jsrmbs.v2i1.128
53. Singla, P.; Sangwan, J.; Garg, S. and Chaudhary, U.(2015). Prevalence and Antibiogram of Multidrug resistant Uropathogenic Isolates of Proteus mirabilis in a Teaching Tertiary Care Hospital. Int. J. Curr. Microbiol. App. Sci., 4(12): 675-682.

Annals of R.S.C.B., ISSN:1583-6258, Vol. 25, Issue 6, 2021, Pages. 12510 - 12529
Received 25 April 2021; Accepted 08 May 2021.

54. Jamel , A. N.; Allami , R. H. and Hamza, S. J. (2020). Molecular study of rpoB gene in proteus mirabilis isolated. From urinary tratect infection from different hospitals in Baghdad .plant archives ,20(1),2379-2382.
55. Alshwaikh, R.M.A.; Al-Sorchee, S.M.A.; Ali, K.A. and Al Beer, W. (2014). Antibacterial activity of parsley and celery aqueous extract on the isolated bacteria from children UTI in Erbil city. Int. J., 2(9): 895-903.
56. Patil, T. (2014). Catheter associated urinary tract infection (cauti) induced nosocomial infection with reference to incidence, duration and organism in a Tertiary Care Teaching Hospital. Education (Asme), 1(4), 211.
57. Chen, B.; Sysoeva, T. A.; Chowdhury, S.; Guo, L. and Nixon, B. T.(2009) ADPase activity of recombinantly expressed thermotolerant ATPases may be caused by copurification of adenylate kinase of Escherichia coli. FEBS J 276, 807-815.
58. Belas, R. (2014). Biofilms, flagella, and mechanosensing of surfaces by bacteria. Trends in microbiology, 22(9), 517-527.
59. Armbruster, C. E.; Mobley, H. L.; and Pearson, M. M. (2018). Pathogenesis of Proteus mirabilis infection. EcoSal Plus, 8(1).