

**How to Cite:**

Kamil, S. A., & Aldahhan, H. A. A. (2022). Analysis of *Proteus mirabilis* proteins by using SDS-page technique in rheumatoid arthritis patients: Comparative study. *International Journal of Health Sciences*, 6(S4), 3544–3552. <https://doi.org/10.53730/ijhs.v6nS4.9837>

## **Analysis of *Proteus mirabilis* proteins by using SDS-page technique in rheumatoid arthritis patients: Comparative Study**

**Shahad Ali Kamil**

Laboratory Investigations Department, Faculty of Science, University of Kufa, Najaf, Iraq

Corresponding author email: [shahadali101@gmail.com](mailto:shahadali101@gmail.com)

**Hawraa A Ali Aldahhan**

Laboratory Investigations Department, Faculty of Science, University of Kufa, Najaf, Iraq

Email: [hawraa.aldahan@uokufa.edu.iq](mailto:hawraa.aldahan@uokufa.edu.iq)

**Abstract**---This study was aimed to detecting difference in proteins separation by SDS-Page for *Proteus mirabilis* isolated from urine of non-Rheumatoid arthritis patients have urinary tract infection , urine from Rheumatoid arthritis patients have urinary tract infection. Two hundred (200) urine sample collected from non RA patient30 urine sample from RA patient. These clinical samples were collected and cultured on blood agar and macConky agar. 32 isolates were identified as *p. mirabilis* by using biochemical test and VITEK 2 system used to complete the identification. The current study used SDS-Page technique for proteins analysis of *Proteus mirabilis* for both RA patient and non RA patient to detect the differences between them and the result appeared that there are no differences in total protein, insoluble proteins and soluble protein at different pH. Values (6.0, 7.0 & 8.0) at 37 c°, also the result shown differences in the cell –free culture supernatants between *Proteus mirabilis* from urine of both rheumatoid arthritis patients and non-rheumatoid arthritis patient in pH.8.0 at 37 c° in 7 h. by band appeared in pH.8.0 at 37 c° in 7 h in RA .and there is no band in non RA at the same condition. In conclusion: *Proteus mirabilis* isolated from urinary tract infection of Rheumatoid arthritis patients shown difference in the cell –free culture supernatants in compare to *Proteus mirabilis* isolated from urinary tract infection of non-Rheumatoid arthritis patient at the same proteins separation conditions.

**Keywords**---SDS-page, rheumatoid arthritis, RA, *Proteus mirabilis*, proteins.

## Introduction

Rheumatoid arthritis (RA) is a chronic systemic inflammatory autoimmune disease that affects around 0.5–1% of the adult population globally. There are 20–50 occurrences per 100,000 each year, with the majority of cases occurring in women over the age of 40. RA is three times more frequent in women than in males, and it mostly affects middle-aged and older adults (Pretorius *et al.*, 2017). *Proteus mirabilis* is the third most frequent cause of urinary tract infection (after *E. coli* and *Klebsiella pneumoniae*), but it is the most dangerous because it causes catheter blockage, bladder and kidney stone development, cystitis, acute and chronic pyelonephritis, and bacteremia (Hasan *et al.*, 2020). *Proteus mirabilis* have many virulence factors play important roles In UTI development such as Toxins like hemolysin and its function of pore formation, biofilm and regulation of pathogenicity, Urease enzyme which causes kidney and bladder stones, and the MR/P fimbriae (Al-Duliami *et al.*, 2011; Schaffer and pearson., 2015) were among the virulence factors found in *Proteus mirabilis* that were important for inflicting UTIs. Exopolysaccharide synthesis and biofilm development are induced by adhesion to the catheter surface or bladder epithelium (Nicolle, 2014). Sera from rheumatoid patients exhibit higher levels of *Proteus mirabilis* IgM antibodies than those from other individuals (Hayder and Aljanaby, 2019b; Kadhum and Hasan, 2019). Through a molecular mimicry mechanism, *Proteus mirabilis* is hypothesized to contribute to the development of RA in susceptible individuals. The ESRRAL amino acid sequence, which is found in *P. mirabilis* haemolysin, and the EQRRAA sequence, which is found in the common epitope of the RA-linked HLADR molecules, have been found to exhibit molecular similarities. There is also a chemical resemblance between the IRRET amino acid sequence found in *Proteus mirabilis* urease enzyme and the LRREI amino acid sequence found in type XI collagen (Ebringer and Rashid ,2006). This study was aimed to detecting difference in proteins separation by SDS-Page for *Proteus mirabilis* isolated from urine from non-Rheumatoid arthritis patients have urinary tract infection , urine from Rheumatoid arthritis patients have urinary tract infection. In order to specialized the role of some bacterial proteins in RA occurrence

## Material and Methods

### Samples collection

A total of 230 urine samples samples were collected during the period from September (2021) to April (2022). These samples were ( 200 urine sample from non RA patient ,30 urine sample from RA patient. All these samples were collected from Al-Sadder Medical City, Al-Hakeem General Hospital. RA patients were identified by clinical and some laboratory analyses( RF, anti-ccp, CRP, and ESR).

### Identification of *Proteus mirabilis*

A cross-sectional study. Out of 230 samples, (32) isolates were identified as *Proteus mirabilis* by routine tests: colony morphology by culturing 37°C for 24 on (blood and macckongy agar) , biochemical tests including; catalase test, oxidase reaction, urease test and also VITEK 2 system used for more identification.

### Blood tests

A 5 millilitres of venous blood were collected, some of it putted into Erythrocyte sedimentation rate (ESR) tube and the remained putted in a clean plain tube and allowable to clot at 37 C° for 10 minutes (for ESR test). Then centrifugeing at 2500 rrpm for 5 mine. until serum separaeted. Serum is storeed at 20 C° until used for the other three tests (RF, CRP, and ACPA).

### SDS-Page

The material used in the SDS-page as in tables 1, 2 and 3.

Table 1: Component of resolving gel and stacking gel

SDS-Page	15%Resolving gel (ml)	5% Stacking gel (ml)
ddH <sub>2</sub> O	2.7	6.95
30% (w/v) Acrylamide	3.8	1.7
3 M Tris-HCl (pH 8.3)	0.95	-
0.5M Tris-HCl (pH 6.8)	-	1.25
10% (w/v) TEMED	0.02	0.02
10% (w/v) SDS	0.1	0.1

Table 2: Component of SDS buffer (5X SDS loading buffer and 1X SDS running buffer)

BUFFER	MATERIA	
5X SDS LOADING BUFFER	Tris-HCl pH 6.8	0.225 M
	Glycerol (v/v)	50%
	SDS (w/v)	5%
	bromophenol blue (w/v)	0.05%
	2-Mercaptoethanol	0.25 M
1X SDS RUNNING BUFFER	Tris	3 g
	SDS	1 g
	Glycine	14.4 g
	dH <sub>2</sub> O	1 liter

Table 3: Staining and destaining solutions

Material	Commassie Blue stain	Destain
Acetic acid	100 ml	100 ml
Methanol	400 ml	400 ml
Coomassie	1.15 g/l	
Brilliant Blue	-	-
dH <sub>2</sub> O to 1 liter		

## **Protein Screening by SDS PAGE**

### **Bacterial culturing**

Freshly prepared 50 ml of BHI or Nutrient broth in 250 ml conical flask was inoculated with 1% of overnight culture. Bacterial broth was left to grow at 37°C with 250 rpm. For total proteins, soluble and insoluble fractions, samples were taken at middle of log phase (7h). For secreted protein screening, samples of bacteria were taken at three different times; starts of log phase (5h), middle of log phase (7h), and at the highest point of log phase (9h). All conditions were taken for each pH values of 6.0, 7.0, and 8.0. All measurements were applied for three replicates and the average was taken.

### **Protein extraction and cell lysate preparation**

#### **Cell collection and storage**

One milliliter of cell culture was centrifuge at 4°C at 5000 rpm for 5 min. The cell pellet was washed twice with 1X phosphate buffer saline (PBS) (Solarbio). The pellet then was frozen at -20°C until use.

#### **Cell lysate preparation**

A 100 ul of 1X PBS was used to suspend the frozen cells, from this point until the end of procedure all conditions was done on ice. Cells was disrupted by sonication 3 cycles each 5 sec. Cells were sonicated on ice using the minimum amplitude value by Sonics Vibra Cell Ultrasonic Processor - VC130 sonicator. Then, the cell lysate was separated from cell debris by centrifugation at 13000 rpm for 30 min at 4°C using cooling centrifuge. The supernatant was separated using new eppendorf tubes, while the debris (non-soluble fractions of proteins) were suspended with 100 ul of 1X PBS.

#### **Acetone precipitation of secreted proteins**

The supernatant of the media was filtered first using 0.22 uM Millipore, and the proteins were precipitate according to the protocol of Acetone precipitation of proteins explained by (Thermo Scientific), as follows:

1. The required volume of acetone was cooled to -20°C.
2. Four times of the protein sample volume of cold (-20°C) acetone was added to the tube of protein sample.
3. The mixture was vortexed and incubated for 60 minutes at -20°C.
4. To precipitate the proteins, the mixture was spined for 10 minutes at 13,000 rpm.
5. The supernatant was disposed, and the acetone was let to be evaporated from the uncapped tube at room temperature for 30 minutes (over-dry pellet may not dissolve properly).
6. Buffer of 1X PBS was used to dissolve protein pellet.

### Protein quantification

The concentration of protein was measured first via 280 nm to roughly estimate protein concentration. Path length for most spectrometers is 1 cm. Concentration (mg/ml) = Absorbance at 280 nm divided by path length (cm.) Typically, a 10 ug was mixed with an appropriate amount of 5X SDS loading buffer and  $\beta$ -mercaptoethanol, and then was boiled for 5 minutes before loading on SDS-PAGE.

### Denaturing gel electrophoresis (SDS-PAGE)

The gel was prepared as described by (Laemmli, 1970). Both resolving and stacking gel were prepared as shown below. The glass plate sandwich was assembled using a holder, and then placed on a rack to pour the gel. The resolving gel was poured firstly between the glass plates, and  $\frac{1}{4}$  of the space was left for stacking gel. This space was filled with 50% isopropanol. Once the gel had polymerized, the stacking gel was poured after discarding the isopropanol and washing twice with water. A comb was inserted, and the gel was left for 20 min. Once the gel had polymerized, the comb was removed, and the gel was transferred to the electrophoresis tank filled with 1X SDS running buffer. Loading dye was prepared and mixed with samples at 1:5 ratio, boiled for 5 min at 100 °C and loaded separately in wells along with a protein ladder. Polypeptides were separated by electrophoresis for 1 h at 150 V. After that, the glass plates were removed, and the gel was stained with Commassie blue stain solution for 1 h. Then, the stain solution was removed, and the gel was de-stained overnight.

### Results and Discussion

In this study the proteins of *Proteus mirabilis* separated by using SDS – page technique. Figure 1 appeared SDS-PAGE analysis of (total protein, insoluble proteins, soluble protein) of Rheumatoid arthritis *P. mirabilis* isolate at different pH values .in which Protein band numbers were indicated on the gel at 37°C. Lane M: represents protein marker (kDa) bands on the left of gel, lanes 1-3: represent total protein, insoluble proteins, soluble protein) of Rheumatoid arthritis *P. mirabilis* strain at pH 6, lanes 4-6: represent total protein, insoluble proteins, soluble protein) of Rheumatoid arthritis *P.mirabilis* strain at pH 7, lanes 7-9: represent total protein, ,insoluble proteins, soluble protein) of Rheumatoid arthritis *P.mirabilis* strain at pH 8.from this figure there are no difference in the number of bands in each three phase and there were the same at the alkaline (pH 6.0), normal (pH7.0) and acidic(pH8.0) gel matrix. Figure 2 show the result of SDS-PAGE analysis of (total protein ,insoluble proteins, soluble protein) of non-Rheumatoid arthritis *P. mirabilis* isolate at the same conditions from the results in figure 2. From figures (1& 2) there were no differences in the bands between these two strains.

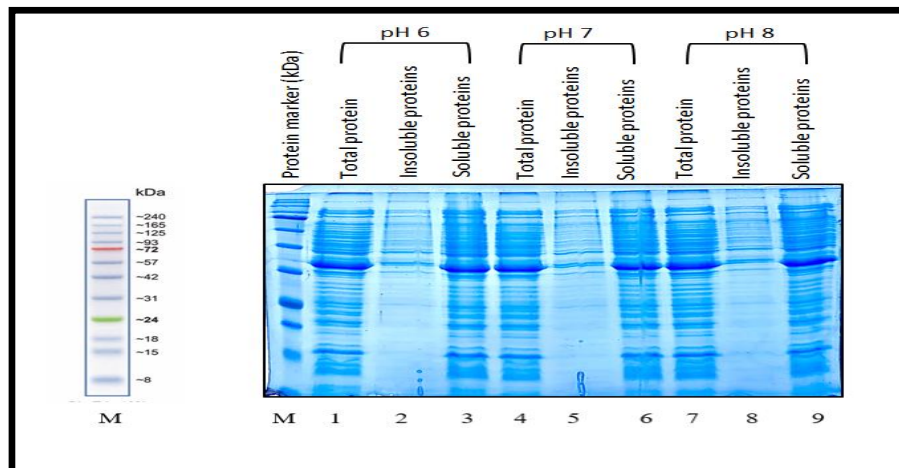


Figure 1: Gelatin-SDS-PAGE (15%) analysis of (total protein, insoluble proteins, soluble protein) of RA *P. mirabilis* isolate at 37 C in different pH (6.0, 7.0, 8.0)

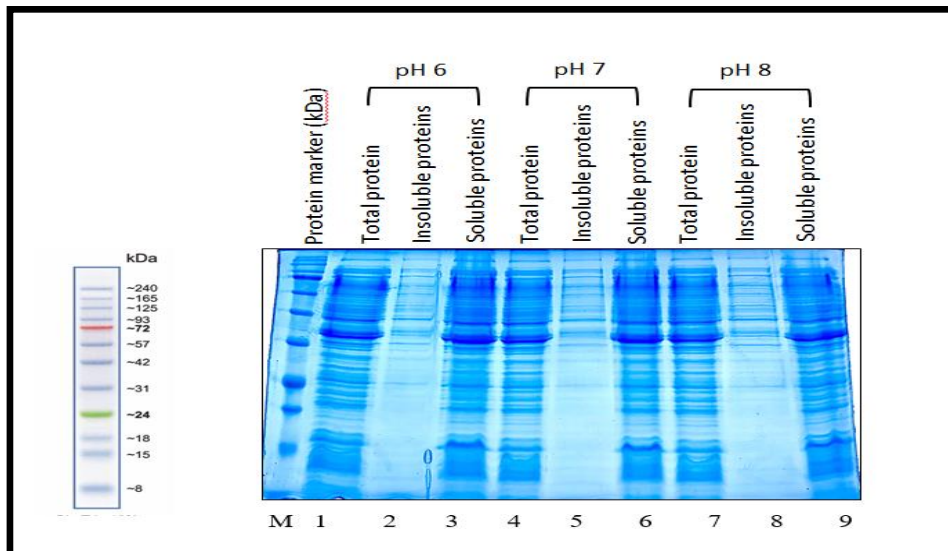


Figure 2: Gelatin-SDS-PAGE (15%) analysis of (total protein, insoluble proteins, soluble protein) of non RA *P. mirabilis* isolate at 37 C in different pH (6.0, 7.0, 8.0)

The results of SDS-PAGE for the cell-free culture supernatants of Rheumatoid arthritis *P. mirabilis* secreted proteins in nutrient broth growth media (NB) in three different pH concentration (6.0, 7.0, and 8.0) at three different time (5h, 7h, 9h) of growth curve were present in figure 3, three bands of secreted proteins were appeared in pH 6.0 at 9h, whereas two bands of proteins were at the same time in pH 7.0, two bands appeared in pH 8.0 at 9 h, one band appeared in pH 8.0 at 7 h, as pointed in stars in 3. Figure 4 appeared the results of SDS-PAGE for the cell-free culture supernatants non Rheumatoid arthritis *P. mirabilis*. Three proteins bands presented in PH 6.0 at 9 h. Two proteins bands in 7.0 pH at 9h. Two bands in pH 8.0 at 9 h from these result 9h is the best time for production deferent proteins in all pH. From these result there are difference in

the cell-free culture supernatants between *Proteus mirabilis* from urine of both rheumatoid arthritis patients and non-rheumatoid arthritis patient in pH.8.0 at 37 c° in 7 h. by band appeared in pH.8.0 at 37 c° in 7 h in RA .and there is no band in non RA at the same condition.

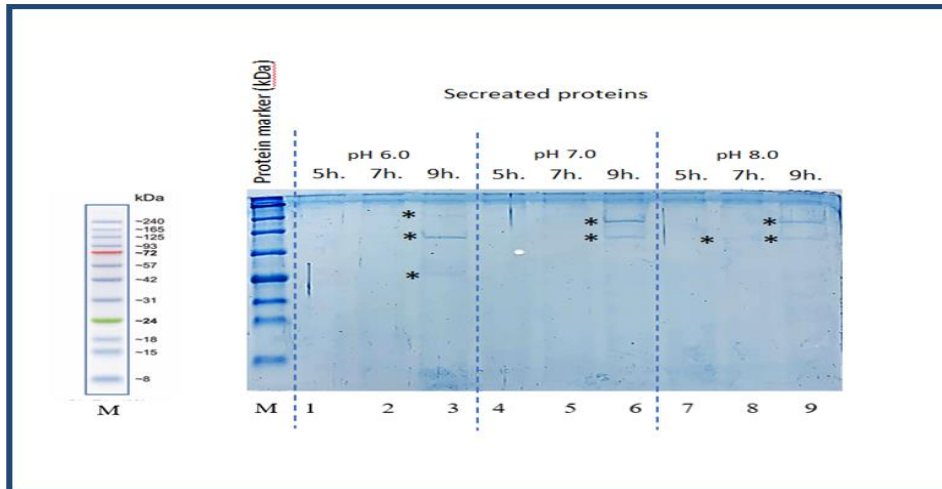


Figure 3: Gelatin- SDS-PAGE (15 %) analysis of (cell-free culture supernatants) for RA *P. mirabilis* isolate at 37 °c in different hours (5, 7, 9) and different pH (6.0, 7.0, 8.0)

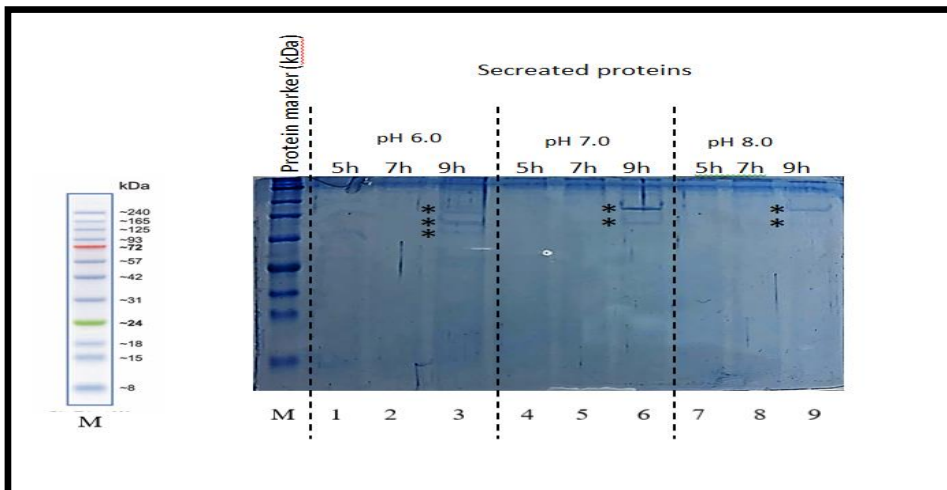


Figure 4: Gelatin- SDS-PAGE (15%) analysis of (cell-free culture supernatants) for non RA *P. mirabilis* isolate at 37 °c in different hours (5, 7, 9) and different pH (6.0, 7.0, 8.0)

Polyacrylamide gel electrophoresis is useful for separating molecules by size and charge and there are many different systems depending on the sample and downstream applications. SDS-PAGE is a very useful tool to separate protein molecules by size. SDS is a detergent that denatures secondary and non-disulfide-linked tertiary structures and coats them with a negative charge that

correlates with their length, allowing molecular weights to be estimated. The percentages of polyacrylamide can be optimized for the size range of molecules present in the sample. Gradient gels can be prepared allowing a greater range of separation in a single gel if both large and small proteins need to be resolved simultaneously. Small proteins will move through the resolving gel more quickly than large proteins. In a previous study Looms *et al.*, (1992) revealed that Analysis of cell lysates and culture supernatants of *P. mirabilis* for proteinase activity after electrophoresis on SDS-polyacrylamide-gelatin gels. Proteinase activity was first detected after 4 h of growth at 37°C, when a 56-kDa proteinase was detected only in cell lysates and a 54-kDa proteinase was detected only in culture supernatants. Over the next 4 h, the internal proteinase continued to be detected only in cell lysates, whereas the secreted proteinase was eventually replaced by two bands of proteinase of 53 and 50 kD. Piccini *et al.*,(1998) studied the SDS-PAGE OMPs profiles of *P. mirabilis* grown in iron-depleted medium and found that several proteins increased their expression under iron restriction compared to iron-replete condition (Piccini *et al.*, 1998). More recently, Lima *et al.* produced a *P. mirabilis* mutant by transposon mutagenesis that was unable to use heme as the unique iron source, and tracked the transposon interruption to an outer membrane heme receptor gene (Lima *et al.*, 2007).

## Conclusion

*Proteus mirabilis* isolated from urinary tract infection of Rheumatoid arthritis patients shown difference in the cell –free culture supernatants in compare to *Proteus mirabilis* isolated from urinary tract infection of non-Rheumatoid arthritis patient at the same proteins separation conditions.

## References

- Al-Duliami, A. A., Nauman, N. G., Hasan, A. R. S., and Al-Azawi, Z. H., 2011. Virulence factors of *Proteus Mirabilis* isolated from patients otitis media in Baquba and it's peripheries. *Diyala Journal of Medicine*, 1(1), pp.69-75.
- Ebringer, A. and Rashid, T., 2006. Rheumatoid arthritis is an autoimmune disease triggered by *Proteus* urinary tract infection. *Clinical and Developmental Immunology*, 13(1), pp.41-48.
- Ebringer, A., Rashid, T. and Wilson, C., 2010. Rheumatoid arthritis, *Proteus*, anti-CCP antibodies and Karl Popper. *Autoimmunity Reviews*, 9(4), pp.216-223.
- Gibofisky, A., 2014. Epidemiology, pathology and diagnosis of rheumatoid arthritis :Asynopsis. *The American journal of managed care*, 20(7 suppl ), pp.S128-35.
- Goronzy, J. and Weyand, C., 2009. Developments in the scientific understanding of rheumatoid arthritis. *Arthritis Research & Therapy*, 11(5), p.249.
- Hasan,T.H.; Al-Harmoosh, R. A. and Al-khilkhali, H. J. B., 2020. Identification of HIV virus in Najaf city, Iraq. *International Journal of Research in Pharmaceutical Sciences*, 11(3), pp.4866-4871.
- Hayder, T. and Aljanaby, A.A.J., 2019. Genotypic Characterization of Antimicrobial Resistance-Associated Genes in *Citrobacter Freundii* Isolated from Patients with Urinary Tract Infection in Al-Najaf Governorate-Iraq. *OnLine Journal of Biological Sciences*, 19(2), pp.132-145.

- Kadhum, H.A. and Hasan, T.H., 2019. The study of bacillus subtilis antimicrobial activity on some of the pathological isolates. *International Journal of Drug Delivery Technology*, 9(2), pp.193-196.
- Laemmli, U. K., 1970. SDS-page Laemmli method. *Nature*, 227, pp. 680-685.
- Loomes, L.M., Senior, B.W. and Kerr, M.A., 1992. Proteinases of *Proteus* spp.: purification, properties, and detection in urine of infected patients. *Infection and immunity*, 60(6), pp.2267-2273.
- Lorenz, W., Buhmann, C., Mobasheri, A., Lueders, C. and Shakibaei, M., 2013. Bacterial lipopolysaccharides form procollagen-endotoxin complexes that trigger cartilage inflammation and degeneration: implications for the development of rheumatoid arthritis. *Arthritis Research & Therapy*, 15(5), p.R111.
- Nicolle, L.E., 2014. Antimicrobial stewardship in long term care facilities: what is effective?. *Antimicrobial resistance and infection control*, 3(1), pp.1-7.
- Piccini, C.D., Barbe, F.M. and Legnani-Fajardo, C.L., 1998. Identification of iron-regulated outer membrane proteins in uropathogenic *Proteus mirabilis* and its relationship with heme uptake. *FEMS microbiology letters*, 166(2), pp.243-248.
- Pretorius, E., Akeredolu, O., Soma, P. and Kell, D., 2017. Major involvement of bacterial components in rheumatoid arthritis and its accompanying oxidative stress, systemic inflammation and hypercoagulability. *Experimental Biology and Medicine*, 242(4), pp.355-373.
- Rashid, T. and Ebringer, A., 2007. Rheumatoid arthritis is linked to *Proteus*—the evidence. *Clinical Rheumatology*, 26(7), pp.1036-1043.
- Schaffer, J.N. and Pearson, M.M., 2017. *Proteus mirabilis* and urinary tract infections. Urinary Tract Infections: *Molecular Pathogenesis and Clinical Management*, pp.383-433.
- Widana, I.K., Sumetri, N.W., Sutapa, I.K., Suryasa, W. (2021). Anthropometric measures for better cardiovascular and musculoskeletal health. *Computer Applications in Engineering Education*, 29(3), 550-561. <https://doi.org/10.1002/cae.22202>