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Sphingomonas Paucimobilis Bacteria Isolation From Different Clinical Samples Of a Nosocomial Infection In Erbil City

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ABSTRACT

Background: Spingomonas paucimobilis is a gram-negative pathogen that causes urinary tract infections, diarrhea, septicemia, and wound infections. Due to the spread of patients with high mortality but low mortality in Spingomonas paucimobilis, it has been isolated from Spingomonas paucimobilis in different clinical samples and is increasing antibiotic resistance all over the world. **Objectives:** The aim of our research was to look at the epidemiology, antibiotic susceptibility series, and pathogenic potential in different clinical samples from Erbil's Rizgary and Raparin hospitals. **Materials and Methods:** A total of 2582 samples were reviewed from different clinical samples from Rizgary Hospital and Raparin Hospital from male and female, we found 24 Spingomonas paucimobilis isolates, identified by using microscopical, morphological, biochemical tests and Vitek2 compact system according to the standard protocol against Ampicillin/Sulbactam, Cefazolin, Ceftazidime, Ceftriaxone, Cefepime, Imipenem, Tobramycin, Ciprofloxacin, Levofloxacin, Trimethoprim. using Vitek 2 compact system. **Results:** 24 total positive results of Spingomonas paucimobilis isolated from 2582 different clinical samples the highest percentage of Spingomonas paucimobilis was isolated from female samples (65%) while from male (35%) wen performing antibiotic susceptibility the highest resistance rate was Trimethoprim (66.66%), followed by To bramycin (50%), Ciprofloxacin (50%) and Levofloxacin (41.66%), respectively in contrast the highest effective antibiotic against Spingomonas paucimobilis was Cefepime (75%), Imipenem (75%), followed by Ceftriaxone (66.66%), Ceftazidime (66.66%), Cefazolin (66.66%), Ampicillin/Sulbactam (66.66%) **Conclusion:** Morbidity attribute to antibiotic resistance to third generation cephalosporin resistant, Spingomonas paucimobilis resistant is significant, if prevailing resistance trends continue, high societal and economic costs can be expected. Better management of antibiotic use, and infection control is needed to avoid infections that caused by drug resistant pathogens like Spingomonas paucimobilis

Introduction

Sphingomonas paucimobilis had first been identified as CDC Group IIk, biotype 1, before being given its own phylogenetic classification throughout 1977, when this was given the name *Sphingomonas paucimobilis* [12]. First *Sphingomonas paucimobilis* infection was discovered in a fisherman with a skin leg ulcer in 1979, as well as the species was identified in pure culture from the wound sample. [3]. This bacterial species was given its own genus, *Sphingomonas*, in 1990, and was designated as the genus' type strain [2]. *Sphingomonas paucimobilis* was once thought to be the only member of the *Sphingomonas* family with clinical significance. However, infection has recently been linked to two other *Sphingomonas* species *Sphingomonas mucosissima* and *Sphingomonas adhesive* [16]. It's Kingdom: Bacteria, Phylum: Proteobacteria, Class: Alphaproteo bacteria, Order: Sphingomonadales, Family: Sphingomonadaceae' [10]. 'The *Sphingomonas paucimobilis* is a gram negative rod shaped, non-spore forming, non-fermentative, strictly aerobic, yellow pigmented, oligotrophic, slow motile with a polar flagellum, oxidase and catalase positive. *Sphingomonas paucimobilis* completely lacks lipopolysaccharides (LPS) that can carry endotoxins, which makes the bacterium almost unique among Gram-negative bacteria. Instead, it possesses at least two different kinds of sphingolipids (where its name derives from). These sphingolipids have a unique sphingoglycolipid with long chain base dihydro sphingosin, ubiquinone 10 (Q-10), and 2-hydroxymyristic acid (2-OHC14:0) and the absence of 3-hydroxy fatty acids' [18]. 'Cultural growth. *Sphingomonas paucimobilis* growth requires at least 48 hours incubation on sheep blood agar. Optimal growth occurs at 30oC in 5% CO or ambient air; it does grow at 37oC but not at 42oC. It grows as a deep yellow colony on tryptic soy and blood agars. It is obligatory aerobic, it grows in brain-heart infusion, thioglycollate, blood culture media and 90% of isolates do not grow on MacConkey agar (10% grow on MacConkey agar and appear as non-lactose fermenters). *Sphingomonas paucimobilis* oxidatively utilizes glucose, xylose, and sucrose. Biochemical test results of interest include the following: esculin hydrolysis positive; motile by wet mount or in motility medium when incubated at 18oC to 22oC (nonmotile when incubated at 37oC; oxidase positive (90%-94% positive); catalase positive; urease negative; and indole negative' [9]. *Sphingomonas paucimobilis* could be present in abundance throughout the natural environment [1]. *Sphingomonas paucimobilis* is also an oligotrophic microbe, which means it can live under low-nutrient conditions. It's also been retrieved from the hospital's plumbing system, respiratory therapy supplies, including surgical devices. *Sphingomonas paucimobilis* nosocomial infections have been identified, and they are thought to be the result of a polluted hospital atmosphere and facilities [14]. While *Sphingomonas paucimobilis* can be spread by state employees' hands. And during outbreak, the hemato-oncology unit thoroughly washed the patient's offices, beds beside appliances, bathroom floors, bathtubs, and sinks, despite the lack of evidence for a single cause. Following the implementation of these steps, the recurrence of *Sphingomonas paucimobilis* infection was successfully prevented. Contaminated solutions are to blame for a lot of *Sphingomonas paucimobilis* infections [23]. Bacteremia, diarrhea, catheter related diseases, meningitis, peritonitis, osteomyelitis, septic arthritis, postoperative endophthalmitis, lung empyema, splenic abscesses, urinary tract

infections, and biliary tract infections have also been linked to *Sphingomonas paucimobilis*. Bacteremia induced by Gram negative bacteria is associated with increased mortality and morbidity rates, especially in hemato-oncology patients. *Sphingomonas paucimobilis* bacteremia, on the other hand, has been identified primarily in individuals of indwelling devices or immunocompromised hosts, especially those with neutropenia. Another study found that it can also induce septic shock [11]. Tetracycline, chloramphenicol, trimethoprim/sulfamethoxazole, carbapenems, and aminoglycosides have all been confirmed to be susceptible to *Sphingomonas paucimobilis*; however, susceptibility to many other antimicrobials such as third generation cephalosporins and fluoroquinolones is variable. Many strains of *Sphingomonas paucimobilis* are vulnerable to aminoglycosides, carbapenems, and SMX-TMP, according to a study. The carbapenem group of antibiotics was shown to be the most effective in treating a variety of infections in a study conducted on an outbreak of *Sphingomonas paucimobilis* in a pediatric hospital. Because of the resistance trends and ease of administration, a fluoroquinolone may be the best option [15]. The World Health Organization recently issued a warning to the public that multidrug-resistant bacteria are on the rise around the world, posing a serious threat to healthcare. Antibiotics can lose their ability to treat diseases if we do not act quickly [7]. Because of the synthesis of chromosomally encoded beta-lactamase, this bacterium is typically immune to penicillin and first-generation cephalosporins. Beta-lactam antibiotics bind to and acylate the active site of penicillin-binding protein (PBP), the enzyme needed for bacterial cell wall biosynthesis. Beta-lactamase is an enzyme that deactivates beta-lactam antibiotics by hydrolyzing the beta-lactam ring. In Gram-negative bacteria, beta-lactamase is typically generated constitutively or through induction by personal communication of a beta-lactam antibiotic with the legal framework [22.]

Methods

Total of 2582 clinical samples were obtained from six separate sites include (mid-stream urine, blood, bronchial wash, seminal fluid, sputum swab and pus swab). Bacterial isolates are characterized by a series of confirming tests after they were collected. Just 24 isolates were classified as such as a result of the analysis (genus and species). During 2017, clinical samples were taken from patients at Rizgary and Raparin hospitals in Erbil, from both male and female patients aged 10 to 79 years. That specimen was immediately inoculated on culture media for microorganism isolation; blood agar, tryptic soy agar and macConkey agar plates were incubated aerobically at 37C for (24-48) hours. Isolated microorganisms were isolated in pure colonies.[17]

Vitek 2 machine antimicrobial susceptibility examination

The Vitek 2 device was used to test all isolates for susceptibility to 13 commonly used antibiotics. The AES is a key component of Vitek 2 technology because it can provide precise "fingerprint" identification of bacterial resistance mechanisms and phenotypes. There

are 64 micro wells on the Vitek 2 card. Each well contains antimicrobials or recognition substrates. Vitek 2 has a detailed menu for identifying pathogens and checking their antibiotic resistance. The Vitek 2 test card is insulated, so aerosols, leaks, and personal contamination are minimized. When compared to microtiter processes, disposable waste is minimized by more than 80%. [17].

Results and Discussion

Just 24 isolates of *Sphingomonas paucimobilis* were reported from a total of 2582 samples obtained in 2017 in Rizgary and Raparin hospitals, with 18 samples coming from Rizgary and 6 from Raparin alone. In a year, both hospitals had almost the same rate of these bacteria, with 0.93% and 0.92% of isolates, respectively, as seen in table 1.

Table 1: Distribution of *Sphingomonas paucimobilis* according to Erbil hospitals.

Sample	No. Samples	No. of <i>S. paucimobilis</i> +ve (%)	No. of <i>S. paucimobilis</i> -ve (%)
Rizgary Hospital	1932	18 (0.93%)	1914 (99.06%)
Raparin Hospital	650	6 (0.92%)	644 (99.07%)
Total	2582	24 (0.93%)	2558 (99.07%)

Sphingomonas paucimobilis prevalence in various clinical samples. Two thousand eight hundred and twenty-two samples were obtained from six separate locations (mid-stream urine, blood, bronchial wash, seminal fluid, sputum swab and pus swab). Out of 2582 samples, 24 (0.93%) were found to be *Sphingomonas paucimobilis* isolates. As seen in table 2, the urine sample proved to be the most dominant specimen.

Table 2: *Sphingomonas paucimobilis* prevalence in various clinical samples

Patients	No. and % of <i>Sphingomonas paucimobilis</i>						Total
	Urine	Blood	Bronchial wash	Seminal Fluid	Sputum	Pus	
	Number and %						
Infected	14 0.54%	1 0.04%	3 0.12%	1 0.04%	4 0.15%	1 0.04%	24 0.93%
Uninfected	2097 81.22%	248 9.60%	93 3.60%	35 1.36%	62 2.40%	23 0.89%	2558 99.07%
Total	2111	249	96	36	66	24	2582

	81.76 %	9.64 %	3.72%	1.39%	2.56%	0.93%	100.00%
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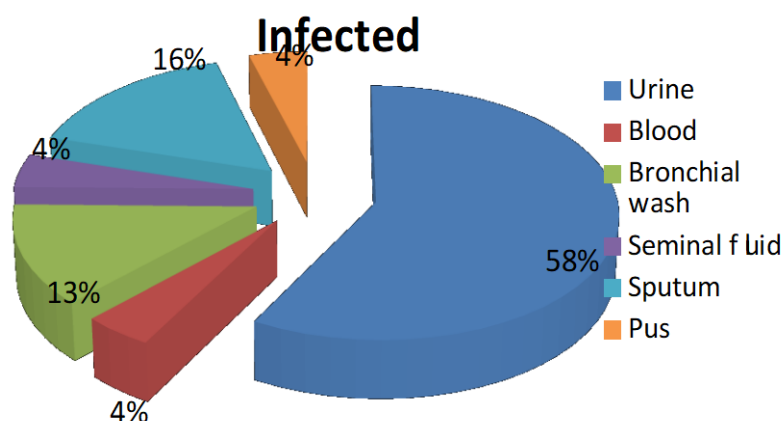


Figure 1: Prevalence in various clinical samples of *Spingomonas paucimobilis*.

Gender differences in *Spingomonas paucimobilis* distribution 24 (0.93%) of the 2582 samples tested positive for *Spingomonas paucimobilis*, the largest proportion of *Spingomonas paucimobilis* among female 16 year old's (1.03%), As seen in the table, female patients had a higher rate of 8 (0.77%) than male patients as in table 3.

Table 3: Distribution of *Spingomonas paucimobilis* relation with genders

Sex	No. Samples	No +ve	% +ve	No. -ve	% -ve
Male	1033	8	0.77%	1025	99.23%
Female	1549	16	1.03%	1533	98.97%
Total	2582	24	0.93%	2558	99.07%

Antibiotics susceptibility patterns tests for *Spingomonas paucimobilis*

Spingomonas paucimobilis isolates were screened for their resistance to ten widely used antibiotics (ampicillin/sulbactam, cefazolin, ceftazidime, ceftriax- one, cefepime, imipenem, tobramycin, ciprofloxacin, levofloxacin, and trimetho- prim). And the results were interpreted

according to standard value by clinical and laboratory standard institute of antimicrobial susceptibility testing (CLSI). It is obvious that *Sphingomonas paucimobilis* isolates showed highest sensitive (75%) to cefepime imipenem, (66.66%) to ampicillin/sulbactam, cefazolin ceftazidime. About resistance the highest resistance (66.66%) to trimethoprim and (50%) to tobramycin and ciprofloxacin as in table 4 and figure 2.

Table 4: Antibiotics susceptibility percentage among *Sphingomonas paucimobilis* isolates.

Antibiotic	No. Sensitive	% sensitive	No. Resistance	% Resistance
Ampicillin/Sulbactam	16	66.66%	8	33.33%
Cefazolin	16	66.66%	8	33.33%
Ceftazidime	16	66.66%	8	33.33%
Ceftriaxone	16	66.66%	8	33.33%
Cefepime	18	75%	6	25%
Imipenem	18	75%	6	25%
Tobramycin	12	50%	12	50%
Ciprofloxacin	12	50%	12	50%
Levofloxacin	14	58.30%	10	41.66%
Trimethoprim	8	33.33%	16	66.66%

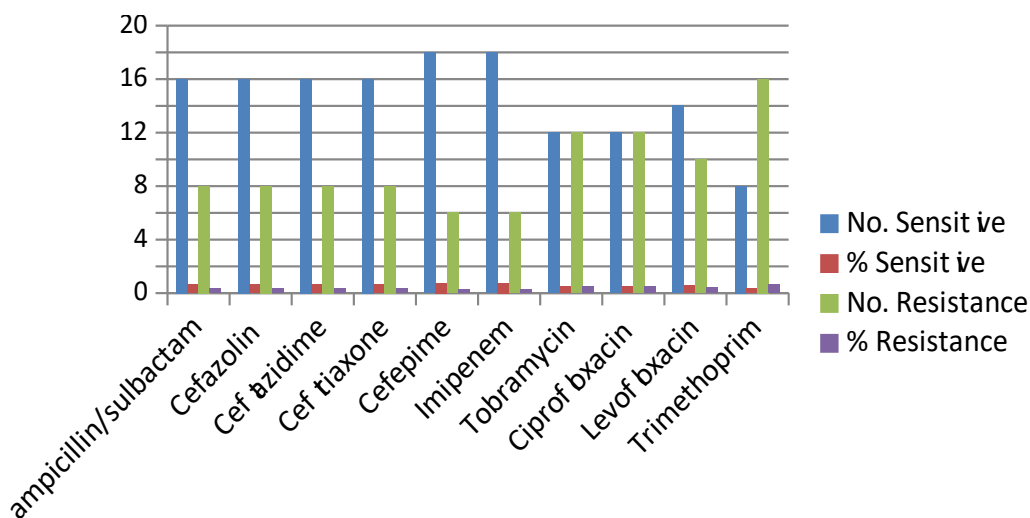


Figure 2: Antibiotic susceptibility percentages were calculated using various antibiotic patterns among *Sphingomonas paucimobilis* isolates

Discussion

Isolates of *Sphingomonas paucimobilis* are found at a high frequency.

Sphingomonas paucimobilis was originally believed to be a non-pathogenic environmental isolates, but it has been linked to a number of infections. Gram-negative bacilli that do not ferment pose a major concern in clinical settings, since they are the most common source of infectious diseases. They are bacterial infections that prey on underlying illnesses. However, bacteraemia/septicaemia caused by polluted solutions, such as distilled water, haemodialysis solvent, and sterile medicine solutions, is a common source of infection with this bacterium. Many cases of pseudobacteraemia and unusable infections have been reported in connection with *Sphingomonas paucimobilis* [20]. All of the bacterial isolates were checked on their diagnosis using a series of laboratory tests to ensure correct findings that the isolates belong to *Sphingomonas paucimobilis*. Microscopical, morphological, and biochemical measurements, as well as the Vitek 2 compact method, are used to identify all *Sphingomonas paucimobilis* isolates. This gram-negative bacterial cells from a smear preparation are rod-shaped, slowly motile with a polar flagellum, and clustered. Blood agar and tryptic soy agar are the standard agars for this bacterium. The colonies were a deep yellow, irregularly shaped, aqueous, and slippery, and they seemed to converge.

Sphingomonas paucimobilis was positive of esculin hydrolysis, that implies it metabolizes esculin in a food medium, producing the dark brown compound esculetin and discoloring the medium. This suggests that the organism is using esculin from the bile esculin agar slant as a carbon source. *Sphingomonas paucimobilis* was found to be oxidase positive, indicating that it produces the cytochrome c oxidase enzyme. A positive test result is a dark purple color that appears in 10 seconds. Catalase testing revealed that the organism was also positive. The presence of oxygen bubbles in the immediate aftermath indicates that the organism is catalase positive. Additional biochemical experiments were carried out.

Sphingomonas paucimobilis was a yellow pigmented, aerobic, non lactose fermenting Gram-negative rod with a polar flagellum that tested negative for indole, citrate, and H₂S in lead acetate. The word "paucimobilis" derives from the fact that only a few bacteria in broth culture are motile. The organism could be cultured on non-selective media such as blood and chocolate agar. It is positive for both oxidase and catalase. It grows best at 30°C, but also at 37°C.[1]

In 2017, 24 of the 2582 samples tested at Rizgary and Raparin Teaching Hospital in Erbil were positive for *Sphingomonas paucimobilis*. 18 isolates were obtained from Rizgary Hospital, while 6 were obtained from Raparin Hospital. In other words, using microscopical, morphological, and biochemical measurements as well as the Vitek 2 compact method, 0.93 percent of the samples obtained in 2017 were classified as *Sphingomonas paucimobilis*. According to Bakir and Ali's analysis [21]. *Sphingomonas paucimobilis* included in 3% of the samples gathered over a year, and this study only collected and focused on throat swabs when dealing with sputum, pus, bronchial wash, seminal fluid, and blood samples.

Furthermore, Ahmed and Ali [13] discovered that *Sphingomonas paucimobilis* was present in 1.4 percent of gram negative bacteria isolated from women with genital tract infection. In therapeutic settings, *Sphingomonas paucimobilis* was quite uncommon. In humans, this bacterium causes two kinds of infections: intermittent or community-acquired infections (bacteremia, urinary tract infection, wound infection, and sometimes meningitis), and nosocomial infections caused by penetration of sterile fluids used in hospitals.[6]

Distribution of *Sphingomonas paucimobilis* according to Erbil hospitals.

Just 24 cases of *Sphingomonas paucimobilis* were reported from a total of 2582 samples obtained in 2017 in Rizgary and Raparin hospitals, with 18 samples coming from Rizgary and 6 from Raparin alone. This variance may be due to sample size, bacterial prevalence varies widely among different areas and communities within the world, bacterial prevalence varies widely among different areas and communities within the country.

The incidence of *Sphingomonas paucimobilis* in different clinical samples.

The bacteria has been isolated from hospital water supplies, respirators, stocked distilled water, blood, wounds, hospital dialysis equipment, patients with meningitis, septicemia, bacteremia, peritonitis, wound infections, soil, river water, deep subsurface sediments, corroding copper pipes, drinking water, and rhizo- sphere.[6]

In 2017, a total of (2582) samples were obtained from six separate sources during the year (urine, Blood, bronchial wash, seminal fluid, sputum, pus). Just (24) were verified *Sphingomonas paucimobilis* after the final validation using vitek 2. In our research, we discovered that the urine sample had the largest number of isolates (58%) followed by (16%) sputum, (13% bronchial wash), (5%) semen, and (4%) pus and seminal fluid samples. Our findings differ from those reported by Toh et al. [19]. who discovered that of the 55 positive patients, the largest number of *Sphingomonas paucimobilis* isolates were found in blood (30.9%), sputum (21.8%), pus (18.2%), and urine (18%). (3.6%). Since *Sphingomonas paucimobilis* is an opportunistic pathogen, it is uncommon to find the organism in clinical samples. Water, sputum, vomit, wounds, bile, cerebrospinal fluid, vagina, and cervix have also been used to isolate it. A wide range of community-acquired and health-care- associated infections have been identified, with catheter-related infection being the most frequent (9), while Kuo et al [22] observed a malignancy incidence of 57.1% and a diabetes rate of 40.5% in 16 cases of *Sphingomonas paucimobilis* bacteremia. This microorganism has been linked to a number of diseases, including sepsis, septic pulmonary embolism, septic arthritis, peritonitis, and endophthalmitis. Unlike other gram negative bacteria, *Sphingomonas* spp. has a low mortality rate and a strong prognosis in cases identified so far. Despite the fact that *Sphingomonas paucimobilis* is a hospital-acquired infection, it should be considered a cause since it is a common part of the flora, particularly in patients with immuno- suppressive diseases and those who have other underlying diseases. As a result, even though it is a low- virulence microorganism, its value cannot be overlooked. More specifically, this organism

has been linked to septic shock in immune-compromised patients, and it has been found more often in recent months.[8]

Distribution of *Sphingomonas paucimobilis* according to gender.

Our results found out that the number of isolates are higher in female samples (67%) than in male samples (33%), in which we compared with result reported by Toh et al [20] in Taiwan who found different in which they had more isolates from male samples, (57.1%) compare with (42.9%) in females, while results reported by Ahmed and Ali [13] showed the presence of *Sphingomonas paucimobilis* 1.4% and isolated only from women with vaginitis we did try to compare with previous researches done in our country but unfortunately there is very little information about this newly species in our country and if there would be present, In their study, they did not have a gender division of the sample. Since our target populations were only patients visiting the hospital randomly in Erbil district, the variations in the number of *Sphingomonas paucimobilis* isolates in male and female may be attributed to sample size, which was higher in female (1549) than in male.(1033)

Antibiotics susceptibility tests for *Sphingomonas paucimobilis*.

Antimicrobial agents have long been the most simple and commonly used treatment alternative for infections caused by a variety of microbes. Microbial populations, on the other hand, have evolved a variety of methods to combat these antimicrobial agents, which is a significant factor in the global spread of antimicrobial resistance. *Sphingomonas paucimobilis* is a common and adaptable human opportunistic pathogen that affects mortality and healthcare costs in both hospitals and the population [8]. Antibiotic resistance tests were conducted on Cefazolin, Ceftazidime, Ceftriaxone, Cefotaxime, Cefepime, Imipenem, Tobramycin, Ciprofloxacin, Levofloxacin, and Trimethoprim from a total of 24 samples of *Sphingomonas paucimobilis* obtained in Erbil. Cefepime and Imipenem susceptibility was 100 percent in *Sphingomonas paucimobilis*. Trimethoprim was found to have the greatest level of resistance, followed by Ciprofloxacin and Tobramycin (71%, 33%, and 33%, respectively).

Jorge et al [5] from Brazil found that 21.7 percent (5 out of 23) of the samples were resistant to Ciprofloxacin, which was close to our findings. Although Ahmad and Ali [13] discovered that samples obtained from Rizgary and Raparin Hospitals in Erbil City in 2015 had the highest percentage of Meropenem and Imipenem resistance but were 100% susceptible to Ciprofloxacin. Trimethoprim, Ciprofloxacin, and Imipenem susceptibility was found in a study by Kelic et al [14] as well as Cefepime, Imipenem, Meropenem, Amikacin, and Tigeracillin susceptibility. Although Amoxicillin/Clavulanic acid and Piperacillin/Tazobactam are the antibiotics of choice for infections caused by *Sphingomonas paucimobilis*, Amoxicillin/Clavulanic acid, Piperacillin/Tazobactam are the antibiotics of choice for *Sphingomonas paucimobilis* infections, as they showed 100% susceptibility in our research. *Sphingomonas paucimobilis*, on the other hand, was found to be (100%) immune to Amikacin, Aztreonam, Clindamycin, and Cefoxitin, according to Ahmed and Ali [13]. Multidrug-resistant bacteria can cause severe nosocomial and community-acquired infections

that are difficult to treat with current antibiotics. Peptide bond forming is disrupted by β -lactam antibiotics, which act as competitive inhibitors of these PBPs. As a consequence, irreversible covalently bound penicilloyl-enzyme complexes with poor cross-linked peptidoglycans form, making bacterial lysis and death easier [22]. Bacterial cell wall biosynthesis is inhibited by β -lactam antibiotics, resulting in cell lysis and death. Gram-negative bacteria have developed a variety of methods to evade the bactericidal effects of β -lactam antibiotics, including the synthesis of β -lactamases, the development of novel PBPs with decreased tolerance for β -lactam antibiotics, limiting β -lactam antibiotic entry by porin mutations, and expelling β -lactam antibiotics out of cells using multi-drug efflux pumps. Growing β -lactamases, enzymes that can hydrolyze the β -lactam ring, is also the most effective of these pathways [17]. Prescription of broad-spectrum antibiotics in an appropriate and diligent manner is critical in preventing the appearance of yet another outbreak of antibiotic resistance in our area. Furthermore, reports on antimicrobial resistance epidemiological investigative results are critical in our area in order to support adequate antibiotic treatment, as well as good infection prevention and clinical care [4]. The rise of ESBLs and MBLs, with their diverse spectrums and unrivaled drug resistance, is posing a clinical threat for clinicians and microbiologists alike [24]. The current study discovered multi-resistance in *Sphingomonas paucimobilis* isolates, demonstrating the need for local or country-based investigations to characterize and detect multi-resistant antibiotics, as well as improve management and control strategies. Furthermore, the use of antibiotic combination therapy against multi-resistant bacteria, as well as good hygiene by hospitalized patients and workers, may significantly reduce the incidence and spread of such cases. Antibiotic resistance patterns may differ due to the bacteria's ecology and physiology, suggesting different modes and pathways of resistance acquisition.

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