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Research Article



## Detection and identification of *Pseudomonas luteola* as newly diagnosis systemic lupus erythematosus in Mosul city, Iraq

Nadia Khalid Mustafa<sup>✉</sup> and Sahar Salim Al-Nakkar

Department of Biology, College of Science, University of Mosul, Mosul, Iraq

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(✉) Corresponding Author: [nadia20scp77@student.uomosul.edu.iq](mailto:nadia20scp77@student.uomosul.edu.iq)

### Abstract

Systemic lupus erythematosus (SLE or lupus) is a chronic autoimmune disease involving multiple organ systems. This study aims to identify uncommon bacteria like *Pseudomonas luteola* as a human pathogen isolate from systemic lupus erythematosus patients in Mosul. This study involved 60 SLE patients who had been diagnosed at Ibn-Sena Educational Hospital in Mosul City from November 2021 to April 2022. Blood and MacConkey agar have been used to culture the samples. 55 samples (91.7%) showed positive results (grew the bacteria). We identified one *Pseudomonas luteola* 1.81% from positive growth of urine culture. It was identified by the biochemical test, API E20, and Vitek-2 system and had its identification verified using 16S rRNA-based molecular methods. The bacteria were resistant to meropenem, ceftriaxone, cefotaxime, gentamycin, doxycycline, ciprofloxacin, and trimethoprim, while they had sensitive to levofloxacin.

**Keywords:** Systemic Lupus Erythematosus, *Pseudomonas luteola*

### Introduction

Lupus, also known as systemic lupus erythematosus, is a chronic autoimmune illness that can affect any part of the body (La Paglia et al., 2017; Dima et al., 2021). Lupus has complex genetic and environmental origins that are only partially understood. Reduced regulatory T cells, increased effector T cells, and greater B cell activation are common outcomes of this altered immunological milieu caused by genetic and environmental variables (Tsang and Bultink, 2021).

Lupus prevalence varies greatly by geographical region, race, and gender, with women having the highest incidence. Women are 9 times more likely than men to develop the condition (Stojan and Petri, 2018).

One of the most common causes of morbidity and mortality in patients with SLE is infection. Around a quarter of all deaths from this condition are caused by infections, and up to half of all patients will suffer from at least one life-threatening infection at some point (Dorgham et al., 2021).

Among Bacterial infections Urinary Tract infections (UTIs) in SLE patients have been reported as the most common primary or secondary infections Women are 5 times more likely to acquire urinary tract infections than males are, and gram-negative bacteria are more likely to cause illness than gram-positive bacteria (El-Raouf et al., 2020). As a result of structural differences between the sexes, bacteria in women have a far easier time entering the bladder (Czajkowski et al., 2021).

*Pseudomonas luteola* is a Gr-, aerobic motile, rod bacteria, oxidase-negative, catalase-positive which is previously known as *Chryseomonas luteola*. It has rarely been reported as causing disease in humans, but it has been reported in a few publications it is important for nosocomial acquired infection, especially among immunocompromised patient that uses corticosteroids, people who use long-term antibiotic therapy, It has been reported as causing agent infection, especially at bloodstream infection (Bacteraemia) peritonitis, wound infection, osteomyelitis, pancreatic abscess, endocarditis, and usual association with prostheses or indwelling vascular catheters. It has been isolated from different clinical

specimens including wounds, Urine, cervix, and throat specimen. (Wyre, 2020; Ben Hmida et al., 2021).

### Materials and methods

Urine samples were collected from 60 diagnosed SLE 60, diagnosis patients of SLE were a target in the time study 10 Males (14-46) years and 50 females (8-76) years from the Nephrology department at Ibn-Sena Educational hospital Mosul, Iraq, between November 2021 and April 2022. All individuals were classified as having lupus according to the 2012 SLICC criteria (Petri et al., 2012).

Urine Samples are cultured at MacConkey and Blood agar and then incubated at 37°C, 18-24 hours. The bacteria were identified depending on bacteriological methods, such as characteristics and appearance of colonies on MacConkey agar and haemolysis on blood agar as well as using gram stain. Biochemical tests such as catalase and oxidase test were done. Then, bacterial isolates were identified by API E20 Kit (Bio-Merieux, France). Vitek-2 (Biomerieux). To confirm the identification, molecular techniques based on 16S rRNA were done.

Geneaid Biotech's (Taiwan) DNA extraction kit was utilized and carried out in accordance with the manufacturer's guidelines. Finally, a nano-droplet instrument was used to analyze the extracted DNA for concentration and purity at 260-280 nm wavelengths.

Success DNA was confirmed by running the DNA extracts on a 1% agarose gel stained with ethidium bromide dye at a voltage of 100 for 1 hour, then the results were shown for electrophoresis using an ultraviolet device at a wavelength of 320 nm (Russell and Sambrook, 2001).

The Kirby-Bauer disc diffusion method was used for the antibiotic susceptibility test, and the antibiotic used was determined by the standards set by the Clinical and Laboratory Standards Institute (CLSI). The antibiotic profile used is listed in Table 1. The colonies of isolated bacteria were inoculated on Mueller-Hinton Agar and the antibiotic discs were put on the surface of the medium and then incubated at a temperature of 37°C for 18-24 hours, the size of the inhibition zone is read on a millimeter, and compared with CLSI.

**Table 1. Antibiotics used in susceptibility test**

	Antibiotics	Abbreviation	Concentration (µg)
1.	Meropenem	MEM	10
2.	Imipenem	IPM	10
3.	Ceftriaxone	CRO	10
4.	Cefotaxime	CTX	30
5.	Gentamycin	CN	10
6.	Doxycycline	DO	10
7.	Ciprofloxacin	CIP	5
8.	Levofloxacin	LEV	5
9.	Trimethoprim	TMP	10

### Results and discussion

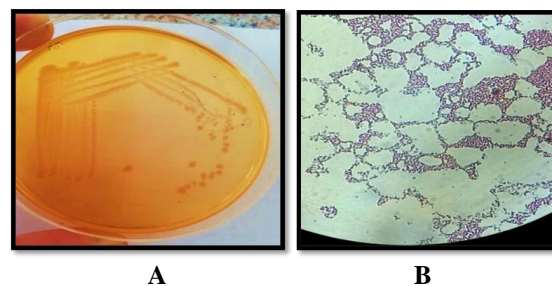
We identified 55 patients 91.7 % appeared to have positive bacterial growth from a total of 60 diagnosed SLE patients, 8 males, and 47 females. The sex ratio revealed a female predominance.

The urinary tract was the most commonly detected site of infection in SLE patients so according to several previous research. Per the reports, bacterial UTIs in those patients have a higher mortality rate. Immunocompromised medications and immunological failure in SLE increase the risk of bacterial infection, flare-ups, and other infectious consequences. Infections can also cause autoimmunity, which worsens the symptoms of SLE. Opportunistic or common bacterial infections with atypical or nonspecific symptoms are common in SLE patients (Dorgham et al., 2021; Li et al., 2020).

According to their phenotype and different method API 20E, Vitek -2, and Molecular technique based on 16S rRNA were used to diagnose bacterially isolated, we Identified

one *Pseudomonas luteola* 1.81% from positive growth of urine culture.

The detected *Pseudomonas luteola* appears as pale to dark yellow smooth colonies on MacConkey agar non-lactose fermenter Fig. 1A and Fig. 1B demonstrates that the gram stain of cultivated colonies revealed tiny Gram-negative rods.



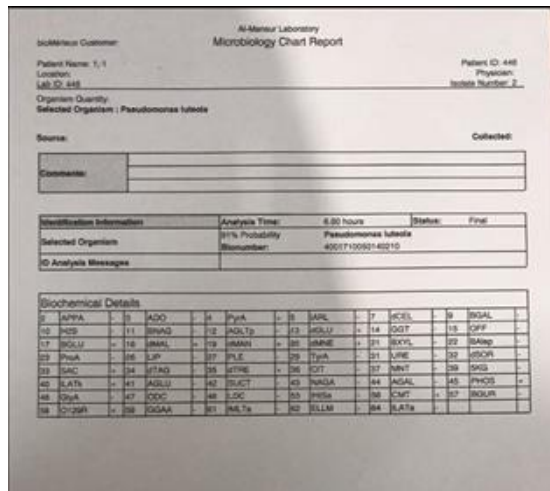
**Fig. 1 A: colonies of *Pseudomonas luteola* on MacConkey agar B: Cells shape of *Pseudomonas luteola* under microscope, 1000X**

**API 20E test and VITEC -2 System**

In this study, we used API 20E test and VITEC-2 system to identify *Pseudomonas luteola* isolate the result shows in Fig. 2 and 3.



**Fig. 2 API 20E test of *Pseudomonas luteola***



**Fig. 3 VITEC-2 result of *Pseudomonas luteola***

**Molecular technique**

Bacteria diagnosed using the molecular method based on 16S rRNA, then the PCR result was sent to Samogen Company in the USA for sequencing. The bacterial isolate showed a 95.05% affinity rate with *Pseudomonas luteola* strain FDAARGOS-637 Fig. 4.

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per Ident	Acc. Len	Accession
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain FDAARGOS_637 chromosome 1 complete sequence</a>	<i>Pseudomonas luteola</i>	1681	10089	95%	0.0	95.05%	4273365	CP044086.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain FDAARGOS_637 chromosome 2 complete sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	919958	CP044085.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain BMC2N21 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	1417	MG956847.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain SJ-49 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	1596	KC301304.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain BCFY_wv1 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	1432	ON202892.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain Pst P11 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	1490	MT045207.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain XFB-BV 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	1388	KC429633.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain NSRC_103146 16S ribosomal RNA partial sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	1462	NR_114215.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain 571 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	1399	HQ172812.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain Marseille 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1681	1681	95%	0.0	95.05%	1459	AF574876.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain RSP10 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1676	1676	95%	0.0	94.95%	1496	KF360652.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain J791 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1674	1674	95%	0.0	94.95%	1402	KY194291.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain J26 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1674	1674	95%	0.0	94.95%	1402	KY194228.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain ZSR24 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1672	1672	94%	0.0	95.18%	1374	KY436208.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain DT5-68 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1672	1672	95%	0.0	95.02%	1393	KM081563.1
<input checked="" type="checkbox"/> <a href="#">Pseudomonas luteola strain 8 16S ribosomal RNA gene partial sequence</a>	<i>Pseudomonas luteola</i>	1670	1670	95%	0.0	94.86%	1442	MN911372.1

**Fig. 4 BLAST result for *Pseudomonas luteola***

*Pseudomonas luteola* was previously known as *Chryseomonas luteola* Rod, non-lactose fermenter, oxidase negative. It was isolated for the first time in 1982 and for the second time in 2021 from patients with lupus erythematosus in Riyadh/Saudi Arabia (Barry, 2021). which are found in natural environments and aren't common as human pathogens, but some Cases have been reported of septicemia, pancreatitis, and endocarditis (Chihab et al., 2004; Alhalimi et al., 2022) reported that *P. luteola* causes disease in human respiratory failure at birth and urinary tract infections UTIs and this study agrees with our study.

**Antibiotic Susceptibility Test**

The results of *P. luteola* resistance against antibiotic in this study is listed in Table 2. The isolate of *P. luteola* shows the bacteria resistance to most antibiotics of the cephalosporine class, aminoglycosides, fluoroquinolones, and carbapenem. This result disagreed with (Otto et al., 2013) which showed that *P. luteola* was susceptible to most antibiotics and agreed with the previous study (Wen et al., 2013).

**Table 2. Antibiotic Susceptibility list**

Sr. No.	Antibiotic	Diameter of inhibition zones (mm)
1	Meropenem	R
2	Imipenem	I
3	Ceftriaxone	R
4	Cefotaxime	R
5	Gentamycin	R
6	Doxycycline	R
7	Ciprofloxacin	R
8	Levofloxacin	S
9	Trimethoprim	R

These results also noted the prevalence of the multidrug resistance (MDR) phenomenon in isolation. This term is applied to bacteria that are resistant to three or more antibiotics belonging to different classes of antibiotics (Santos et al., 2020; Sweeney et al., 2018).

There are two reasons that may explain the acquisition of multi-resistant bacteria. The first reason is a result of the presence of the antibiotic in the bacterial environment. It causes genetic mutations that lead to the conversion of bacteria from sensitive to resistant, or the acquisition of many resistance genes by bacteria.

The phenomenon of horizontal gene transfer through the presence of resistant plasmids (R-plasmids) or transposon genes, as bacteria may possess structural properties that enable them to naturally resist some antibiotics Gram-negative bacteria, which are distinguished from Gram-positive bacteria by the presence of the outer envelope of the cell wall, that stands as a barrier without permeability. Many antigens attach to the cell and thus confer the characteristic of resistance against these (Hasan and Al-Harmoosh, 2020).

### Conclusions

In this study, we highlighted unique bacteria as human pathogens such as *P. luteola* a New diagnostic bacteria that cause UTIs in SLE patients in Mosul city. The bacteria were detected using the Vitek-2 system and a molecular technique based on 16S rRNA.

### Conflict of Interest

The author hereby declares no conflict of interest.

### Funding support

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