Morphological and molecular identification of multi-antibiotic resistant bacteria in the wound site of diabetic ulcers

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Abstract. Risqiyyah W, Narulita E, Rofiqoh A, Ludfi AS, Iqbal M. 2022. Morphological and molecular identification of multi-antibiotic resistant bacteria in the wound site of diabetic ulcers. Biodiversitas 23: 663-670. The study aimed to determine the characteristics of diverse species of bacteria found in diabetic ulcers and the inhibition of antibiotics against these bacteria. The method used is Gram staining for morphological characterization, identification using 16S rRNA gene, and pour plate for antibiotic resistance test. The morphological characterization result showed that the colonies had white color, circular shape, flat elevation, entire edge, small size, and basal shape with Gram negative type of bacteria. Another result showed yellow, greenish, pelliculid, and green color, irregular shape, raised elevation, undulate and lobate edge, medium size, coccus and Gram positive type of bacteria. Query cover of molecular identification showed 73-100% and 77.61-96.77% for the result of similarity identification with Alcaligenes faecalis, Shigella flexneri, Enterococcus faecalis, Proteus mirabilis, and Acinetobacter seohaensis. The identification and antibiotic resistance testing showed that the bacterial species found in diabetic ulcers was A. faecalis strain NRBC 13111, which was resistant to all tested antibiotics, except A. faecalis strain NRBC 13111 from the UB 2.3K and A. faecalis strain NRBC 13111 from the sample UB 3.4M. Enterococcus faecalis strain ATCC 19433 was resistant to ceftriaxone, ceftazidime, clindamycin and metronidazole, intermediates to cefoperazone, and sensitive to ciprofloxacin. Proteus mirabilis strain JCM 1669 was resistant to clindamycin and metronidazole, sensitive to ceftriaxone and ciprofloxacin, intermediates to ceftazidime and cefoperazone. Proteus mirabilis strain ATCC 29906, A. seohaensis strain SW-100 and S. flexneri strain ATCC 29903 were resistant to all tested antibiotics.

Keywords: Alcaligenes faecalis, antibiotics, diabetic ulcers, resistance

INTRODUCTION

Diabetes is characterized by a state of hyperglycemia that can occur due to decreased insulin secretion or impaired insulin activity. Diabetes mellitus is classified into type 1 diabetes mellitus, known as insulin dependent, in which the pancreas fails to produce insulin characterized by a lack of insulin production and type 2 diabetes mellitus, known as non insulin dependent, due to the body’s inability to effectively use the insulin produced by the pancreas. Diabetes mellitus which accounts for 90% of cases worldwide is type 2 diabetes which is known as non-insulin dependent (Sudoyo et al. 2007). The number of people with diabetes in the world was 463 million in 2019, Indonesia ranks seventh in the world with around 10.7 million people with diabetes and is predicted to increase to 16.2 million in 2045 (International Diabetes Federation 2019). East Java Province ranks the ninth highest diabetes prevalence in Indonesia (Kominfo 2015). One of the districts with quite high cases of diabetes 2 is Jember district as evidenced by data on the number of patient visits in 2015 (Dinkes Jember 2015) and increased from January to December 2016, reaching 10,941 (Sasmita et al. 2019). The prevalence of diabetes continues to increase in several districts in the district of Besuki (Riskesdas 2018). The highest number of diabetes mellitus cases was at the Putrang Public Health Center which had 371 cases (Sasmita et al. 2019). It is known that the prevalence of diabetes mellitus in Banyuwangi Regency from 2013-2018 has increased, from 1.5% to 2%. Bondowoso Regency also increase from 1% in 2013 to 1.5% in 2018. Meanwhile, Situbondo Regency increased from 2% in 2013 to 3% in 2018 (Riskesdas 2018). This triggers an increasing number of patients makes an ulcer complications of peripheral neuropathy complications and it causes a damage to nerve cells and blood vessels (Ghotaswii et al. 2018). One of the contributing factors is its control against bacterial infections (Rahmadilyani and Muhlisin 2008). Bacterial infections occur due to high glucose levels which are strategic places for various bacteria to breed (Sri and Setyawan 2016), such
as *Staphylococcus aureus* and non-haemolytic *Staphylococcus* sp. (Donastin et al. 2019). Control of bacterial growth in diabetic ulcer patients is generally given empirically (Donastin et al. 2019). One thing that needs to be considered in choosing empiric therapy is the type of bacteria (Katarnida 2014). The type of antibiotic used to treat infection must be appropriate and wise because the microorganisms that infect patients with diabetic ulcers are very diverse. The precise use of antibiotics in treatment will provide better therapeutic results, reduce the number of antibiotic resistance, reduce the incidence of amputations, and reduce mortality rates (Sari et al. 2018). Therefore, there is a need for further studies on bacterial resistance found in diabetic ulcer patients to antibiotics commonly prescribed by doctors such as ceftriaxone (Agistia et al. 2017), ceftazidime, cefoperazone, metronidazole, ciprofloxacin (Sari et al. 2018) and clindamycin (Sugiyono and Padmasari 2019) to determine their inhibition against bacteria. Hence, identifying macro and micro is very necessary to know the characteristics and species of bacteria (Rinanda 2011). The present study deals with morphological and molecular identification of multi-antibiotic resistant bacteria in the wound site of diabetic ulcers.

**MATERIALS AND METHODS**

**Isolation of bacteria**

Bacteria were isolated from sample of patients with diabetic ulcers at Dr. Soebandi Hospital and Diabetics Clinic at Jember, East Java, Indonesia and performed wound care with inclusion criteria, having ulcer grade I or II. Isolated bacteria were grown onto nutrient agar (NA) plate, then continued grown onto maltose salt agar (MSA), eosin methylene blue agar (EMBA) and King’s B medium (Table 1). Observations on macro and micro characteristics was determined based on color, colony shape, elevation, edge, size, and shape/type of Gram bacteria (Table 2). tests for antibiotic resistance of bacteria isolated from diabetic ulcers were carried out using disk paper, six antibiotics with 3 replications, and ddH2O as a negative control.

Based on the result of bacterial growth on three kinds of medium, sample UB 1.1- UB 5.8 grow well on maltose salt agar, eosin methylene blue agar and King’s B agar, and the other samples grow on two or one kind of medium with different colors each on medium.

**Isolation and extraction of Bacterial DNA**

The bacteria were grown in nutrient broth (NB) medium for 24 hours at 37°C which then 1000 µL aliquot was retrieved and centrifuged at 4°C, 1500 rpm for 10 minutes. The obtained supernatant contained DNA transferred to other eppendorf tubes, added with 180µL digestion buffer, 20µL proteinase K and mixed by vortex. Two hundred microliters lysis/binding buffer was added then. Five hundred microliters volumes of cold absolute ethanol and washing buffer were added, then centrifuged for 1 minute in 1000 g. The pellet was dried and resuspended in 25-200 µL elution buffer (Zimbro et al. 2009).

**DNA amplification and quantification**

The 16S rRNA genes were amplified by using the universal primer of 27F 5'- GAG AGT TTG ATC CTG GCT CAG -3' and 1495R 5'- CTA CGG CTA CCT TGT TAC GA -3'.The first PCR amplification process was conducted using gradient temperature to obtain optimum temperature for each primer pairs. The gradient temperature setting was based on melting temperature (Tm) of each primer at 5°C below of Tm with 35 cycles. The PCR condition was pre-denatured at 95°C for 1 minute, denaturation at 94°C for 45 seconds, followed by 35 cycles annealing temperature at 53°C for 30 seconds, and extension at 72°C for 2 minutes, and final extension at 72°C for 10 seconds (Sunar et al. 2014). The amplification products were separated on 1.2% agarose gel stained with 3 µg/mL ethidium bromide using 1kb DNA ladders as molecular-weight size marker (Tilahun et al. 2018).

**Table 1.** Bacterial growth on maltose salt agar, eosin methylene blue agar, and King’s B agar medium

| Sample | Maltose salt agar | Eosin methylene blue agar | Kings’ B agar |
|--------|------------------|---------------------------|---------------|
| UB 1.1 | White            | Pink Purplish             | White         |
| UB 1.2 | White            | Pink Purplish             | Pellucid      |
| UB 2.3 | White            | Pink purplish             | Pellucid      |
| UB 3.4 | White            | Pink purplish             | White         |
| UB 4.5 | White            | Pink purplish             | White         |
| UB 4.6 | White            | Pink Purplish             | Pellucid      |
| UB 5.7 | White            | Pink Purplish             | Pellucid      |
| UB 5.8 | White            | Pink Purplish             | Pellucid      |
| UB 6.15| White            | Pellucid                  | -             |
| UB 6.16| -                | -                         | Pellucid      |
| UB 7.17| -                | -                         | Pellucid      |
| UB 7.18| -                | -                         | Pellucid      |
| UB 8.9 | Yellow           | -                         | -             |
| UB 8.10| Yellow           | -                         | -             |
| UB 9.11| -                | Purplish pink             | -             |
| UB 10.12| -               | Purplish pink             | -             |
| UB 11.13| Putih           | Pellucid                  | -             |
| UB 12.14| White           | Pellucid                  | -             |
| UB 13.19| -                | -                         | Pellucid      |
| UB 13.20| -                | -                         | Pellucid      |
| UB 14.21| -                | -                         | Pellucid      |
| UB 14.22| -                | -                         | Pellucid      |

Note: - (not growing).
Table 2. Macroscopic and microscopic bacterial morphological analyses

| Sample  | Color  | Colony shape | Macro characteristics | Cell shape/type of gram bacteria |
|---------|--------|--------------|-----------------------|----------------------------------|
| UB 1.1M | Yellow | Circular     | Flat                  | Entire Small Coccus (GN)         |
| UB 1.1E | White  | Circular     | Raised               | Entire Small Coccus (GN)         |
| UB 1.1K | White  | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 1.2M | White  | Circular     | Raised               | Entire Medium Bacilli (GN)       |
| UB 1.2E | White  | Circular     | Flat                 | Entire Medium Coccus (GN)        |
| UB 1.2K | White  | Circular     | Flat                 | Entire Medium Coccus (GN)        |
| UB 2.3M | White  | Circular     | Flat                 | Entire Small Coccus (GN)         |
| UB 2.3E | White  | Circular     | Raised               | Entire Point Bacilli (GN)        |
| UB 2.3K | White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 3.4M | White  | Circular     | Flat                 | Entire Medium Bacilli (GN)       |
| UB 3.4E | White  | Circular     | Raised               | Entire Small Coccus (GN)         |
| UB 3.4K | White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 4.5M | White  | Circular     | Flat                 | Entire Medium Bacilli (GN)       |
| UB 4.5E | White  | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 4.5K | White  | Circular     | Raised               | Entire Medium Bacilli (GN)       |
| UB 4.6M | Yellow | Circular     | Flat                 | Entire Small Coccus (GN)         |
| UB 4.6E | Yellow | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 4.6K | White  | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 5.7M | White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 5.7E | White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 5.7K | White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 5.8M | White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 5.8E | White  | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 5.8K | White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 6.15M| White  | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 6.15E| White  | Circular     | Raised               | Entire Medium Bacilli (GN)       |
| UB 6.16K| White  | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 7.17K| White  | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 7.18K| White  | Circular     | Raised               | Entire Medium Bacilli (GN)       |
| UB 8.9M | Golden | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 8.10M| White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 9.11E| White  | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 10.12E| White | Circular     | Flat                 | Entire Small Coccus (GN)         |
| UB 11.13M| Green | Circular     | Raised               | Entire Small Bacilli (GN)        |
| UB 12.14M| Green | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 12.14E| Yellow| Circular     | Raised               | Entire Point Bacilli (GN)        |
| UB 13.19K| White | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 13.20K| White | Circular     | Flat                 | Entire Small Bacilli (GN)        |
| UB 14.21K| Pellucid| Circular | Flat                 | Entire Small Bacilli (GN)        |
| UB 14.22K| White | Irregular    | Raised               | Entire Lobate Bacilli (GN)       |

Note: GN- Gram negative; GP- Gram Positive.

RESULTS AND DISCUSSION

The selected samples were UB 1.2E, UB 2.3K, UB 3.4, and UB 4.5M showed the similarities with *Alcaligenes faecalis* strain NRBC 13111 observed by morphological characteristics in the form of *Bacillus*, including Gram-negative thype of bacteria (Table 2). Thats four bacterial samples had different characters from other samples, which were both grown in white on MSA medium, purplish-pink on EMBA, and on white King’s B (Tabel 1). *Alcaligenes faecalis* strain NRBC has a tolerance to high salt content of almost 10%. However, the growth was not as much as when the salt concentration was below 7% (Suhartati et al. 2018). *Alcaligenes faecalis* can grow in EMBA differential selective medium. EMBA contains eosin Y as a pH indicator and inhibits the growth of Gram-positive bacteria. The inability of *A. faecalis* to ferment sucrose and glucose makes the colony color purplish pink (Omer et al. 2017). This medium is used to confirm the presence or absence of *Pseudomonas aeruginosa* based on its fluorescence. A positive result indicates the presence of luminescence green-yellow fluorescent pigment on *Pseudomonas aeruginosa* when placed under a UV lamp with a wavelength of 366 nm (Quinn et al. 2004). Case infection *A. faecalis* in diabetic ulcers has rarely been reported in the literature. There were only 4 cases worldwide in 1952, 1997, 2019, and 2020 (Sommeng et al. 2019). Based on the molecular identification showed the similarities until 96.77% with *A. faecalis* strain NRBC 13111 (Table 3).

Sample UB 6.15E is known to have similarities with *Shigella flexneri* strain ATCC 29903 observed by molecular identification (Table 3). *Shigella flexneri* is a Gram-negative Bacilli. Based on the results of macroscopic morphological observations, *S. flexneri* also grew on white
MSA medium and clear colored EMBA medium. The growth of \textit{S. flexneri} on MSA medium is due to \textit{S. flexneri} having a tolerance of salt levels up to 8% to still survive on MSA medium (Huang 2020). \textit{Shigella flexneri} is a bacteria that cannot ferment lactose, so when grown in EMBA medium, it will be clear or colorless. \textit{Shigella flexneri} grown dimedium time EMBA, large colony size and colorless (Zaika et al. 2002). In general, \textit{S. flexneri} is a bacterium that does not have flagella, is aerobic, does not form spores, causes diarrhoea and dysentery. Its habitat is in the digestive tract with infection through the mouth. The morphological characters were clear colony color, raised elevation, entire edge, medium colony size, and smooth surface. This is in accordance with the observations that have been made (Table 2). \textit{Shigella flexneri} has also been found in the bloodstream of patients with uncontrolled diabetes mellitus. If these bacteria survive long enough in large numbers, it can cause serious infections (Power and Johnson 2009).

Sample UB 7.17K (\textit{Enterococcus faecalis}) was a facultative anaerobe, had a white colony color, around colony shape, a smooth surface (Khamid and Mulasari 2012). This was also suitable for observing morphological results (Table 4). Samples UB 11.13M and UB 12.14M based on molecular identification were similar to \textit{Proteus mirabilis} bacteria but different strains. UB 11.13M was similar to \textit{P. mirabilis} strain JCM 1669 while UB 12.14M was similar to \textit{P. mirabilis} strain ATCC 29906. \textit{Proteus mirabilis} was a Gram-negative Bacilli bacteria (Table 2). Sample UB 13.20K was known to have similarities with \textit{Acinetobacter seohaensis} strain SW-100 (Table 3), with morphological characteristic was a Gram negative-Bacilli bacteria (Table 2).

The antibiotics resistance test results showed that all \textit{A. faecalis} strain NRBC 13111 were resistant to all tested antibiotics. \textit{Enterococcus faecalis} strain ATCC 19433 and \textit{P. mirabilis} strain JCM 1669 are resistant, intermediate and sensitive to several antibiotics. \textit{Proteus mirabilis} strain ATCC 29906, \textit{A. seohaensis} strain SW-100 and \textit{S. flexneri} strain ATCC 29903 were resistant to all the antibiotics tested (Table 4) (Figure 1).

Sample UB 7.17K is similar to bacteria \textit{E. faecalis} strain ATCC 19433. \textit{Enterococcus faecalis} is coccius-shaped and includes Gram positive bacteria. The growth of \textit{E. faecalis} on King’s B medium other microbes could be grown other then \textit{Pseudomonas aeruginosa}. \textit{Enterococcus faecalis} also did not grow in MSA medium because the tolerance limit for NaCl was 6.5% only (Ninan et al. 2016). \textit{Enterococcus faecalis} is a facultative anaerobe, has white colony color, rounded colony shape, smooth surface (Ahmad et al. 2002). This is also in accordance with the observations (Table 2). \textit{Enterococcus faecalis} can grow at a high pH of 4.8-9.6, with no spores, and non-motile. These bacteria can cause urinary tract infections and are found in diabetic ulcers, but their abundance is about 8% (Khamid et al. 2012).

**Table 3. Molecular identification of bacteria causing diabetic ulcers**

| Sample   | Species                                | Max score | Query cover (%) | E-value | Identity (%) | Accession number | Length (bp) |
|----------|----------------------------------------|-----------|-----------------|---------|--------------|-----------------|-------------|
| UB 1.2E  | \textit{Acaligenes faecalis} strain NRBC 13111 | 1956      | 100             | 0.0     | 96.77        | NR_113606.1     | 1462        |
| UB 2.3K  | \textit{Acaligenes faecalis} strain NRBC 13111 | 2132      | 99              | 0.0     | 95.15        | NR_113606.1     | 1462        |
| UB 3.4M  | \textit{Acaligenes faecalis} strain NRBC 13111 | 422       | 73              | 3e-117  | 77.61        | NR_113606.1     | 1462        |
| UB 4.5M  | \textit{Acaligenes faecalis} strain NRBC 13111 | 1884      | 97              | 0.0     | 90.86        | NR_113606.1     | 1462        |
| UB 6.15E | \textit{Shigella flexneri} strain ATCC 29903 | 1803      | 100             | 0.0     | 91.80        | NR_026331.1     | 1530        |
| UB 7.17K | \textit{Enterococcus faecalis} strain ATCC 19433 | 1229      | 90              | 0.0     | 83.97        | NR_115765.1     | 1483        |
| UB 11.13M| \textit{Proteus mirabilis} strain JCM 1669 | 2100      | 96              | 0.0     | 95.46        | NR_113344.1     | 1465        |
| UB 12.14M| \textit{Proteus mirabilis} strain ATCC 29906 | 915       | 90              | 0.0     | 85.24        | NR_114419.1     | 1497        |
| UB 13.20K| \textit{Acinetobacter seohaensis} strain SW-100 | 1960      | 99              | 0.0     | 92.20        | NR_115299.1     | 1493        |

**Table 4. Measurement results of antibiotic clear zone against bacteria that cause diabetic ulcer**

| Sample | Antibiotics treatment | Paper disk conc. * (µg/mL) | Inhibition zone criteria | Average (mm) | Result |
|--------|-----------------------|----------------------------|--------------------------|--------------|--------|
|        |                       | S            | I               | R            |        |
| \textit{Acaligenes faecalis} strain NRBC 13111 | CAX        | 30           | ≥ 23            | 20-22        | ≤ 19   | 0      | R      |
| (UB 1.2E) | CAZ | 30           | ≥ 21            | 18-20         | ≤ 17   | 0      | R      |
|         | CFP        | 75           | ≥ 21            | 16-20         | ≤ 15   | 0      | R      |
|         | CDM        | 2            | ≥ 21            | 15-20         | ≤ 14   | 0      | R      |
|         | MTZ        | 44           | ≥ 21            | 16-21         | ≤ 16   | 0      | R      |
|         | CP         | 5            | ≥ 21            | 16-20         | ≤ 15   | 0      | R      |
|         | K(-)/ddH₂O | -            | -               | -             | 0      | -      | -      |
|                      | CAX | CFP | CDM | MTZ | CP | K(-)/ddH2O |
|----------------------|-----|-----|-----|-----|----|------------|
| *Alcaligenes faecalis* strain JCM 1669 (UB 2.3K) | 30  | 75  | 2   | **5** | 5  | -          |
|                     | ≥ 23| ≥ 21| ≥ 21| ≥ 21| ≥ 21| -          |
|                     | 20-22 | 16-20 | 15-20 | 16-21 | 16-20 | ≤19        |
|                     | 14.83 | 12.06 | 0    | 0    | 0    | R          |
| *Alcaligenes faecalis* strain JCM 13111 (UB 3.4M) | 30  | 75  | 2   | **5** | 5  | -          |
|                     | ≥ 23| ≥ 21| ≥ 21| ≥ 21| ≥ 21| -          |
|                     | 20-22 | 16-20 | 15-20 | 16-21 | 16-20 | ≤19        |
|                     | 21.86 | 10.4 | 0    | 0    | 0    | R          |
| *Shigella flexneri* strain ATCC 29903 (UB 6.1SE) | 30  | 75  | 2   | **5** | 5  | -          |
|                     | ≥ 23| ≥ 21| ≥ 21| ≥ 21| ≥ 21| -          |
|                     | 20-22 | 16-20 | 15-20 | 16-21 | 16-20 | ≤19        |
|                     | 7.56  | 2.83  | 0    | 0    | 0    | R          |
| *Enterococcus faecalis* strain ATCC 19433 (UB 7.17K) | 30  | 75  | 2   | **5** | 5  | -          |
|                     | ≥ 23| ≥ 21| ≥ 21| ≥ 21| ≥ 21| -          |
|                     | 20-22 | 16-20 | 15-20 | 16-21 | 16-20 | ≤19        |
|                     | 14.33 | 2.83  | 0    | 0    | 0    | R          |
| *Proteus mirabilis* strain JCM 1669 (UB 11.13M) | 30  | 75  | 2   | **5** | 5  | -          |
|                     | ≥ 23| ≥ 21| ≥ 21| ≥ 21| ≥ 21| -          |
|                     | 20-22 | 16-20 | 15-20 | 16-21 | 16-20 | ≤19        |
|                     | 26.23 | 4.23  | 0    | 0    | 0    | S          |
| *Proteus mirabilis* strain ATCC 29906 (UB 12.14M) | 30  | 75  | 2   | **5** | 5  | -          |
|                     | ≥ 23| ≥ 21| ≥ 21| ≥ 21| ≥ 21| -          |
|                     | 20-22 | 16-20 | 15-20 | 16-21 | 16-20 | ≤19        |
|                     | 17.97 | 12.93 | 0.38 | 0    | 0    | R          |
| *Acinetobacter seohaensis* strain SW-100 (UB 13.20K) | 30  | 75  | 2   | **5** | 5  | -          |
|                     | ≥ 23| ≥ 21| ≥ 21| ≥ 21| ≥ 21| -          |
|                     | 20-22 | 16-20 | 15-20 | 16-21 | 16-20 | ≤19        |
|                     | 0    | 0    | 0    | 0    | 0    | R          |

Note: *Standard of antibiotic concentration according to (CLSI 2017); ** Concentration according to (Fabanyo et al. 2017).
Figure 1. Antibiotics resistance: A. *Alcaligenes faecalis* strain NRBC 13111 (UB 1.2E); B. *Alcaligenes faecalis* strain NRBC 13111 (UB 2.3K); C. *Alcaligenes faecalis* strain NRBC 13111 (UB 3.4M); D. *Alcaligenes faecalis* strain NRBC 13111 (UB 4.5M); E. *Shigella flexneri* strain ATCC 29903 (UB 6.15E); F. *Enterococcus faecalis* strain ATCC 19433 (UB 7.17K); G. *Proteus mirabilis* strain JCM 1669 (UB 11.13M); H. *Proteus mirabilis* strain ATCC 29906 (UB 12.14M); I. *Acinetobacter seohaensis* strain SW-100 (UB 13.20K).

(1) CAX; (2) CAZ; (3) CFP; (4) CDM; (5) MTZ; (6) CP

UB 11.13M and UB 12.14M samples have similarities with bacteria *P. mirabilis* but different strains. UB 11.13M looks like *P. mirabilis* strain JCM 1669 while UB 12.14M has similarities with *P. mirabilis* strain ATCC 29906. *Proteus mirabilis* is a Gram-negative bacterium in the form of a bacilli, does not form spores, is facultatively anaerobic, moves with flagella and is a pathogenic bacterium in the intestines of both humans and animals. This bacterium is often found in wound infections and is a urogenital pathogen. This is because *P. mirabilis* has a virulent gene that causes it to become pathogenic. The presence of *P. mirabilis* in diabetic ulcers is quite high at 17.5% (Putri et al. 2018).

Sample UB 13.20K is known to have similarities with *A. seohaensis* strain SW-100. This bacterium is a gram negative bacterium in the form of a bacillus. This *A. seohaensis* can be isolated from seawater (Nur et al. 2016) and there are no studies previously found *A. seohaensis* in diabetic ulcers or wound infections. This can happen because a diabetic ulcer is a strategic place for the proliferation of various kinds of bacteria so that when the ulcer is still in the treatment process, it can be contaminated with bacteria from the air, surrounding objects and from the closest people, especially if the ulcer is not bandaged for a long time.

The types of bacteria found in diabetic ulcers may vary and differ in each region because bacteria are microorganisms that are easy to mutate so that they can form new strains with different characteristics such as resistance to antibiotics. This is evidenced by the results of antibiotic resistance tests (Table 4) and (Figure 1) showing that *A. faecalis* strain NRBC 13111 is known to be resistant to all the antibiotics tested, except *A. faecalis* strain NRBC 13111 from UB sample 2.3K which is sensitive to ciprofloxacin and *A. faecalis* strain NRBC 13111 derived from UB sample 3.4M intermediate to ceftriaxone. This is reinforced by the case of diabetic ulcers with the infection *A. faecalis* in 2019 and known that *A. faecalis* was resistant to antibiotics ciprofloxacin, ceftriaxone, and ceftazidime (Ahmad et al. 2002).

*Shigella flexneri* strain ATCC 29903 is known to be resistant to all antibiotics which was tested and
strengthened by research conducted by Ninan et al. 2016. *S. flexneri* was resistant to cefazidime and ceftriaxone. *Enterococcus faecalis* strain ATCC 19433 is resistant to ceftriaxone, cefazidime, clindamycin and metronidazole, intermediate to cefoperazone, and sensitive to ciprofloxacin. *Proteus mirabilis* strain JCM 1669 and *P. mirabilis* strain ATCC 29906 had different results even though the bacteria were the same. *Proteus mirabilis* strain JCM 1669 is resistant to clindamycin and metronidazole, sensitive to ceftriaxone and ciprofloxacin, intermediate to cefazidime and cefoperazone. *Proteus mirabilis* strain ATCC 29906 *A. seohuensis* strain SW-100 and *S. flexneri* strain ATCC 29903 were resistant to all tested antibiotics.

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