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Isolation and Characterization of *Proteus mirabilis*

A Research
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Introduction

Proteous mirabilis. part of the enterobacteriaceae family of bacilli, is gram-negative facultative anaerobe with an ability to ferment maltose and inability to ferment lactose , Proteous mirabilis also has swarming motility and the ability to salf elongate and secrete a polysaccharide , when in contact with solid surfaces , this allow for attachment and easy motility along surfaces (e.g.medical equipment) . then flagella of Proteous mirabilis are what allow colonization , but it also has been associated with its ability to form biofilm and is suggested to contribute to resistance to host denfenses and certain antibiotic , proteous is found abundantly in soil and water and although it is part of the normal human intestinal flora (along with klebsiella species and Escherichia coli) , it has been known to causes serious infectious in humans

General character Proteous mirabilis

These bacteria are described as short gram-negtive bacilli diameter ranges from 0.3-1 micrometer and length (0.6-6.0) micrometer. These bacteria are character as an aerobic , that grows under ther conditions of air it is actively moving and is not made up of spinner (abbot,2007.2000 coker etal..,) these bacteria are not made up of capsules . it contain fimberae as well as containing flagellae . and anegative oxidase test assay to vogus proskour test

Urease production

This enzyme is important virulence factor for these bacteria it is a metallo enzymes becauses contains nickel (Ni) whose presence is necessary to form an active site of the enzyme (li etal ., 2004) . the urease enzyme is produceal from many bacteria strains that causes urinay tract infections such as : proteous spp, Klebsiella spp, Ecoli , staphylococcus spp.

(nielubowiscz and mobley ,201011). Proteous bacteria are the main pathogens of VRE , which play a major role in the colonization process . (Jacobsenetal .,2008: dattelbaum.etal ., 2003) several studies have shown that proteous bacterial have a high potential to produce a large amount of urease enzyme other than other bacteria (kosikowska and berlieki, 2011 Gendlina etal 2002). These bacteria are caused by this enzyme lower urinary tract infection but less so this confirmed by chalvicz and his group (2011) in Poland that there were an complicated infection by proteous mirabilis bacteria in the rate of 3-4 %

(2002a) morbeley amd Li (2002) urease enzyme is of cytoplasmic enzymes to proteous bacteria .

(pili) fimbriae

Of the most important factor on the surface of the cell , a variety of polyps are called fimbriae that have arole in

adherence to the surface of the host cell. Adherence is known as the attack and colonization of the Pathogen

organism of host tissue cells. It is the first step in bacteria pathogenesis (Emdyetal.,2003) in the adhesion process , the interaction and correlation between the superficial molecules on the surface of the pathogen are called the adhesion , or called adhrsive factor (bodeletal , 2016, khandellwal etal 2009) or may be called colonization factors (zhou etal 2001) these adhesives are associated with superficial receptor which it is attached , it islocated on the host cell surface. It adhesives are usually fatty proteous , or sugary proteins , their receptor it is only sugar of many like . sugar of mango (tororaetal 1998) the pilli are external stricture on the surface of the cell wall.

Virulence factors :-

The flagellum of P.mirabilis is crucial to its motility a characteristic that helps the organisms colonization . the flagellum has also been linked to the ability of P.mirabilis to form biofilms , aiding in the bacteria's resistances to defenses of the host and select antibiotics, P.mirabilis also relise on its pili for adhesion to avoid being flushed out of the urinary tract system. Important to P.mirabilis is ureases , responaible for raising the PH and consequently making it easier to thrive increase PH allows stone formation to take place. On occ asion the stone fill the

entire renal pelvis . also inflammatory response system and pore.forming hemolysine.

Pathogenicity :-

The interaction between P.mirabilis and the host defense (immune system) determine the resultant infection , proteous species have an extracy to plasmic oute memberane , like other gram-negtive bacteria , which contains lipoproteins , polysaccharides , lipopolysaccharides , and a lipid bilayer . Different component of this host membrane interact with the host and host defens mechanisms to determine the organisms virulence . additionally , the size of the inoculum has apoitive correlation with level of infection . attachment of P.mirabilis to has tissue depends on the activity of its fimbriae or (pili) which are tiny projection on the bacterium surface the tips of these fimbriae also contain certain compound and polysaccharides that allow for attachment to specific sites in the host organisms (e,g endothelium of the urinary tract) or other manim surface (e.g. medical devices)

Once proteous species attact to the target site , a cascade of events is initiated in the host cell, in including interleukin (IL 6) and (IL-8) secretion in addition to species also proteous urease , which has been shown to be associated with an increased risk of pyelone phritis and upper UTIs ,

proteous species also hydrolyze urea to ammonia , thereby alkalinizing the urine , through the production of urease and ammonia proteous can proteous an environmental where it

can survive. Additionally , alkaline urine will decrease the solubility of both organic and inorganic compounds , encouraging precipitation and strurite (e.g. magnesium ammonium phosphate and calcium carbonate-apatite) stone formation , like other gram-negtive bacteria , proteus species release endotoxin (part of the gram-negtive bacterial cell wall) when invading the bloodstream , theraby triggering additional host inflammatory response which can ultimately resulting in sepsis or systemic inflammatory response syndrome (SIRS), a severe condition with a 20% to 5 % associated incidence of mortality.

***flagella and motility ***

Flagella they are longmesopolamia hair-like appendes and a hollow spiral , and emerge from the outer surface of the cell and the use of bacteria for ,movement . and bonding , flagella are one of the most virulence factor of these bacteria (Siddiqui, 2003,volketal..1997) however , the distribution of flagella on the surface of the bacterial cell is used to diagnose and classify the bacteria the location of the flagella and thir number are specific to each race . there

are race that have only one polar line and are called monotrichous while genders suuounded by flagella are called pertrichous as in the escheria bacteria , which have between 6-10 peripheral flagella hite the proteous miabils bacteria possess more than 100 flagella peritoneal location , as the flagella help the bacteria to rise and enter the upper urinary tract and contrary

to the trend of the blood strean leading to the occurrence of infectionas, (forbes etal . 1998 , volk etal 1997) the main components of flagella is a protein called flagellin (umpierezetal , 2013). P.mirabilis bacteria has two genes responsible for the generation of two falagenes fla A and fla B (Hatt and Rather , 2008 , Mangesetal 2000)

The presence of these flagella on the surface of the pathogene bacteria , oppprtunism facilitates the process of colonization and spread of infection from the primary sites (Armbruster and mobley , 2012, Rather, 2005) P.mirabilis bacteria them to invade the urinary tract , increase their morbidity and make them able to invade the urethra, bladder , ureter kidneys (Jacobsen etal 2008 , Liaw etal 2003 , liaw etal 2001) . this P.mirabilis bactria phenomenon swarming is one of the most important viralences factors of the these bacteria , which is known as the movemental of bacteria in waves it start from the edge of the original colony by flagella . this phenomenon is

crealy observes in the normal steel medium . the sex of the proteous family specices (Ratheri,2005, liaw etal 2003 , Gueetal 2001) in this phenomenon , growth appears in concentricrings forming what the waves of the sea are like on blood agar dishes (verstraeten etal , 2008 . Koneman etal 1997).

haemolysine

Haemolysine are extract cellular enzymes produces by bactria that work on the analysis of red blood cell by making holes in the cell membrane of the vary bacteria producing causes of blood on the abilityed analysis of diffenent types of erythroctytes for examples, erythrocytes humans , sheep, rabbit , and also different in type analysis caused by (liaw etal . 2000) there are four types of decomposition erytheroctes, decomposition full of the type of beta , B-haemolysis appears in areas clear transparent colors on the colonial bacterial , decompositium in part of the type of alpha a- haemolysis shows in area green shinines about colonical bacteria , either types three it is gamma colonisl only . type the fourth to analyze the blood (brook etal , 2001). There are relationship document between the production of blood and fury bacteria through their effectiveness toxicity to cells epithetial lining derices

urology represent by the kidney causing damage to cell and lead to domage tissue (liaw etal , 2000 , baun and focareta , 1991)

***biofilm formation ***

Is the gatherining micro bilogy and adhesion surface cell of the host in an environmental of water and be surrounded extercellular polymers , and this polymers she is a sugars multiple they contribute to this membrane is an injury and resistance to antibiotics , and adhesion bacteria on surfaces

is on of the steis initial which lead to configure the membrane vital (kokare ectal 2009, lynch etal , 2003) . as the lead polymeric materials abroad cellular to install the membrane vital it provides protection cells located inside of the environmental conditions inappropriate such as UV. And the change in PH , and shoch osmasies and drough , materials and toxic (de charvalho,2007) represents the membranes vital medical problem serious as it can be bacteria constituent membranes vital of colonization of applanced and medical as a catheter is an important sources pollution and bacteria that causes disease (morrisetal , 1999 , Casterton etal , 1999)

***B-lactam antibiotics ***

B-lactam the name is called on all antibiotics containing the B-lactam ring in their composite (wilke etal , 2005) this group of antibiotic's is the most widely used sice its

discovery because of its high effectiveness . representing 60% of all antibiotics used for infections causes by gram negative bacteria (liver more and woodord , 2006) these antibics include , carbapenem , monobactam (wilke etal 2005). These groups differ in the nature of the additional ring related to the ring of B-lactam in the total penicillins we find that additional ring is (5-thiozolidin).

Materials and Methods

Laboratory equipment and tools :Materials

Table (3-1): The equipment and laboratory tools used in the study and the thereof manufacturers

Manufacturing	Equipment	No.
Biomerieux (France)	Api 20 E tapes to diagnose gram-negative bacteria with their reagents	1
Al-Hani (USA)	Disposable petri dishes	2
Superestar (India)	Test tubes	3
Gallenkamp (England)	Incubator	4
NUMIT,OEM available China	Calipers	5
Concord (Lebanon)	Refrigerator	6
Bioneer (Korea)	Electrophoresis	7
Fisons (Japan)	Distiller	8
Hoelezean (Germany)	Thermocycler PCR	9
Olympus (Japan)	Centrifuge	10
Memmert (Germany)	Water bath	11
Superestar (India)	Slides and cover slides	12
Gallenkamp (England)	Hot plate	13

Eriotti (Italy)	Electric oven	**14**
Cruma (Spain)	Laminar flow cabinet	**15**
Sony (Japan)	Digital camera	**16**
CYAN (china)	Vortex	**17**
Olympus (Japan)	Light microscope	**18**
Superestar (India)	Disposable Syringes	**19**
SterellinLtd.(England)	Sterilized cotton Swabs	**20**
MUV (Taiwan)	UV-Trans illuminator	**21**
Hoelezean (Germany)	PH meter	**22**
Hermle (Germany)	Cooling centrifuge	**23**
Gallenkamp (England)	Autoclave	**24**
Sartorius (USA)	Sensitive balance	**25**

Chemicals and Biologics

Table 3.2: Chemical and Biologics Used in the Study.

Manufacturing	Biological Materials	No.
Biolife (Italy)	Agarose	**1**
BDH (England)	Agar	**2**
	Crystal violet	**3**
	α-naphthol	**4**
	Hydrogen peroxide H_2O_2	**5**
	Gelaten	**6**
	$_4H_2SO$	**7**
	HCL	**8**
	Safranine	**9**
	Methyl red	**10**
Bio BASIC INC /USA	Methene blue	**11**
Nerk (Germany)	Ethidium bromide	**12**

BDH (England)	Sulfate Sodium Dodecyl (SDS)	**13**
	Dipotassium hydrogen phosphate	**14**
	Monopotassium hydrogen phosphate	**15**
	Tetra methyl paraphenylen diamine dihydro chloride	**16**
	Barium chloride	**17**
	Ferric chloride	**18**
	Sodium chloride	**19**
	Glycerol	**20**
	Urea Solution	**21**
	Potassium hydroxide	**22**
	Iodin	**23**
	Potassium iodid (KI)	**24**

3.1.3: Cultures Media Table (3-3): The 3.1.3: Cultures Media used in the study and the manufacturers thereof. used in the study and the thereof manufacturers .

Manufacturers	**Purpose**	**Cultures Media**	**No.**
Himedia (India)	Use this medium to investigate portabilityIsolates to produce hemolysis enzyme and the observation of Swarming phenomenon	Blood agar base	**1**
	Use to investigate bacterial susceptibility to the production of urease enzymes	Urea ager base	**2**
	To detect the andol ring	Pepton water broth	**3**
Oxoid	General growth	Nutrient broth	**4**

(England)	Used as a transport medium and activating isolates	Brain-Heart infusion agar	**5**
Himedia (India)	Used to investigate bacterial susceptibility to production For the DNase	DNase	**6**
Oxoid (England)	It was used to investigate bacterial susceptibility to the production of phenyl pyrofic acid from Phenylalanine	Phenylalanine ager	**7**
Himedia (India)	Used as a diagnostic medium	chrom agar	**8**
	Used to describe it as an electoral medium for negative bacteria And the differentiation between fermented and non-fermented bacteria of lactose sugar	MacConkey agar	**9**
	Used to detect complete or partial analysis of sugars and production of carbon-like acetylene	Methyl red vogas-Proskaour broth	**10**
	General growth	Nutrient agar	**11**
	To investigate bacterial susceptibility to biomembrane formation	Tryptone soya broth	**12**
Oxoid (England)	It is used to detect fermented bacteria as a source of carbon	Simmon's citrate agar	**13**
Himedia (India)	It is used to examine the sensitivity of bacteria to antibiotics	Muller hinton ager	**14**
Oxoid (England)	Used to investigate sugar fermentation and production of H2S	Triple-sugar iron	**15**

Antibiotics

Table (3-4): Antiviral tablets used in the examination and concentration of sensitivities and the prepared company.

Manufacturers	Dish concentration in microgram	Code	Named of antibiotic	Type of antibiotic
Bioanalyse (Turkyi)	20/10	AMC	Amoxcillin / Clvulanic acid	β-lactam / β-lactamase inhibitor combination
	10	P	Penicillins	Penicillins
	30 30	CTX CL	Cefotaxime Cephalexin	Cephems
	10 10	IMP MEM	Impenem Meropenem	Penems

Oxoid (England)	10 30 10 30 30 10	CN AK TOB K NET S	Gentamycin Amikacin Tobramycin Kanamycin Netilmicin Streptomycin	Aminoglycosides

Methods

Sterilization Methods

All prepared agglomerative and non-autoclave media and dispensers were sterilized at the autoclave at 121 ° C under 1 hour air pressure for 15 minutes. For glass, they were combined with the Oven at temperature168 ° C for one hour and a half, and materials and fluids that are affected by the heat were sterilized by filters . (Benson, 2002) with a diameter of 0.22 millipore (millipore filters)

preparation solutions

:Normal Saline

Prepare to dissolve 0.85 g of NaCl in 90 ml of water

100 ml in the ground water and then sterilized with the catheter for 15 minutes and kept at 4 ° C until use

. (Macfaddin, 2000)

3.2.3: Preparation of reagents

Oxidase reagent

Prepare Oxidase dissolving 0.1 ml of Tetramethyl -P-phenylalanine diamine dihydrochloride in 10 ml of distilled water.The detector is used in an empty bottle. The detector is used to investigate the bacterial susceptibility to enzymatic production . (Baron et al., 1994)

Kovac's reagent:

In 75 ml Para-Dimethyl amino benzaldehyde, dissolve 5 g of a substance Of isoamyal alcohol and 25 mL of HCL. The reagent is kept in an empty bottle in the refrigerator, and the reagent is used in the test of the production of the ethanol (Forbes et al., 2007).

Methyl red reagent:

Prepare the detector by dissolving 0.1 g of the red dye in 300 ml of ethyl alcohol with 95% concentration and then complete the volume to 500 ml using distilled water (Macfaddin, 2000).

Voges-Proskauer reagent

The detector consists of:

A-α-nephthol detector, which was present by dissolving 5 g of material in 100 ml Of absolute ethyl alcohol to 5% concentration.

B. The potassium hydroxide solution, which is present by

dissolving 40 g of material in 100 ml of distilled water, becomes 40% (700%, Forbes et al.

Ferric chloride

Prepare to dissolve 10 g of chlordic chloride in 100 ml of distilled water. I will use

. (Macfaddin, 2000) Phenyl alanine deaminase reagent for the detection of anemia

DNase reagent DNA

Prepare the detector by adding 8.4 HCL of the reservoir slowly to 80 mL to the dew water, then complete the volume to 100 mL of distilled water to obtain 1 mL of HCL. The detector will be used to investigate the ability of the bacteria to produce the DNA enzyme

. (Macfaddin, 2000)

Culture Media

Ready Culture Media

I attended the transplantation communities used in the study according to the instructions of the manufacturer, which is installed on the packaging and was combed with the dispenser at 121 ° C for 15 minutes. Prepare the center of the solid blood and

sterilize and leave until it reaches 50 ° C and add 5% Of human blood and then pour into sterile Petri dishes and kept in the refrigerator for use (Macfaddin, 2000).

Structural Culture Media

1- Urea agar

Prepare the base of the sterilized sterilized urea base according to the manufacturer's instructions. Then add 5 mL of the 20% urea solution after 50 ° C coolant, and then distribute the medium

On tubes placed diagonally to harden, and used to investigate bacterial susceptibility to the production of urease enzymes.

. Gelatine agar medium

Prepare the center by dissolving 6 g of gelatin in 500 ml of nutritious broth and then spread in

Glass tubes with 5 ml per tube, sterilized by the catheter and kept in the refrigerator for use. Use the medium to investigate isolates capable of producing gelatin enzymes (Atlas et al.

. (1996)

3. Sugar Fermentation Medium

The center of Phenol red broth was to adjust the pH to 7.2 and then sterilize

In the bacterium, add 1 ml of 1% diabetic solution to each of the various sterile diuretics filtered by fine filters with 0.22

micrometers, and the sugars used are glucose, fructose, lactose, mannose, kaltose, maltose

6.2.3:

Isolation and Identification of isolated bacteria

The developing bacterial isolates were identified on both the center of the solid blood base and the solid maconucleic base on the following bases:

Cultural characteristics: 1.6.2.3

Pmirabilis colonies were initially identified by appearance traits

Including the shape, size and color of colonies. Pmirabilis colonies were concentrated on the center of the solid blood base, as well as pale colonies

Non-fermented lactose sugar on the solid MacConkey base medium. Cultures, growth characteristics, growth or non-growth of developmental colonies were studied

(Macfaddin, 2000). Pmirabilis was also identified on the center of Chrome-based steel

Which he considers to be a diagnostic medium.

Microscopic characteristics: 2.6.2.3

The study examined the microbial properties of bacterial colonies by taking a swab of these growing colonies on the culture media and installing them and dyeing them in a gram to observe the shape, collect the bacterial cells, interact with the dye and examine them under the optical microscope

Biochemical tests

Catalase test

Transfer a portion of a 24-hour-old infant colony to a clean glass slide and place a drop of 2H2O hydroxide solution with 3% concentration (para. 2-3-2-3) on the colony. The result was positive gas bubbles

. (Brown, 2007) Oxygen

Oxidase test

The test was performed by placing part of the colony on a filter sheet saturated with oxidase reagent (§ 1.3.2.3), and the violet color during 30-15 seconds was positive evidence

. (Forbes, 2007) test

Urease test

The tubing was cleaned in the center of the elliptical medium in a method of staining and planning and the tubes were incubated at 37 ° C for 24 hours. Changing the medium from yellow to pink A test positive evidence is that anthrax is a bacterium by the bacteria (Benson, 2002).

Indol test

Rinse the center of the peptone water to be tested and then incubate the medium at 37 ° C for 24 hours and then add a few drops of Kovac's reagent

(Fig. 3.3.2.3), and that the appearance of a red ring indicates the positive test (Macffaddin, 2000).

Methyl test

MR-VP Media was incubated with a bacterial plant, incubated at 37 ° C for 48-24 hours and after the end of the incubation period, 5 drops of the red reagent were added (para. 4.3.2.3) and the red appearance of the tube The result is positive and indicative of acid production, where the yellow color remains negative (Collee et al.

Voges - Proskaur

The MR-VP was tested with the bacterial plant, incubated at 37 ° C for 48-24 hours, then 0.5 mL of vanfethol reagent solution and 0.2 mL of potassium hydroxide solution (3.2.3.5) were added to each Tube with stirring and then leave for 10-15 minutes. The appearance of pink indicates the positive test, which refers to partial glycolysis and the production of Colle et al., Acetyl methyl carbonyl. (1996)

Citrate utilization test

This test was used to investigate the ability of bacteria to consume jackets that are a source of carbon. The Simmon citrate agar medium was injected with bacterial colonies to be tested and incubated at 37 ° C for 48-24 hours. Turn the center color

From green to blue is a sign of positive testing, ie, the consumption of jackets for jackets

. (Winn et al., 2006)

Triple - Suger iron agar and gas production test

The bacterial susceptibility to glycoside, lactose, sucrose and hydrogein was detected by refining the Triple-Suger iron in a method of staining and plotting on the liquid surface of the bacterial plant and then incubating at 37 ° C for 24 hours, changing the medium color from red to Yellow in the deep part is a positive result for glucose fermentation only, while the whole color change from red to yellow is a positive result for the fermentation of lactose and sucrose. The gas is also in the form of bubbles below the center. In the case of bacteria forming hydrogen gas, In the bottom of the tube

Gelatinase liquification test:

This test was performed to detect the ability of the bacteria to produce the gelatinase enzyme, which acts as a gelatin solution. The tubes were centrifuged in a sterile manner and incubated at 37 ° C for 24 h. The gelatin was investigated after placing the medium tubes in the refrigerator at 4 ° C for half H, the occurrence of maladaptation is an indication of the effectiveness of an enzyme before

10-7-2-3Sugar Fermentation test:

The center of modernized sugar preparations (3.2.4.2.3) in modern Victoria is growing at the age of 24 years

H and incubated at 37 ° C for 5 days. The transformation of the medium from red to yellow indicates the fermentation of sugars (7002, Forbes et al.

11.7.2.3: Test for the displacement of the amino acid group from phenylalanine

This test will be used to investigate the ability of the bacteria to displace the 2NH amino acid group phenylamine and release phenyl pyruvic acid and ammonium 3NH. Rude

And then incubate at 37 ° C for 24 h. After that, 5-4 drops of ferric chloride solution were added with a concentration of 10% on the surface of the medium, while the tubing was rotated in a circular way to immerse

The center surface of the detector indicates that the change of center color to green indicates that the result is positive (1997, Koneeman et al.).

12-7-2-3DNase enzyme test:

In the center of the DNA analyzer, incubate the 18-hour-old bacteria in patches and incubate the dishes at 37 ° C for 24 h after incubating the dish with the DNA analyzer (3.2.3.7). A transparent area around the inoculation area after immersion of the dish indicates the response of the test (1996, Colle et al.

13.7.2.3: Conservation and maintenance of bacterial isolates

The tubes containing the bacteric acid-fed nutrient medium to be stored and incubated at 37 ° C for 24 h were then incubated at 4 ° C and the conservation process was repeated to maintain and prevent isolates and avoid contamination (Sambrook et al., 1989). Bacterial isolates were kept for a long time by adding 15%

calcerol to the liquid nutrient medium and kept at -20 ° C and renewed every 6 months (NCCLS)

. (2003)

8.2.3: Investigation of some of the severity factors of isolates 1-8-2-3 under study Haemolysin production test:

The method in Senior and Hughes (1988) was followed to investigate bacterial susceptibility

Isolating the hemolinesin and determining the susceptibility of this enzyme to enzymatic

analysis on the identification of four groups of human blood as follows:

1. Discard 5 ml of blood sample withdrawn using sterilized plastic tubes containing a blood clotting blocker to get rid of the plasma and obtain red blood cell deposits.

2 - Wash the cells twice consecutive with saline saline solution with the installation of cells by centrifugation after each wash.

3. Red blood cells were used to prepare the center of the solid blood base. The isolates were planted in a planing manner on the medium and incubated at 37 ° C for 24 hours to detect bacterial

susceptibility to blood analysis by forming a translucent aura around the developing colonies.

Results and discussion

3 - 1 : Isolation of bacteria *P.mirablis* And diagnosis

The current study has shown that only 49 isolates of the P.mirablis species have been studied through the study of some culturing, microbiological, and chemical tests, as follows:

Cultural characteristic: 1.1.3

Developing colonies on MacConkey Agar appeared colonies

Pale blue, medium-sized, smooth, non-fermented, lactose-free. No odor of bacterial growth, similar to the smell of moldy fish . swarrming on blood agar, which is a primary diagnostic characteristic of these bacteria, Figure (4-1). The bacteria were then identified on the chrom agar medium and colonies of brown color appeared in Fig.

Microscopic characteristic : 2-1-3

The results of the microscopic examination showed that the cells of the bacteria isolated by the bacillus spores are Gram- negative to the chromium, not composed of spores

3.1.3: Biochmical Test

The developing isolates were identified on the center of the Agar chromium based on the chemical tests where table (3-1) was shown. Response of all isolates for both the catalytic test, the red-methyl test, the gelatin test, and the jacket consumption test as the sole source of carbon if the blue-to-green color change was observed as a result of changing the color of the prothemol to blue to increase the pH.

While P.mirablis fermented both sucrose and glucose and the formation of a black deposit on the medium indicating the production of hydrogen sulfide gas

The isolation response was differentiated with DNase were showed that 32 positive isolates were detected. A translucent halo formed around the bacterial colony after the addition of the HCL DNase analyzer, while the isolates were all given a negative result for both the oxidase and Fox-

The isolates under study also showed that they did not become an endol ring if the positive result resulted in the formation of a red ring in the layer alcohol Isoamyl alcohol as a result of the decomposition of amino acid tryptophan and indole formation

Schedule) 3 - 1 (Demonstrates the diagnostic biochemical tests of bacteria *P.mirablis*

The result	Type of test
+	**Alcatelis**
-	**Oxidase**
-	**Fox- Procauer**
+	**Gelatinase**
-	**Andol**
+	**Red-methyl**
+	**Explain Aldnyiz**
+	**Consumption of jackets**
+	UrizThey are H2S
+	Growth on West
Acid / Alkaline	TSI

+Positive - Negtive

3.2: Numbers and percentages of isolation of P.mirablis bacteria

49 isolates were diagnosticed by isolating 28.82% of the total number of samples collected from patients with urinary tract infection. The current study also showed that the percentage of female genital infections was higher than in males (Table 4.2) 33 (67.19%), while in males the ratio was (16. 3265%).

3.3: Isolation and diagnosis of P.mirablis bacteria

Urinary tract infections are of great importance. Therefore, the current collection of samples in the current study of patients has been infected with urinary tract infection. P.mirablis is one of the most common types of urticaria in urinary tract infection. The isolates of the bacteria under study were first diagnosed through a study In some microorganisms and microorganisms, the agar samples were planted on the MacConkey Agar medium to obtain pale, medium-sized colonies with smooth, non-fermented, lactose-like surfaces with a smell similar to the smell of rotting fish. P.mirablis is used as a negative bacteria And distinguishes them From the rest of the bacterial species positive for the color of Kram and Swarrming also appeared on the blood center Agar, which is a primary characterization of these bacteria and as in Figure (4-1) and this result is consistent with what is mentioned

The results of the microscopic examination showed that the cells of the isolated bacteria are in the form of chromosomes negative to the form of chromium and non-composed and non-composed of spinach

As for the biochemical tests used as supplementary tests for the initial diagnosis of P.mirablis, the purpose of which is to confirm the diagnosis of the species of bacteria being studied. The results shown in Table 4.1 showed the response of all isolates under study for catalysis

Indicating the ability to produce the catalase enzyme if the positive result of the emergence of air bubbles directly after the addition of hydrogen peroxide H2O and the result was positive to test the consumption of jackets as the sole source of carbon

if the change in the color of the medium from green to blue due to change the color of bromothaimeol to blue to increase PH

The isolates were also positive for the red-methyl test and negative test for the Vox-Proskauer because the Acetyl-Methyl Carbinol was not synthesized from the molecular breakdown of sugar

As for the examination of indoles, the result was negative for the isolates under study. This test is used to distinguish between the sex of the other P.mirablis and the rest of the species sex mutants if positive result is a red ring as a result of the analysis of amino acid tryptophan and its transformation on the Andole also gave isolates bacteria negative result For oxidase testing because of its inability to produce oxidase

All isolates showed their ability to ferment sugar, sucrose, and glucose, and gave gas and black spray to the medium, indicating the production of hydrogen sulfide

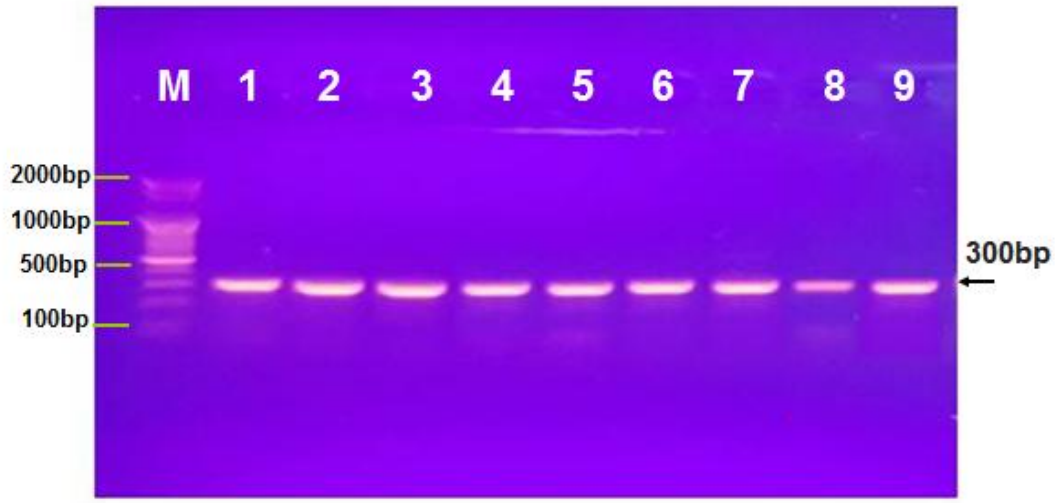


Figure 1: Agarose gel electrophoresis image that shown the PCR product of 16S ribosomal RNA gene in Proteus mirabilis isolates. Where M: Marker (2000-100bp), lane (1-9) some positive PCR amplification at (300bp) PCR product size.

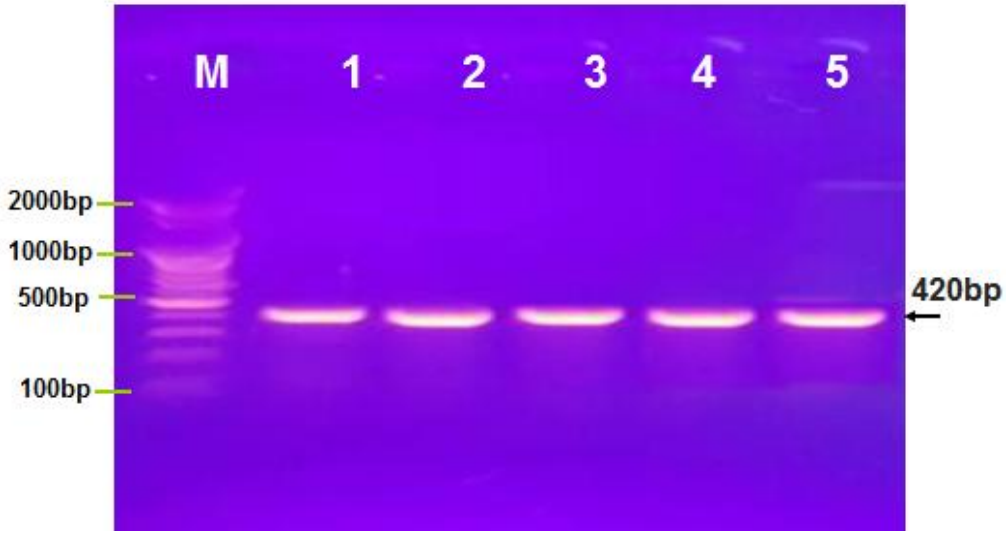
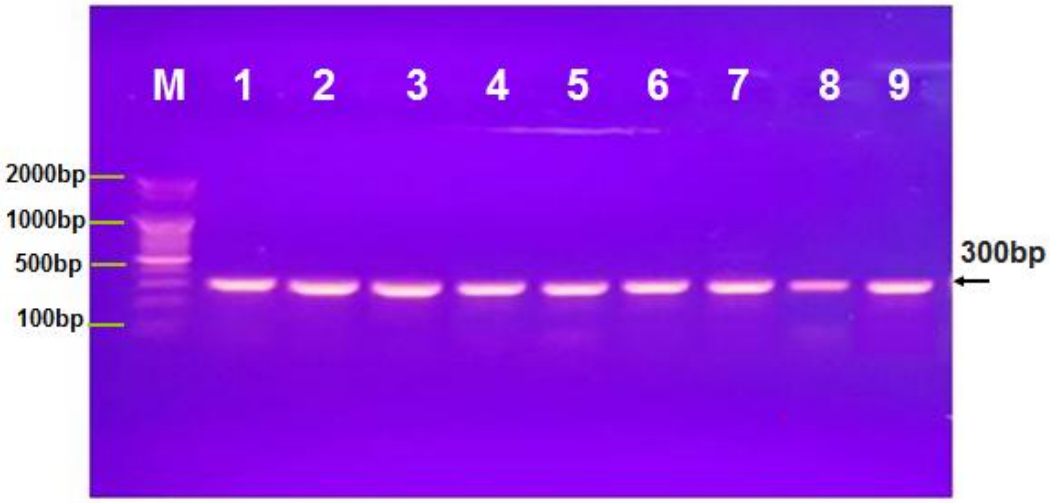


Figure 3: Agarose gel electrophoresis image that shown the PCR product of virulence factor UreC gene in Proteus mirabilis isolates. Where M: Marker (2000-100bp), lane (1-5) only positive PCR amplification at (420bp) PCR product size.



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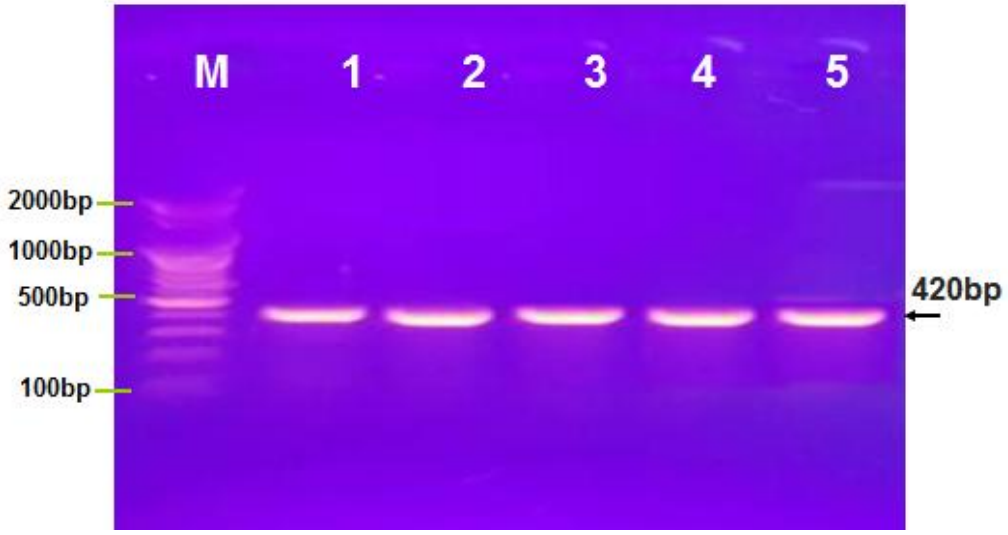

Figure 3: Agarose gel electrophoresis image that shown the PCR product of virulence factor UreC gene in Proteus mirabilis isolates. Where M: Marker (2000-100bp), lane (1-5) only positive PCR amplification at (420bp) PCR product size.