

## Study of Virulence Factors in Urease-Positive Bacteria Isolated from Urinary Tract Infections Clinical Specimens

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The study included investigation virulence factors that product from urease positive bacteria species isolated from urinary tract infection. a total of 60 clinical specimens, taken from urine sample had been cultured and identified pure isolates result show like *Klebsiella pneumonia* ( $n = 5$ , 8.3%), *Staphylococcus.sp* ( $n = 3$ , 5%) and *Pseudomonas.aeruginosa* ( $n = 1$ , 1.6%). Most isolated of urease positive bacteria were negative gram stains excepted *Staphylococcus sp.* was positive gram stain. All isolates gave positive result of catalase test, while coagulase, oxidase, indole, Simmon citrate and kligler test was performed for all isolated gave variable result. Also all urease positive bacteria were identified by using VITEK-2 system. *Klebsiella.pneumonia*, *Staphylococcus.aureus* and *Pseudomonas.aeruginosa* non fermentation for lactose sugar test while *Staphylococcus.Saprophyticus* had ability lactose fermentation. Antibiotic susceptibility for all urease positive bacteria results showed that all highly sensitive towards (Amikacin, Gentamycin, Imipenem, Norfloxacin, Ciprofloxacin and Vancomycin) while showed low sensitivity towards (Ceftriaxone, Cefotaxime, Nalidixic acid and Nitrofurantoin). In addition all bacteria produce urease showed had Clear difference in another virulence factors like (DNase, Lipase, Protease).

**Keywords:** Virulence Factors, Urease positive bacteria, UTI, Clinical specimens.

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Urinary tract infection (UTI) caused by different Gram-negative bacteria is the most common infections. About 150 million patients are suffering UTI each year worldwide. Antibiotics resistance in uropathogens bacteria are a major global attention which can lead to failure of treatment, genetically modified of bacteremia, increase for requirement therapy, and lengthening of hospital accommodation UTI is the second infectious disease after the respiratory disorder. Catheterization process is the main risk factor of urinary tract infection<sup>1-3</sup>. The high risk of UTI at women's pregnancy. They are common, reminder diagnosis and treatment as even occasional bacteria can be related with reverse pregnancy result. Complications of embryo include early

labour, stillbirth and depressed birth weight. The pyelonephritis is a maternal complication with has a high return rate<sup>4</sup>. The urease positive bacteria are five species with urease activity (*Proteus.mirabilis*, *Pseudomonas.areginousa*, *Klebsiella pneumoniae*, *Enterobacter.cloacae* and *Staphylococcus aureus*) were subsequently isolated from different patient clinical samples<sup>5</sup>. Coagulase-negative staphylococci (CNS) are a many of Gram-positive bacteria. They are consisting of 16 Subspecies are known to cause human infection. Makeup about 9% of nosocomial infections. The species are most recurrent causes diseases in humans are *Staphylococcus epidermidis*, *Staphylococcus. haemolyticus* and *Staphylococcus. Saprophyticus*. Whereas *Staphylococcus aureus* is the best known and has been frequently implicated in the etiology of a series of infections and intoxications in humans<sup>6-8</sup>. *Pseudomonas. aeruginosa* has many types of virulence factors that have important roles in the pathogenicity of this

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bacterium. The major virulence factors are toxin A, alkaline protease (aprA), elastase, and have many exoenzymes. Two phospholipases C enzymes may also have roles in the hydrolysis of phospholipids in lung tissue<sup>9,10</sup>. The potency virulence factors of bacterial pathogenesis associated with the disease pathway, including swarming, growth rates, fimbria expression, flagella, and the production of hemolysins, ureases, proteases, lipase in addition Dnase have been described in many studies<sup>11</sup> Lipopolysaccharide (LPS) on their exterior is the essential composition of the outer membrane and exclusive of the central virulence factors of the microorganism It composed of a polysaccharide part, containing an O-specific chain (O-antigen, O-PS), and a core region, as well as a lipophilic region, termed lipid A, which anchors the LPS to the bacterial outer membrane<sup>12-14</sup>. *Staphylococcus epidermidis* and other CONS are infected bacteria, it contains the extracellular products like protease, DNase, lipase, hemolysis and external enzymes (exoenzymes) probably responsible for human cell tissues degradation and spreading of a disease caused by these germ *organism*<sup>15-17</sup>.

## MATERIALS AND METHODS

### Samples

Sixty samples were obtained from AL Ramadi teaching hospital for Maternity and Children from the period June to August /2018. Collected from suspected patients suffering from urinary tract infection in a different age.

### Collection and Culture Samples

All specimens were collected in sterilized recommended containers. The urine specimens were run by centrifuge machine at 1500 rpm for 5 minutes. The discarded of the supernatant then cultured the precipitate by streaking on MacConkey agar, blood agar then incubated at 37°C for 24-48 hour<sup>18</sup>.

### Laboratory diagnosis

It was included microscopic examination of the growing bacterial cells were carried out by transferring a pure single colony to glass slide and procedure gram staining<sup>19</sup>. The selected colonies undergo biochemical tests to diagnose the species level and as follows:

#### Catalase test

Application of this test to differentiate

between staphylococci and streptococci bacteria. Transfer single colony by age (18-24) hour to slide and add few drop of 3% hydrogen peroxide. Immediate bubble formation indicating a positive test<sup>20</sup>.

#### Oxidase test

Pick up some colonies from the growth media by the sterile wooden sticks loop into the moistened filter paper by 1% oxidase reagent. The change in the color of the zone to purple color after (30) second indicating for a positive result.

#### Coagulase test (slide method)

Was rapid diagnosis used as the following: add two drops of sterile saline on each area of the divided slide. Then transfer the bacterial colonies to make a suspension. And was treated with undiluted plasma. Observed the result by clumping within 10-20 second of the bacterial suspension was positive result<sup>21</sup>.

#### Mannitol Fermentation test

Mannitol salt agar (MSA) was used for investigation staphylococci pathogenic types mainly *Staphylococcus aureus*. Furthermore, inoculated with a clear colony of growing bacteria and incubated at 37°C for 24 hours. the ph phenol read indicator (MSA) through changed to a yellow color mean positive result<sup>22</sup>.

#### Indole test

The peptone water inoculated by single colony form isolated bacteria, incubated for 24-48 hours at 37°C added after that 0.5ml of Kovacs reagent. And recorded the result<sup>23</sup>. **2.3.6 Citrate utilization test:** (Simmon's citrate agar) bacterial inoculated in agar and incubated for 24-48hours at 37°C the sodium carbonate generated were change the indicator color (bromothymol blue) from green to blue color<sup>23</sup>.

#### Kligler iron agar (KIA)

All isolated species was inoculated and incubated at 37°C for overnight, change in color phenol-red indicator from red to yellow color means a positive result<sup>24</sup>.

#### Vitek 2 system for identification of bacteria

The bacterial isolates from urine were identified by Vitek 2 compact System. The selected cards were used depended upon result of the gram stain and conditions growth of the microorganism to be tested<sup>25</sup>.

#### Sugar Fermentation

Broth Fermentation Tests were used to

differentiate between microorganism ability to carbohydrate fermentation by a metabolic process. Each kind of following sugar: glucose, fructose, sucrose, maltose, lactose, xylose, mannose and sorbitol were placed in tubes containing 5 ml Purple Broth Base medium, the results appear after 24-48 hour of incubation at 37°C<sup>24,26</sup>.

#### Antibiotic Susceptibility test

To determined antimicrobial agent we used Muller – Hinton (MH) agar by Kirby-Bauer Disk diffusion technique. Transferred the bacterial growth and spread by sterile glass spreader L - shape. The antibiotics disc was placed. The plates were incubated for 24 hours at 37 °C. And the determined result by measuring the millimeter zone inhibition<sup>27</sup>.

#### Statement of some virulence Factors

##### Determination of DNase test

Prepared DNase agar according to the instruction of Manufacture Company. Inoculated the plates from growth clear colonies of bacterial isolated and incubated for 18-24 hour at 37°C, the plate flooded with 1N HCl reagent and leave the plates for few minute reactions by absorbing the Hydrochloric acid. The positive reaction indicates through a clear zone surrounding growth colonies on the DNase agar<sup>28</sup>.

##### Determination of Urease test

Inoculating the isolated bacteria onto the urea agar slant tubes prepared according to the instruction of Manufacture Company and incubated at 37°C for 18-24 hours, observe any change of color in the inoculated urea agar to a pink color is a positive result of urease bacterial activity<sup>23</sup>.

##### Determination of Lipase production

Inoculating the isolated bacteria into lipase activity agar Tributryin agar and the growth colonies had the opacity after 24-48hour incubation time at 37°C defined the positive result test<sup>29</sup>.

##### Determination of Protease production

Detected of protease production test on skim milk (SM) agar. The agar was prepared according to the instruction of Manufacture Company. The isolated bacteria were inoculated and the clear zone surrounding colonies through hydrolysis of casein main component in milk is a positive reaction<sup>30</sup>.

## RESULTS AND DISCUSSION

A total of sixty clinical patients samples were investigated as urinary tract infection tabulated samples from urine culture. Also, the samples distributed as the gender group female and male. 28% from all cultures are the positive percentage as shown in Table 1<sup>31</sup>. The risk factors estimation which associated with kinds of culture samples and appeared the urine sample cultures is more represented because of many acquired urinary tract infection<sup>32</sup>.

Statistical analysis revealed the age and gender was considered as a risk factor. The age is more significant risk factors. As well as another studies they were reported such as linked to the surgical process, a hospital long time stay, and used the urinary devices<sup>33,34</sup>.

We studied important virulence factors in urease positive bacteria species which causes

**Table 1.** The percentage of risk factors associated with a patient infected.

Variables	Groups	Number Patient	Cultures +ve		Cultures -ve	
			N	%	N	%
Samples	Urine	60	17	28.33	43	71.66
Age	≤ 2 year	39	11	28.20	28	71.79
	>2 year	21	6	28.57	15	71.42
Gender	Male	20	5	25	15	75
	Female	40	12	30	28	70
Total		60	17	28%	43	72%

different urinary inflammation for the human being. In this study, the specimens were collected from different patients represented to AL Ramadi Teaching Hospital for Maternity and Children. The microscopical examination for all isolates bacteria were done by Gram stain. The results showed the most predominant isolated bacteria pathogen was *Escherichia coli* ( $n = 8$ , 13.3%) the second major causative microorganism *Klebsiella.pneumonia* ( $n = 5$ , 8.3%) followed by *Staphylococcus.sp* ( $n = 3$ , 5%) and *Pseudomonas aeruginosa* ( $n = 1$ , 1.6%) Table 2.<sup>19</sup>

**Table 2.** Number and percentage of bacteria isolated from UTI patients

Bacterial Isolates	Number and percent of bacteria isolates (from 60 patients)		
	Number	Gram Stain	%
<i>E. coli</i>	8	-	13.3
<i>Klebsiella pneumonia</i>	5	-	8.3
<i>Staphylococcus aureus</i>	2	+	5
<i>Staphylococcus Saprophyticus</i>	1	+	
<i>Pseudomonas aeruginosa</i>	1	-	1.6
Negative culture result		43	
Total		60	

**Table 3.** Biochemical tests of bacterial isolated.

bacterial isolated	Biochemical test					
	Catalase	Coagulase	Oxidase	Indole	Citrate	Kligler Iron
<i>Klebsiella.pneumonia</i>	+	-	-	+	+	+
<i>Staphylococcus.aureus</i>	+	+	-	-	-	+
<i>Staphylococcus.Saprophyticus</i>	+	-	-	-	-	+
<i>Pseudomonas.aeruginosa</i>	+	-	+	-	+	-

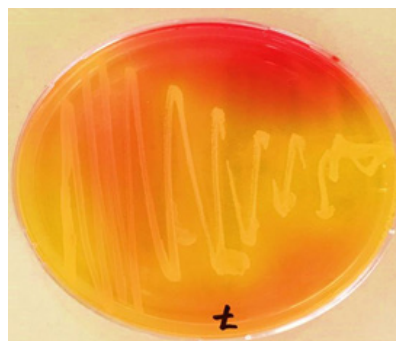
to a positive result for kligler iron test and a negative result for Simmon citrate and indol test. *Staphylococcus.Saprophyticus* species gave positive result for catalase test and kligler iron.

*Pseudomonas aeruginosa* gave positive result for catalase and oxidase test that is differentiates test for *Pseudomonas sp.* these results agreement with the study of [10] who found that all *Pseudomonas aeruginosa* isolated

The results constant with the previous studies of feedback in which *E. coli Pseudomonas aeruginosa Klebsiella pneumonia* and others were the major causative pathogen isolated from urinary tract disorder patients<sup>31</sup>.

Table 3 showed all isolates of bacteria species were given a different result for biochemical identification. *Klebsiella.Pneumonia* gave positive result for catalase, indole, Simmon citrate and kligler iron test while gave negative result for oxidase and coagulase test.

*Staphylococcus aureus* gave positive result for catalase test; coagulase test is indicator test for *Staphylococcus. aureus* from another *Staphylococcus sp.* and had capability to ferment mannitol salt agar to produce large yellow zone colonies like a golden stain (Fig. 1)<sup>35</sup>. In addition



**Fig. 1.** *Staph.aures* isolated (golden stain) on Mannitol salt agar

positive reaction for Simmon citrate while negative reaction for kligler iron test, indole test and glucose fermentation test (Fig 2). Additionally for identification of isolated bacteria we used Vitek 2 compact system and the percentage of bacterial tested was ranged from (85-97%),

Table 4 showed all isolated were fermented some types of sugar through change the indicator color as a positive result for sucrose, fructose,

xylose, and sorbitol. *Klebsiella pneumonia* gave positive fermentation for glucose, maltose, mannose and helpless to lactose fermentation test this results in agreement with [36].

While *Staphylococcus aureus* had various result for sugar type's fermentation glucose, maltose and mannose. All isolates were unable to ferment lactose exception *Staphylococcus saprophyticus* and had ability less for glucose and maltose fermentation. (Fig: 2) Also *Pseudomonas aeruginosa* gave positive result for maltose fermentation and negative result to ferment glucose, lactose and mannose.

Seventeen selected isolates from urine samples were tested for resistance toward ten antibiotics and only urease positive bacteria were tested Table 5. In all urinary tract infections studies, *Klebsiella pneumonia* is present<sup>37</sup>. and the sensitivity test result show with Amikacin, Norfloxacin, Gentamycin and Ciprofloxacin, sensitive which is agreement with the previous data of studies<sup>38</sup> while resistance for Cephalosporines group (Ceftriaxone and Cefotaxime).

*Staphylococcus aureus* and *Staphylococcus Saprophyticus* isolated show clear sensitive result aminoglycoside antibiotic group with (Amikacin, Gentamycin) and good sensitive for Imipenem, Vancomycin<sup>39</sup> Beta-lactam antibiotics Ceftriaxone, Cefotaxime are

actually often multi-resistant with staphylococcal infections<sup>40</sup>.

In this project, *Pseudomonas aeruginosa* showed the variable sensitive result for Amikacin and Gentamycin. Imipenem agent gave quite broad sensitive reaction against *Pseudomonas aeruginosa*.

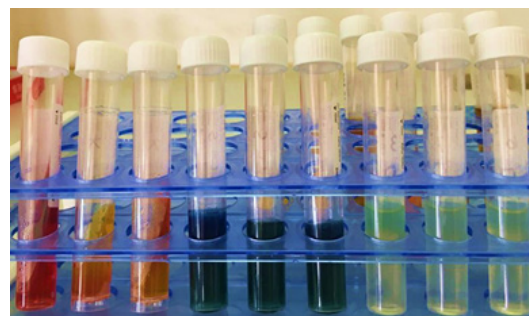


Fig. 2. Biochemical test for Urease +ve isolated bacteria

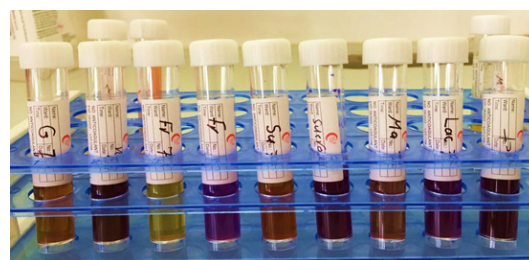


Fig. 3. Sugar fermentation test for Urease +ve bacteria isolated

Table 4. Sugar fermentation for the bacterial strain

Bacterial Strain	Sugar Fermentation							
	Glucose	Lactose	Sucrose	Maltose	Fructose	Xylose	Mannose	sorbitol
<i>Klebsiella pneumonia</i>	+	-	+	+	+	+	+	+
<i>Staphylococcus aureus</i>	+	-	+	+	+	+	+	+
<i>Staphylococcus saprophyticus</i>	-	+	+	-	+	+	+	+
<i>Pseudomonas aeruginosa</i>	-	-	+	+	+	+	-	+

+: positive result; - : negative result.

Table 5. Antibiotic sensitivity test

Urease PS bacteria Isolated	Antimicrobial agent									
	AK	IPM	VA	CRO	CTX	NOR	NA	CIP	NI	GM
<i>Klebsiella pneumonia</i>	++	-	-	R	R	+++	+	+++	R	+
<i>Staphylococcus aureus</i>	++	++	++	-	R	-	-	-	-	+
<i>Staphylococcus Saprophyticus</i>	++	+++	++	R	R	-	-	-	-	++
<i>Pseudomonas aeruginosa</i>	++	+++	-	+++	++	++	R	R	R	+

AK \Amikacin, IPM \ Imipenem VA \Vancomycin , CRO \Ceftriaxone, CTX\Cefotaxime, NOR \Norfloxacin, NA \Nalidixic acid, CIP\Ciprofloxacin , NI\Nitrofurantion, GM \Gentamycin,

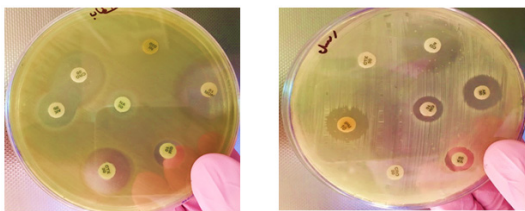
this results in consistent with<sup>41</sup> The major reason for resistance Aminoglycoside antibiotic group through Aminoglycoside-modifying enzymes (AMEs) secretion<sup>42</sup>(Fig. 4).

Results in table 6 showed all isolated bacteria were positive for Urease test because of the most predominant clinical samples from urine (Fig. 5). *Staphylococcus aureus*. produces many types of a virulent factor. Showed of all species isolated production DNase (Fig. 6) and Lipase (Fig. 7)<sup>17</sup>. Lipase activity has main properties for nutrition of the bacteria. The correlation between lipase extracellular activity and pathogenicity of staphylococci is a very equal role in patients with the *Staphylococcus aureus* infections also interfering with phagocytosis process<sup>43</sup>. while the isolated gave a negative result with Protease

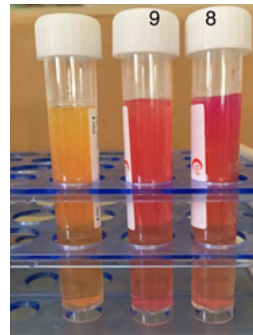
**Table 6.** Virulence factors which produce by Urease PS bacterial isolated from UTI samples

Bacterial Isolates	Virulence Factor			
	DNase	Urease	Lipase	Proteases
<i>K. pneumonia</i>	-	+	-	-
<i>S. aureus</i>	+	+	+	-
<i>S. saprophyticus</i>	-	+	-	-
<i>P. aeruginosa</i>	-	+	+	+

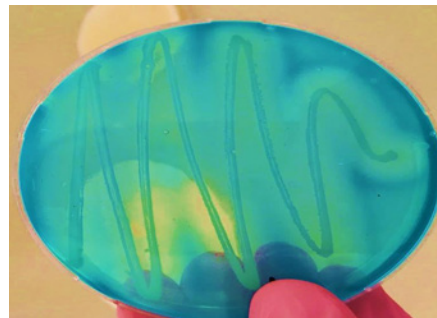
*Pseudomonas aeruginosa* isolated release a large number of factors showed cultured positive result for media used Lipase and exactly for protease enzyme factor (Fig. 8)<sup>44</sup>. while gave negative result for DNase, from the result appear various production of protease enzyme depending on the source isolation. The enzyme has importance role for the resistance of phagocytosis by neutrophils inhibition and destroys surface-bound IgG and C3.



**Fig. 4.** Antibiotic Resistance Test for *Staphylococcus sp.* and *Pseudomonas sp*



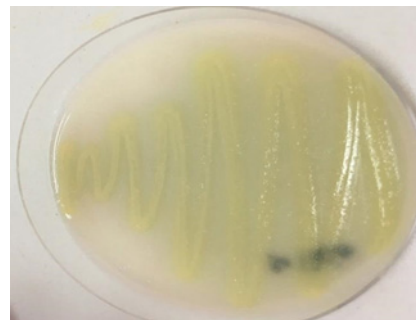
**Fig. 5.** Urease Positive test



**Fig. 6.** DNase positive result for *Staph.aureus* isolated



**Fig. 7.** Lipase test for *Staph.aureus* isolated



**Fig. 8.** Protease test for *Pseud.aeruginosa*



## CONCLUSION

The study appears different bacterial isolation from urinary tract infection in different parameter. The female patients are more prone to infection from male. Antibiotic sensitivity for bacterial isolated appeared bigger variation result compare with bacteria in same species. The variation in produce virulence factors depended on the species types but all isolated were positive for urease test because the urine contains urea composed.

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