Pro Index | Journal of Integrity in Ecosystems and Environment
Volume: 2 Issue: 8 Year: 2024
ISSN: 3030-3729 | http://journals.proindex.uz

# Pathogenic Bacteria in Wastewater from Medical City, Baghdad

**Dr. Thana Majid Zayer Noor**
Department of Biology Science, Mustansiriyah University, POX 46079, Iraq-Baghdad

***Abstract:*** Bacterial contaminants have a direct impact on overall human health. This study focuses on microbial pathogens in sewage discharged from four main municipal stations of BMC/ Baghdad Medical City into the Tigris River. Sensitivity tests were conducted on six isolates against common antibiotics used by Iraqi patients as initial screening tests. The study aimed to characterize prevalent pathogenic and environmental isolates from the Tigris River, receiving sewage water from Baghdad Medical City. Over four months in 2023, two Gram-positive and four Gram-negative bacterial groups were identified in wastewater isolates. Findings highlighted biochemical reactions and antibiotic resistance mechanisms using VITEK 2 Compact Mechanism in the recovered isolates. Two dominant microbial strains in BMC wastewater Klebsiella pneumoniae and Staphylococcus aureus accounted for 34% of a total 106 isolates each. Both strains showed resistance to commonly tested antibiotics with percentages 76% and 62% respectively. Staphylococcus hominis and Pseudomonas putida were a least dominant bacterial groups in sewage samples, accounting for 6.6% each from a total of 106 isolates. However, they showed resistance with percentages 61% and 17% respectively. Each of Ralstonia pickettii and Rhizobium radiobacter accounted for 7.5% and 9.4% from a total of 106 isolates each and were sensitive to most tested antibiotics, with percentage 92% and 90% to respectively. Significant differences ($P < 0.0001$) between the total number of isolates conducted at BMC compared to the control were achieved. Alongside, significant differences between each isolated bacterial group at BMC and UTR were recorded, with results as follows: Staphylococcus hominis, Staphylococcus aureus, Klebsiella Pneumoniae, Rhizobium radiobacter, Ralstonia pickettii, and Pseudomonas putida with ($P < 0.000008$, 0.000001, 0.000019, 0.000003, 0.00004 and 0.00046), respectively.

***Key points:*** BMC/ Baghdad Medical City, UTR/ Upstream Tigris River, Staphylococcus aureus, Staphylococcus hominis, Klebsiella pneumoniae, Pseudomonas putida, Rhizobium radiobacter and Ralstonia pickettii. VITEK. MIC, CLSI.

**Introduction:** Water plays an essential and major role for most forms of life on Earth. It also holds a fundamental importance in metabolic processes within living organisms (1). Therefore, the contamination of water by released wastewater from industrial and hospital sectors, containing pathogenic microbes, is considered a negative indicator for water quality (2). The presence of pathogenic microbes in wastewater from health institutions is a significant concern (3, 5). Wastewater quality, typically rich in easily decomposable organic materials, creates ideal conditions for the rapid growth and reproduction of microbes (13), leading to increased contamination of the original water with pathogenic organisms, particularly if present in excessive quantities beyond permissible limits (4,5). The wastewater generated by the Medical City in Baghdad, housing seven medical hospitals, directly flows into the Tigris River (6,7,8). Due to the natural environment, microbial organisms isolated from this wastewater have distinct characteristics compared to those from various other environments (5). The release of small amounts of drugs into drainage channels by the hospital community, due to insufficient awareness among patients and health workers, contributes to an increased pathogenic load in the same hospitals. These factors collectively contribute to the development of bacterial resistance to common antibiotics, especially in developing countries like Iraq (6,19). Antibiotic resistance is a significant issue that Iraq is currently grappling with, necessitating realistic solutions for the near future (6, 7). The significance of this study lies in isolating and characterizing pathogenic microbes causing common diseases and investigating their resistance to commonly prescribed antibiotics by health workers in the same hospital community.

**Study Area:** Study Area: Baghdad Medical City, established in 1961, is located in the Bab al-Muadham area, in the middle of the Baghdad governorate. The institute has a limited capacity of 1000 beds (5,7), yet it accommodates 2212 normal resident patients, doubling its designed capacity (6). The institute conducts over 380 operations monthly (5). Four sewage stations affiliated with this health institute were selected. The first station is a sewage control station located over 450 meters from Baghdad Medical City. The second station discharges pollutants, as sewage water, directly into the Tigris River from Baghdad Medical City. The third station supports the second station, and the fourth station is located 1600 meters away from the third one (7,8). Sewage samples were collected in triplicate from the four stations and from upstream Tigris River weekly, over four months, at depths ranging from 15 to 30 cm from water surfaces.

**Samples Collections:** Sewage water samples from the four stations at Baghdad Medical City and Upstream Tigris River were collected under aseptic conditions using sterilized bottles with a 100 ml capacity. Four samples were collected for each month in triplicate. All samples were transferred directly to the laboratory, stored in a dry and clean place, and subjected to serial dilution and culture techniques using nutrient agar, as described by (10). Only 100 microliters from each sewage sample were diluted up to $10^{-6}$ and cultured on nutrient agar.

**Bacterial Culture Conditions:** Nutrient agar, MacConkey, tryptone soya agar and blood agar prepared according to the manufacturer's instructions, was used for culturing techniques. It was poured into plates and used for culturing techniques (10).

**Isolating Bacteria from Sewage Water Samples at Baghdad Medical City & and Upstream Tigris River:** Using VITEK 2 Compact machine, microorganisms were isolated from wastewater samples. To achieve different microbial colonies the serial dilution techniques were applied to all collected samples. Only 100 microliters from the sixth dilution were transferred to the prepared agar previously (Nutrient agar, MacConkey, tryptone soya agar and blood agar) than, spread using spreaders, plates incubated at 35°C- 37°C for 24 to 48 hours. After incubation, individual colonies were examined, sub cultured until pure colonies were obtained and used for the suspension preparation for the ID Cards. Pure colonies were also stored in 20% glycerol in the freezer for further tests (7,8,11).

**Bacterial Culture Characterization:** Under the microscope examination, all samples were observed and characterized based on cell features and morphology.

a) **Characteristics of Cell Morphology:** Size, shape and color are the primary features of bacterial cell morphology, all samples were closely examined through light microscopic observation. The bacterial cell margin was employed to determine the colony's shape, categorized as cocci, round, or rod-shaped. The color of the bacterial cell was identified under microscopic observation as well (9,10,11).

b) **Biochemical Characteristics:** Unknown cultures were identified through biochemical tests using the VITEK 2 Compact Machine with a 30-card capacity system, employing fluorogenic methodology for all isolated bacterial cells. The available test kits include ID-GP (gram positive cocci), ID-GN (gram negative bacillus), AST-GP (gram positive susceptibility), and AST-GN (gram negative susceptibility). Biochemical test was applied to a prepared bacterial suspension through transferring three ml of saline water into a tube. In the saline tube, a colony was selected and suspended. After entire mixing, checked the optimal density with Densi CHECK. The tube and the ID card were then placed in the cassette machine. All suspension samples and cards were set in a cassette. After loading the cassette into the instrument, the fill button was pressed. Visual and audible indicators signaled the completion of the fill process, and then the loaded cassette was transferred to the loading station, followed by closing the load door. When the indicator started flashing, the cassette from the loading station removed, and the remove status was clicked (11,12).

c) **Assay of Antibiotic Susceptibility:** The VITEK 2 Compact Machine was employed to assess antibiotic susceptibility, determining the susceptibility of some selected isolates. The area of interest in this study is the resistance of bacterial isolates inhabiting sewage water at Baghdad Medical City. The antibiotic susceptibility test was applied on 10-20% of the selected isolates from sewage water at **BMC**.

d) **Data analysis:** The collected wastewater samples data from Baghdad Medical City and upstream Tigris River were organized in tabular format and subsequently presented. conducted data statistically analyzed using Graph Pad Prism 8 via applying Two-way ANOVA and t-test where it is necessary. The susceptibility of the identified bacteria to common antibiotics was determined using Minimum Inhibitory Concentration (MIC). All (MIC) results were reported as resistant, intermediate, or sensitive, following interpretive standards set by the Clinical and Laboratory Standards Institute (CLSI). The MIC value was measured in (µg/mL) and interpretive results for the bacterial cells classified as resistant had values greater than or equal to 160 and lower than or equal to 8. Bacterial cells with values between 16 to 64 were considered intermediate, while values greater than or equal to 0.25 and lower than or equal to 20 were considered sensitive. The table below provides the values corresponding to the interpretation of MIC.

**Table 1: Interpretation of minimum inhibitory concentrations in a qualitative manner.**

| **Minimum inhibition concentration (µg/mL) in numbers** | **Minimum inhibition concentration (µg/mL) expression** | **Minimum inhibition concentration (µg/mL) interpretation** |
|---|---|---|
| <= 0.25 to <= 20 | S | Sensitive: the organism is restricted by serum drug concentration that is attained by the standard dosage |
| 16 to 64 | I | Intermediate: the organism is only inhibited when exposed to a maximum recommended dosage. |
| >= 8 to > = 160 | R | Resistant: the organism exhibits resistance to the serum drug levels typically attained |

**Results:**

**Table 2: Wastewater samples and their physical characteristics.**

| **Samples collected from BMC** | **Color** | **Turbidity** | **Average Temperature** | | | | **pH** | **Total solids** |
|---|---|---|---|---|---|---|---|---|
| | | | **September** | **October** | **November** | **December** | | |
| Station one | A= light gray | Low | 41°C | 38°C | 36°C | 28°C | 8.12 | 397 mg/l |
| Station two | B= medium | Medium | 39.8°C | 38.1°C | 35.3°C | 27.8°C | 7.34 | 514 mg/l |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | gray | | | | | | | |
| Station three | C= medium gray | Medium | 40°C | 39°C | 35°C | 27.1°C | 7.69 | 543 mg/l |
| Station four | D= gray brown | Medium | 41.2°C | 38.3°C | 36.1°C | 28°C | 8.02 | 621 mg/l |

**BMC** = Baghdad Medical City

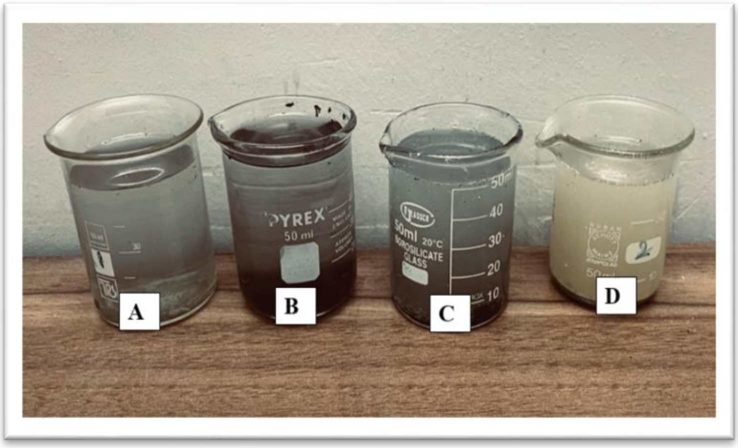


**Figure 1: Collected samples from four stations at Baghdad Medical City. A= Station one, B = Station two, C = Station three and D = Station four.**

Table 2: displays the physical characteristics of wastewater samples collected from four Baghdad medical city stations. Station one (A) exhibits light gray color and low turbidity, suggesting a low particle presence (25). Stations two and three (B and C) show medium gray color and medium turbidity, indicating a specific form of pollution. Station four (D) displays a gray-brown color and medium turbidity suggesting the presence of a supportive medium for transporting pathogenic microorganisms (26). Across all stations, the samples exhibit high alkalinity due to particulates and the type of dissolved organic and inorganic compounds. Average temperatures were suitable for both environmental and pathogen bacteria growth (25, 26). The average of total solids indicates concerns about the hardness, chloride, iron, alkalinity, sulfate, manganese, nitrate, and general salt content at station one, while stations two, three, and four should raise a concern about other salts like lithium, bromide, aluminum, other metals, and the water potential for scale formation (27). Samples images collected from upstream Tigris River were not displayed. The color and pH of the collected samples were colorless, with an average pH 7.2 for most samples, serving as control alongside samples collected from sewage at Baghdad Medical City.

**Table 3: Isolates collected at Baghdad Medical City over four months.**

| **Wastewater samples at Baghdad Medical City 2023** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **September four stations** | | **October four stations** | | **November four stations** | | **December four stations** | |
| **W1 & W2** | **W3 &W4** | **W1 & W2** | **W3 &W4** | **W1 & W2** | **W3 &W4** | **W1 & W2** | **W3 &W4** |
| Total 12 samples | | Total 12 samples | | Total 12 samples | | Total 12 samples | |
| 25 isolates | | 30 Isolates | | 22 Isolates | | 29 isolates | |
| 2 | *Staphylococcus hominis* | 2 | *Staphylococcus hominis* | 1 | *Staphylococcus hominis* | 2 | *Staphylococcus hominis* |
| 7 | *Staphylococcus aureus* | 9 | *Staphylococcus aureus* | 11 | *Staphylococcus aureus* | 10 | *Staphylococcus aureus* |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 10 | *Klebsiella pneumoniae* | 11 | *Klebsiella pneumoniae* | 6 | *Klebsiella Pneumoniae* | 10 | *Klebsiella pneumoniae* |
| 2 | *Rhizobium radiobacter* | 4 | *Rhizobium radiobacter* | 1 | *Rhizobium radiobacter* | 3 | *Rhizobium radiobacter* |
| 2 | *Ralstonia pickettii* | 3 | *Ralstonia pickettii* | 1 | *Ralstonia pickettii* | 2 | *Ralstonia pickettii* |
| 2 | *Pseudomonas putida* | 1 | *Pseudomonas putida* | 2 | *Pseudomonas putida* | 2 | *Pseudomonas putida* |
| Highest frequent bacteria identified as *Klebsiella pneumoniae* | | Highest frequent bacteria identified as *Klebsiella Pneumoniae* | | Highest frequent bacteria identified as *Staphylococcus aureus* | | Highest frequent bacteria identified as *Klebsiella pneumoniae, Staphylococcus aureus* | |

Table 3: presents data on the isolates collected at Baghdad Medical City from September to December, amounting to a total 106 isolates from four stations. *Klebsiella Pneumoniae* had the highest number of isolates in September, October and December with 10,11, and 10 respectively. *Staphylococcus aureus* showed the highest frequency in November with 11 isolates. *Staphylococcus hominis* had only one isolate in November, maintaining similar numbers for the other three months. The highest number of *Rhizobium radiobacter* isolates occurred in October, totaling four. *Ralstonia pickettii* differed from *Pseudomonas putida* in October, with *Ralstonia pickettii* having the highest number (three isolates) compared to the other months. *Pseudomonas putida,* on the other hand, recorded the lowest number of isolates, with only one in October, while maintaining two isolates per month in the other months (See Figure 2).

**Table 4: Isolates collected at Upstream Tigris River over four months.**

| Upstream Tigris River samples 2023 | | | | | | | |
|---|---|---|---|---|---|---|---|
| **September upstream** | | **October upstream** | | **November upstream** | | **December upstream** | |
| **W1 & W2** | **W3 &W4** | **W1 & W2** | **W3 &W4** | **W1 & W2** | **W3 &W4** | **W1 & W2** | **W3 &W4** |
| Total 12 samples | | Total 12 samples | | Total 12 samples | | Total 12 samples | |
| 28 isolates | | 24 Isolates | | 21 Isolates | | 24 isolates | |
| 7 | *Staphylococcus hominis* | 3 | *Staphylococcus hominis* | 6 | *Staphylococcus hominis* | 5 | *Staphylococcus hominis* |
| 1 | *Staphylococcus aureus* | 1 | *Staphylococcus aureus* | - | *Staphylococcus aureus* | - | *Staphylococcus aureus* |
| 1 | *Klebsiella pneumoniae* | 1 | *Klebsiella pneumoniae* | - | *Klebsiella Pneumoniae* | - | *Klebsiella pneumoniae* |
| 7 | *Rhizobium radiobacter* | 6 | *Rhizobium radiobacter* | 4 | *Rhizobium radiobacter* | 8 | *Rhizobium radiobacter* |
| 6 | *Ralstonia pickettii* | 8 | *Ralstonia pickettii* | 4 | *Ralstonia pickettii* | 6 | *Ralstonia pickettii* |
| 6 | *Pseudomonas putida* | 5 | *Pseudomonas putida* | 7 | *Pseudomonas putida* | 5 | *Pseudomonas putida* |
| Highest frequent bacteria identified as *Staphylococcus hominis* | | Highest frequent bacteria identified as *Ralstonia pickettii* | | Highest frequent bacteria identified as *Pseudomonas putida* | | Highest frequent bacteria identified as *Rhizobium radiobacter* | |

Table 4: presents data on isolates collected from Upstream Tigris River between September and December, totaling 97 isolates. Both *Staphylococcus aureus* and *Klebsiella Pneumoniae* followed the same pattern, each recording only one isolate in both September and October, with no isolates recorded for the other two months. The number of isolates for the other four genera varied by month, with *Staphylococcus hominis* recording the highest number in September (7 isolates) *Rhizobium radiobacter* in December (8 isolates), *Ralstonia pickettii* in October (8 isolates), and *Pseudomonas putida* in November (7 isolates) (See Figure 2).

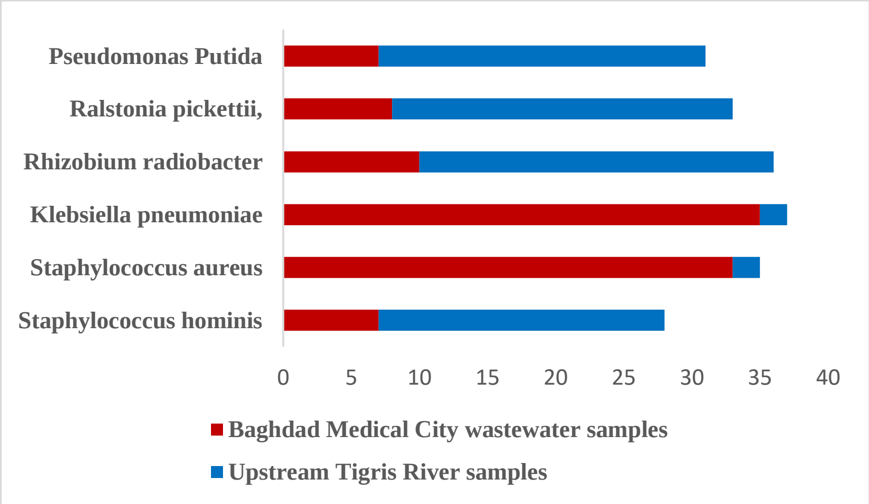


**Figure 2: Total bacterial isolates from both disposed wastewater at Baghdad Medical City and Upstream Tigris River.**

The total collection of bacterial isolates at Baghdad Medical City was 106. This count exceeded the isolates from Upstream Tigris River, which totaled 97. Pathogenic bacteria dominated wastewater samples at Baghdad Medical City with *Staphylococcus aureus* and *Klebsiella Pneumoniae* each representing 34%. *Staphylococcus hominis* and *Pseudomonas putida* recorded each, 6.6%. While *Rhizobium radiobacter* and *Ralstonia pickettii* had 9.4% and 7.5% respectively. In contrast, bacterial groups from Upstream Tigris River displayed notably different percentages. *Staphylococcus aureus* and *Klebsiella Pneumoniae* had the lowest at 2.06% each, with other groups ranging from 21.6% to 25.7%. Two-way RM ANOVA revealed significant differences (P <0.0001) between samples, with a total variation percentage of 1.549. Error bars were not displayed, and multiple t-tests confirmed significant p-values for each bacterial group (see table 5).

**Table 5: Significant p-values between isolated bacterial groups at Baghdad Medical City and Upstream of the Tigris River.**

| Bacterial Isolates | Number of total isolates at Baghdad Medical City | Number of total isolates from Upstream Tigris River | t-test / P-Value |
|---|---|---|---|
| *Staphylococcus hominis* | 7 | 21 | P= 0.000008 |
| *Staphylococcus aureus* | 37 | 2 | P= 0.000001 |
| *Klebsiella Pneumoniae* | 37 | 2 | P= 0.000019 |
| *Rhizobium radiobacter* | 10 | 25 | P= 0.000003 |
| *Ralstonia pickettii,* | 8 | 24 | P= 0.00004 |
| *Pseudomonas putida* | 7 | 23 | P= 0.00046 |

**Table 6: The morphological traits of pathogenic and environmental bacteria recognized in wastewater samples and Upstream Tigris River.**

| Characteristics | | | | Diagnoses of highest frequent identified bacteria/VITEK Compact 2 |
|---|---|---|---|---|
| **Colony Color of isolated Bacteria** | **Colony Shape / incubation periods** | **Lactose fermentation** | **Gram Stain** | |
| pink on MacConkey | dome-shaped with mucoid aspects and 3 to 4 mm after 24 hours incubation at 30 or 37°C | lactose fermenter | Gram negative | **BMC1/ UTR1**= *Klebsiella pneumoniae* |
| not pigmented to light white on nutrient agar | smooth, circular, convex 1 to 2 mm after 48 hours at 27°C | non-lactose fermenter | Gram negative | **BMC2/ UTR2** = *Rhizobium radiobacter* |
| dark white or beige on tryptone soya agar | smooth, glistening domed 1 to 2 mm after i 24 hours incubation at 30 to 37°C | non-lactose fermenter | Gram negative | **BMC3/ UTR3** = *Ralstonia pickettii* |
| transparent color and not mucoid on nutrient agar | smooth entire edges with a round shape 1 to 2 mm after 24 hours incubation at 30 to 35°C | non-lactose fermenter | Gram negative | **BMC4/ UTR4** =*Pseudomonas putida* |
| white or tan in color not hemolytic on blood agar | small round in shape and 1 to 2 mm after 24 hours incubation at 30 or 35°C | lactose fermenter | Gram positive | **BMC5/ UTR5** = *Staphylococcus hominis* |
| gold-colored colonies on tryptone soya agar | sharp edges round, convex, and 1 to 4 mm after 24 hours incubation 18 to 24 hours at 37°C | lactose fermenter | Gram positive | **BMC6/ UTR6** = *Staphylococcus aureus* |

**BMC= Baghdad Medical City and UTR = Upstream Tigris River**

Table 6: displays the bacteria isolated at Baghdad Medical City and collected samples from Upstream Tigris Revier. Across the four sewage treatment stations at **BMC**. *Klebsiella penumoniae* and *Staphylococcus aureus* were the most commonly isolated bacterial groups, identified at with probabilities of 97% and 99% respectively. On the other hand, bacterial groups were distributed quite similarly and identified as *Staphylococcus hominis, Rhizobium radiobacter, Ralstonia picketti,* and *Pseudomonas putida,* with probabilities of 99%, 98%,98% and 96%, respectively. Bacterial isolates from **UTR** were used as control to compare the number of isolates for each genus from two different places (**BMC** and **UTR**). Both *Klebsiella penumoniae* and *Staphylococcus aureus* were the least commonly isolated bacteria, identified with probabilities of 98% and 99% respectively. The other bacterial groups were in somehow similar regarding the number of isolates and identified as *Staphylococcus hominis, Rhizobium radiobacter, Ralstonia picketti*, and *Pseudomonas putida* with probabilities of 99%, 97%,97%, and 95%, respectively.

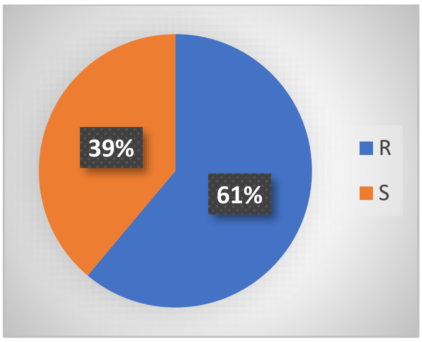


**Figure 3: Percentage of antibiotic sensitivity results of *Staphylococcus hominis* against twenty-one antibiotics. R = resistant and S = sensitive.**

Figure 3 above displays the outcomes of antibiotic susceptibility tests conducted on *Staphylococcus hominis* isolated at **BMC**. The data reveals that the *Staphylococcus hominis* isolated, is responsive to seven antibiotics recording a percentage of 39%, namely Levofloxacin, Moxifloxacin, Linezolid, Vancomycin, Tetracycline, Nitrofurantoin, and Rifampicin. Moreover, it demonstrates resistance sensitivity recording a percentage of 61% of Gentamicin, Tobramycin, and Clindamycin, Benzylpenicillin, Piperacillin/Tazobactam, Oxacillin, Erythromycin, Teicoplanin, Tetracycline, Fusidic Acid and Trimethoprim/Sulfamethoxazole. On the other hand, each of Inducible Clindamycin Resistance, Fosfomycin and Mupirocin showed a negative result.

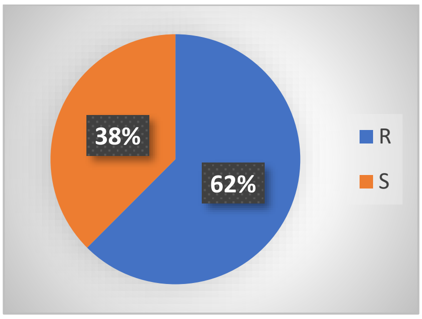


**Figure 4: Percentage of antibiotic sensitivity results of *Staphylococcus aureus* against twenty-one antibiotics. R = resistant and S = sensitive.**

Figure 4 above illustrates the results of antibiotic susceptibility tests conducted on *Staphylococcus aureus* isolated at **BMC**. The data indicates that the isolated *Staphylococcus aureus* isolated is susceptible to six antibiotics recording a percentage of 38% against each of Gentamicin, Levofloxacin, Moxifloxacin, Linezolid, Tetracycline, and Nitrofurantoin. Additionally, it exhibits resistant recording a percentage of 62% against each of Tobramycin and Tetracycline, Benzylpenicillin, Piperacillin/Tazobactam, Oxacillin, Erythromycin, Clindamycin, Teicoplanin, Vancomycin, Fusidic Acid, Rifampicin, and Trimethoprim/Sulfamethoxazole. On the other hand, each of Inducible Clindamycin Resistance, Fosfomycin and Mupirocin showed a negative result.

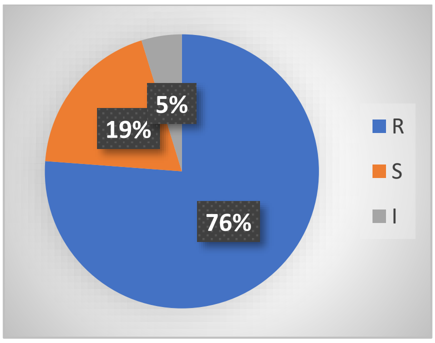


**Figure 5: Percentage of antibiotic sensitivity results of *Klebsiella pneumoniae* against twenty-four antibiotics. R = resistant, S = sensitive and I = intermediate.**

Figure 5 above illustrates the results of antibiotic sensitivity testing conducted on *Klebsiella pneumonia* isolated at **BMC**. The data indicates *Klebsiella pneumoniae* displays resistance to all commonly tested antibiotics, recording a percentage of 76% against each of Ampicillin, Amoxicillin/Clavulanic Acid, Ticarcillin, Piperacillin, Piperacillin/Tazobactam, Cefazolin, Cefuroxime, Cefoxitin, Cefixime, Ceftazidime, Ceftriaxone, Cefepime, Ertapenem, Imipenem, Amikacin and Gentamicin. Nevertheless, it exhibits sensitivity with a percentage of 19% to each of Ciprofloxacin, Levofloxacin, Tigecycline, Trimethoprim/ Sulfamethoxazole and showed intermediate sensitivity with a percentage of 5% to Nitrofurantoin exclusively. On the other hand, each of ESBL and Amoxicillin showed a negative result.

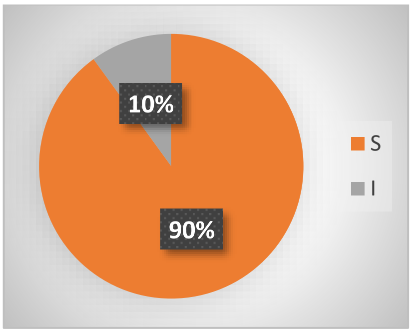


**Figure 6: Percentage of antibiotic sensitivity results of *Rhizobium radiobacter* against ten antibiotics. S = sensitive and I = intermediate.**

Figure 6 above presents the outcomes of antibiotic sensitivity testing conducted on *Rhizobium radiobacter* isolated at **BMC**. The results reveal that the isolated *Rhizobium radiobacter* displays sensitivity to widely used antibiotics recording a percentage of 90% against Piperacillin/Tazobactam, Cefazolin, Ceftriaxone, Cefepime, Gentamicin, Ciprofloxacin, Tigecycline, Levofloxacin and Trimethoprim/ Sulfamethoxazole. Additionally, it demonstrates intermediate sensitivity recording a percentage of 10% against Ceftazidime exclusively.

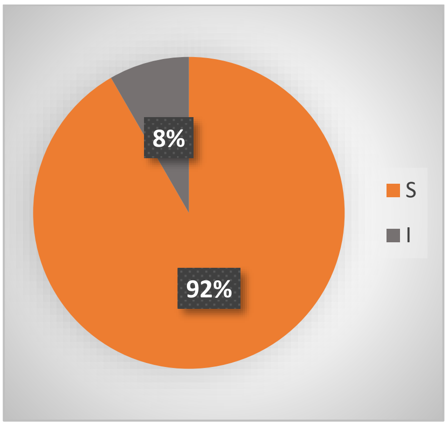


**Figure 7: Percentage of antibiotic sensitivity results of *Ralstonia pickettii* against twelve antibiotics. S = sensitive and I = intermediate.**

Figure 7 above showed the results of antibiotic sensitivity testing conducted on *Ralstonia pickettii* isolated at **BMC**. The data indicates that the isolated *Ralstonia pickettii* is sensitive to commonly used antibiotics recording a percentage of 92% Piperacillin/Tazobactam, Cefazolin, Ceftriaxone, Cefepime, Imipenem, Gentamicin, Ciprofloxacin, Tigecycline, Levofloxacin, Trimethoprim/ Sulfamethoxazole and Amikacin. Additionally, it showed intermediate sensitivity recording a percentage of 8% Ceftazidime exclusively.

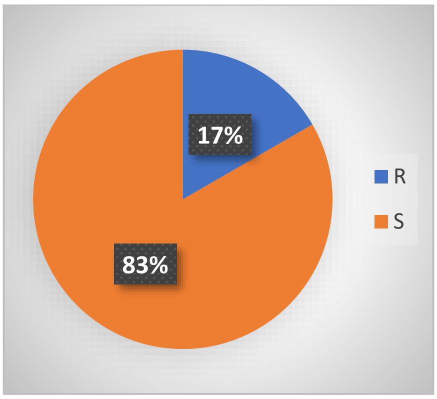


**Figure 8: Percentage of antibiotic sensitivity results of *Pseudomonas putida* against fourteen antibiotics. R = resistant and S = sensitive.**

Figure 8 above presents the results of antibiotic sensitivity testing conducted on *Pseudomonas putida* isolated at **BMC**. The data indicates that the isolated *Pseudomonas putida* is sensitive to commonly used antibiotics recording a percentage of 83% to each of Piperacillin/Tazobactam, Ceftazidime, Cefepime, Imipenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline, Levofloxacin, and Trimethoprim/ Sulfamethoxazole. Additionally, it exhibits resistance sensitivity to only two antibiotics, specifically Cefazolin and Ceftriaxone recording a percentage of 17%. On the other hand, each of ESBL, Ampicillin and Nitrofurantoin showed a negative result.

**Table 7: Biochemical reactions using VITEK test for gram-negative isolates isolated at BMC and UTR.**

| Bacterial isolates | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 |
|---|---|---|---|---|---|---|---|---|
| BMC1/UTR1 | - | + | + | - | + | + | - | + |
| BMC2/UTR2 | - | + | - | + | + | - | - | - |
| BMC3/UTR3 | - | + | - | + | + | - | - | - |
| BMC4/UTR4 | - | - | - | - | - | - | - | - |
| **Bacterial isolates** | **T9** | **T10** | **T11** | **T12** | **T13** | **T14** | **T15** | **T16** |
| BMC1/UTR1 | - | + | + | + | + | + | + | + |
| BMC2/UTR2 | - | + | - | - | + | + | + | + |
| BMC3/UTR3 | - | + | - | - | + | + | + | + |
| BMC4/UTR4 | - | + | + | - | - | - | - | - |
| **Bacterial isolates** | **T17** | **T18** | **T19** | **T20** | **T21** | **T22** | **T23** | **T24** |
| BMC1/UTR1 | + | - | + | - | + | + | + | + |
| BMC2/UTR2 | - | - | - | - | + | - | - | + |
| BMC3/UTR3 | - | - | - | - | + | - | - | + |
| BMC4/UTR4 | - | + | + | - | - | + | - | - |
| **Bacterial isolates** | **T25** | **T26** | **T27** | **T28** | **T29** | **T30** | **T31** | **T32** |
| BMC1/UTR1 | + | - | + | + | + | - | + | - |
| BMC2/UTR2 | + | + | + | - | - | - | - | - |
| BMC3/UTR3 | + | + | + | - | - | - | - | - |
| BMC4/UTR4 | - | - | - | + | - | - | + | - |
| **Bacterial isolates** | **T33** | **T34** | **T35** | **T36** | **T37** | **T38** | **T39** | **T40** |
| BMC1 | + | - | + | + | + | - | + | - |
| BMC2 | - | - | - | - | - | - | - | - |
| BMC3 | - | - | - | - | - | - | - | - |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **BMC4** | + | - | - | - | - | - | - | + |
| **Bacterial isolates** | **T41** | **T42** | **T43** | **T44** | **T45** | **T46** | **T47** | |
| **BMC1/UTR1** | + | - | + | - | - | - | - | |
| **BMC2/UTR2** | + | - | - | - | - | - | - | |
| **BMC3/UTR3** | - | - | - | - | - | - | - | |
| **BMC4/UTR4** | + | - | + | - | + | - | + | |

**BMC1= Klebsiella penumoniae ssp penumoniae, MBC2= Rhizobium radiobacter, BMC3= Ralstonia pickettii, BMC4= Pseudomonas putida**

**T1**=APPA, **T2**= ADO, **T3**= PyrA, **T4**= IARL, **T5**= dCEL, **T6**= BGAL, **T7**= $H_2S$, **T8**= BNAG, **T9**= AGLTp, **T10**= dGLU, **T11**= GGT, **T12**= OFF, **T13**= BGLU, **T14**= dMAL, **T15**= dMAN, **T16**= dMNE, **T17**= BXYL, **T18**= BAlap, **T19**= ProA, **T20**= LIP, **T21**= PLE, **T22**= TyrA, **T23**= URE, **T24**= dSOR, **T25**= SAC,**T26**= dTAG, **T27**= dTRE, **T28**= CIT,**T29**= MNT, **T30**= 5KG,**T31**= ILATk,**T32**=AGLU,**T33**= SUCT, **T34**= NAGA, **T35**= AGAL, **T36**= PHOS, **T37**= GlyA,**T38**= ODC, **T39**= LDC, **T40**= IHISa, **T41**= CMT, **T42**= BGUR, **T43**= O129R, **T44**= GGAA, **T45**= IMLTa, **T46**= ELLM, **T47**= ILATa

**Table 8: Biochemical reactions using VITEK test for gram-positive isolates isolated from BMC**

| **Bacterial isolates** | **T1** | **T2** | **T3** | **T4** | **T5** | **T6** | **T7** | **T8** |
|---|---|---|---|---|---|---|---|---|
| **BMC5/UTR5** | - | - | - | + | + | + | - | - |
| **BMC6/UTR6** | - | - | - | + | - | + | - | - |
| **Bacterial isolates** | **T9** | **T10** | **T11** | **T12** | **T13** | **T14** | **T15** | **T16** |
| **BMC5/UTR5** | - | - | - | + | - | - | - | - |
| **BMC6/UTR6** | - | - | - | + | - | - | - | - |
| **Bacterial isolates** | **T17** | **T18** | **T19** | **T20** | **T21** | **T22** | **T23** | **T24** |
| **BMC5/UTR5** | + | - | - | - | - | + | + | + |
| **BMC6/UTR6** | + | - | - | - | - | - | + | + |
| **Bacterial isolates** | **T25** | **T26** | **T27** | **T28** | **T29** | **T30** | **T31** | **T32** |
| **BMC5/UTR5** | - | + | + | - | + | + | + | + |
| **BMC6/UTR6** | - | - | - | - | + | + | - | + |
| **Bacterial isolates** | **T33** | **T34** | **T35** | **T36** | **T37** | **T38** | **T39** | **T40** |
| **BMC5/UTR5** | - | - | - | - | - | + | - | + |
| **BMC6/UTR6** | + | + | + | - | - | + | - | + |
| **Bacterial isolates** | **T41** | **T42** | **T43** | | | | | |
| **BMC5/UTR5** | + | - | + | | | | | |
| **BMC6/UTR6** | + | - | + | | | | | |

**BMC5= Staphylococcus hominis spp, BMC6= Staphylococcus aureus**

**T1**=AMy, **T2**= PIPLC, **T3**= dXYL, **T4**= ADHI, **T5**= BGAL, **T6**= AGLU, **T7**= APPA, **T8**= CDEX, **T9**= AspA, **T10**= BGAR, **T11**= AMAN, **T12**= PHOS, **T13**= LeuA, **T14**= ProA, **T15**= BGURr, **T16**= AGAL, **T17**= PyrA, **T18**= BGUR, **T19**= ALaA, **T20**= TyrA, **T21**= dSOR, **T22**= URE, **T23**= POLYB, **T24**= dGAL, **T25**= dRIB,**T26**= ILATk, **T27**= LAC, **T28**= NAG,**T29**= dMAL, **T30**= BACI,**T31**= NOVO,**T32**=NC6.5,**T33**= dMAN, **T34**= dMNE, **T35**= MBdG, **T36**= PUL, **T37**= dRAF,**T38**= O129R, **T39**= SAL, **T40**= SAC, **T41**= dTRE, **T42**= ADH2s, **T43**= OPTO

Table 7 presents the results of biochemical reactions for gram-negative bacteria, while table 8 depicts biochemical reactions for gram-positive bacteria using VITEK 2 Compact Machine. The study involved isolating and characterizing bacterial populations at **BMC** and **UTR** area in triplicate. This led to the characterization of the most frequently isolated genera, along with their antibiotic sensitivity. The investigation identified bacterial genera, including *Staphylococcus hominis, Staphylococcus aureus, Klebsiella pneumoniae*, *Rhizobium radiobacter*, *Ralstonia pickettii,* and *Pseudomonas putid.*

**Discussion:** In the present study, the altogether 192 samples collected from the four distinct wastewater stations at **BMC** and **UTR** exhibit diverse physical characteristics (14). The isolates were identified based on phenotypic characteristics and biochemical reactions, utilizing VITEK 2 diagnosis (11,12). Wastewater contains a mixture of bacterial pollutants, simple and complex organic compounds, and chemical compounds from hospitals as medical waste (13). This blend serves as a conducive medium for microbial growth due to nutrient availability. Microbes in such environments may develop characteristics harmful to humans, such as antibiotic resistance (14). The 192 samples collected from Upstream Tigris River were used as a control to discern the differences in the number of pathogenic bacterial groups. These groups, although similar, were found in both places with varying numbers of isolates (5, 6 and 7). Station one at **BMC**, produces a light grey color typical of fresh sewage, while older sewage appears as medium grey due to anaerobic activities (36). The samples are characterized by a basic pH, conducive to the thriving of microorganisms within a pH range of 6.5 to 8.5 (37), Promoting the growth and proliferation of numerous microorganisms. Despite this, certain microorganisms can endure extreme pH levels and flourish in both acidic and basic environments. Factors contributing to the abundance of the isolated spacious in this study from wastewater include high turbidity. Turbidity, associated with increased bacterial densities. (38, 39), provides food and shelter for pathogens and environmental microorganism. If not addressed, the causes of high turbidity can facilitate the regrowth of microorganism in the water. Wastewater samples from stations two and three at **BMC**, exhibit a medium gray color and medium turbidity, while station four samples at **BMC**, display gray-brown color with medium turbidity. The variation in color and turbidity of sewage may be attributed to differences in water usage among various hospitals, where some have open sewage, exposing them to the various forms of contamination (36 and 39). Both collected samples at **BMC** and **UTR** were statistically different in terms of isolates counts with ($P < 0.0001$). The variation in number of pathogenic bacteria could be belong to many reasons for example the origin of contamination introduction of contaminated sewage into Tigris River that were originally originated at **BMC** brings up a diverse range of bacteria associated with humans, potentially leading to a higher presence of pathogenic strains (5,6,7 and 8). This source is likely responsible for a greater number of pathogenic isolates compared to the relatively cleaner Upstream River. In addition to that, availability of nutrients may create a more conducive environment for the growth of pathogenic bacteria (25 and 26). Consequently, this can lead to elevated concentrations of pathogenic strains in contrast to the natural conditions of the Upstream River (8). The direct discharge of sewage produced at **BMC** can significantly influence the composition of bacteria because pathogenic bacteria linked to human waste may thrive in these conditions, resulting in a higher count. The inadequate treatment of sewage and dispose it directly to Tigris River may harbor a greater diversity and abundance of pathogenic bacteria. Conversely, the Upstream River might maintain a more natural microbial balance with fewer pathogenic strains (6 and 7). The variation of ecological factors between both places were samples collected may selectively support the growth and survival of specific pathogenic bacteria, contributing to the observed differences (27). This study suggests that proper disposal of medical pollutants, avoiding direct discharge into wastewater sewers leading to the Tigris River, can reduce antibiotic resistance observed in microbes isolated from wastewater (15). The presence of some pathogenic isolates in wastewater emphasizes the importance of purifying and disinfecting wastewater at **BMC** to preserve human health (13). Susceptibility testing, is a technique used to assess antibiotic effectiveness against bacterial isolates, may lack comprehensiveness when applied to 10-20% of isolate from a group of collected isolates belonging to the same genera (52). However, such a limitation stems from various factors. First, resource constraints, encompassing limited time and high facility costs, can make testing of 10-20% of selected isolates as a challenge in this research. In such scenarios, opting to test a representative isolate becomes a practical approach (53). Second, it serves as a preliminary screening, occasionally, testing 10-20% of isolates initially can function as a screening step. If the tested isolates demonstrate sensitivity, it suggests a higher probability that other isolates will respond similarly (54). The third reason supporting sensitivity testing in this study is the homogeneous source. Since the isolates originate from a relatively homogenous environment, with consistent conditions, assuming similar sensitivity profiles becomes more plausible. To address bacterial infections, antibiotics are commonly used. However, bacterial resistance to antibiotics indicates a change in the microbes' response mechanism, emphasizing the need for effective antibiotic management (16).

The bacterial group ***Staphylococcus hominis*** a facultatively anaerobic, gram-positive bacterium, is typically found on the human skin and is generally considered benign. However, individuals with a low immune response may occasionally experience infections leading to conditions such as bacteremia endocarditis, and septicemia (23). *Staphylococcus hominis* recorded totaling count 7 over the four months at **BMC**. While isolates from **UTR** recorded totaling count 21 over the four months with September having the highest count at 7 isolates. *Staphylococcus hominis* showed a significant difference regarding the isolate's numbers compared to the same bacterial groups isolated from **UTR** with ($P < 0.00008$). The primary mode of transmission for *Staphylococcus hominis* is through direct or indirect contact, particularly with contaminated objects or individuals (24).

The bacterial group ***Staphylococcus aureus*** is responsible for many human diseases, including soft tissue infections and the formation of pus in certain wounds associated with secondary infections. *Staphylococcus aureus* is commonly known for causing skin and tissue infections of the skin and some tissues in humans. While infection with *Staphylococcus* itself is not typically dangerous, except in cases of open wounds or first and second-degree burns, the presence of this bacteria can lead to septicemia, a potentially fatal infection (23). In this study, this isolate recorded the same highest count like *Klebsiella pneumoniae* among other isolates, totaling 37 over the four months at **BMC**, with November having the highest count at 11 isolates. *Staphylococcus aureus* showed a significant difference regarding the isolate's numbers compared to the same bacterial groups isolated from **UTR** with ($P < 0.000001$).

The bacterial group ***Klebsiella pneumoniae*** isolated from sewage water at **BMC** exhibited a significant difference in terms of isolates numbers compared to the same bacterial groups isolated from **UTR** with ($P < 0.000019$). *Klebsiella pneumoniae* is responsible for many diseases associated with the human body (30, 32). This bacterial group recorded 37 counts over the four months at **BMC** samples, October was the highest with 11 isolates. In contrast, the same bacterial group isolated from **UTR** recorded 2 counts and only one for each September and October. When *Klebsiella pneumoniae* infects the human lungs, it could result in pneumonia. Similarly, if the same bacteria are present in the bloodstream, it can cause a blood infection. This type of bacteria could also lead to a severe infection of the urinary system if present, sometimes causing multiple urinary system infections (17).

The bacterial group ***Rhizobium radiobacter*** isolated from sewage water at **BMC** presents a significant difference in terms of isolates numbers compared to the same bacterial groups isolated from **UTR** with ($P < 0.000003$). *Rhizobium radiobacter* is an aerobic, gram-negative bacterium, shares close genetic ties with various plant pathogens found globally in soil (20). This isolate recorded totaling count 10 over the four months, with October having the highest count at 4 isolates at **BMC**. In contrast, from **UTR** the isolate recorded totaling count 25 over four months, with December having the highest count at 8 isolates.

The bacterial group ***Ralstonia pickettii*** isolated from sewage water at **BMC** displayed a significant difference regarding the isolate's numbers compared to the same bacterial groups isolated from **UTR** with ($P < 0.00004$)**.** *Ralstonia pickettii* considered as an opportunistic, gram-negative pathogen known for its low virulence (21). This isolate recorded totaling count 8 over the four months, with October having the highest count at 3 isolates at **BMC**. In contrast, from **UTR** the isolate recorded totaling count 24 over four months, with October having the highest count at 8 isolates. It is commonly found in hospital settings and has the potential to cause bacteremia. This bacterium is associated with the contamination of medical products and primarily infects immunosuppressed patients. Its main habitat is soil, leading to common diseases known as bacterial wilt (35).

The bacterial group ***Pseudomonas putida*** categorized as a specialized aerobic member of the fluorescent group within *Pseudomonas* species, is renowned for its role as a fish pathogen and its ability to form colonies in the human throat. Additionally, it serves as an infrequent cause of soft tissue and skin infections. It is regularly associated with an immunocompromised state or trauma. In specific instances, it may lead to bacteremia arising from soft tissue and skin infections, with immobility, malnutrition, and peripheral vascular disease identified as risk factors (22). This isolate recorded totaling count 7 over the four months, with October having the least count with only one isolate at **BMC**. In contrast, from **UTR** the isolate recorded totaling count 23 over four months, with November having the highest count at 7 isolates. *Pseudomonas putida* showed a significant difference regarding the isolate's numbers compared to the same bacterial groups isolated from **UTR** with ($P < 0.00046$).

According to (57), 10 - 20% of each bacterial group linked to the same isolation source and time underwent an antibiogram to achieve an initial comprehensive assessment. Testing a subset enhances the likelihood of capturing potential variations in antibiotic resistance within the larger group leading to more robust data guiding antibiotic treatment decisions. In this study, the isolates collected at **BMC** were screened initially as follows seven isolates were chosen from each *Staphylococcus aureus* and *Klebsiella pneumoniae* which represent 20% of the total isolates count underwent an antibiogram test. Additionally, *Staphylococcus hominis* and *Pseudomonas putida* represented by two isolates each, were also tested. *Rhizobium radiobacter* and *Ralstonia pickettii* followed the same pattern with two isolates each subjected to the test. Initial screening occurred per genus. Subsequently, the observed results revealed an interesting antibiogram resistance result for each of *Klebsiella pneumoniae, Staphylococcus aureus*, *Staphylococcus hominis and Pseudomonas putida 76%, 62%, 61% and 17% respectively.* Promoting further investigations on each genus alone. *Staphylococcus aureus* showed resistant to commonly prescribed antibiotics at **BMC** by medical staff, and increased resistance against Tobramycin and Tetracycline (see Figure 4), these findings match with the findings of (28 and 33). *Staphylococcus hominis,* showed resistant to commonly prescribed antibiotics at **BMC** by medical staff, and increased resistance against Gentamicin, Tobramycin and Clindamycin (see Figure 3). These findings match with the findings of (59 and 60). *Staphylococcus* species isolated from sewage water may exhibit noteworthy resistance, particularly towards diverse antibiotics, especially penicillin. Certain strains display resistance to multiple drugs as well. *Staphylococcus aureus* is expected to show higher resistance levels, and findings from *Staphylococcus hominis* cannot be safely generalized to *Staphylococcus aureus*. In this study, both stains kept separated even in testing (56).

*Klebsiella pneumoniae* reported to possess one or more Ambler class B-lactamases. Classes A, B, C, & D all have been reported in *K. pneumoniae* or in other species. In this study some of these antibiotics were tested; cefpodoxime, cefotaxime, ceftriaxone, ceftazidime, and aztreonam (for ESBLs) and cefoxitin (for AmpC B-lactamases) (55 and 56). Conducted results indicate that *Klebsiella pneumoniae* showed remittance to most common antibiotics prescribed at **BMC** by medical staff (see Figure 5) belongs to the blakPC genes within *Klebsiella pneumoniae* (29,30). These genes are predominantly transported on plasmids, resulting in reduced sensitivity or resistance to nearly all beta-lactam antibiotics. The worldwide control of bacterial diseases faces a substantial challenge due to the dissemination of these resistance genes (31).

*Rhizobium radiobacter* showed sensitivity response to most tested antibiotics (see Figure 6) and intermediate response against Ceftazidime exclusively. While findings for (61) revealed that the isolated *Rhizobium radiobacter* from patients with peritonitis in a peritoneal dialysis showed sensitive results against Ceftazidime and used as treatment. This bacterium has been linked to systemic diseases in humans, encompassing peritonitis, urinary tract infections, cellulitis and myositis (20,34). Regarding *Rhizobium radiobacter*, the species itself contains different species with different susceptibility profiles (21,35). Further investigations need to be considered in future.

*Ralstonia pickettii* displayed sensitivity to most tested antibiotics and intermediate resistance to only Ceftazidime (see Figure 7). This bacterium showed resistance to Ceftazidime as findings in (62). *Pseudomonas putida* showed sensitivity to commonly tested antibiotics and resistance against Cefazolin and Ceftriaxone exclusively (29) (see Figure 8). *Ralstonia* and *Pseudomonas* are widely distributed in soil and intrinsic resistance within their species is expected. However, *Pseudomonas putida* is usually more susceptible than other *Pseudomonas* species, especially the notorious *aeruginosa* species.

Despite the geographical distance between sampling points, the identification of *Staphylococcus aureus* and *Klebsiella pneumoniae* in the total 192 samples (106 at **BMC** isolates) implies a widespread presence of these strains in hospital wastewaters. The isolated species were suggested to have potential cause of infections in humans. ***Staphylococcus aureus,*** commonly found in human and animal skin and nails, can act as an opportunistic pathogen, causing various infections due to the production of virulence factors. This isolate is prevalent in sewage across all four stations, suggests its ability to multiply in this environment (40). Given that fecal matter is a significant component of domestic sewage and a source of most human pathogens in wastewater (41), disease-causing organisms from humans can enter the wastewater especially from patients at hospitals or individuals carrying diseases (37). While ***Klebsiella pneumoniae*** is responsible on pneumonia, meningitis, urinary tract infections, and bloodstream infections (12,17). Many infections are linked to water exposure, either through symbiotic relationship with these bacteria or the consumption of contaminated food and water. This isolate is present in sewage across all four stations. This could explain the presence of these isolates in hospital sewage, possibly originating from clinical and environmental contamination (42). Therefore, the Tigris River area where samples were collected poses a high risk of contamination with ***Staphylococcus aureus*** and ***Klebsiella pneumoniae*** when sewage water combines with Tigris River water. ***Staphylococcus hominis*** found in sewage across all four station is associated with furunculosis, infected cysts, and vulvar infections, ***Pseudomonas putida, Rhizobium radiobacter and, Ralstonia pickettii*** were found abundantly in hospitals sewage at all four stations. These isolates likely come from the surrounding environment, including soil, air and water. Introductions into the hospital sewage system may occurred through contaminated surfaces, equipment or personal activities (43). Certain hospitals procedures using water or similar solutions could harbor these bacteria, and improper disposal practices afterward may contribute to their presence in sewage. The bacteria may also originate from normal flora of patients admitted to hospitals and the waste generated from patient care, for example bodily fluids or the use of medical equipment (44). Accidental spills or improper disposal of laboratory waste might further introduce these bacteria into hospital wastewater. The complex network of sewage systems plays a role in the transportation and survival of various microorganism, with factors like pH, temperature, and nutrient availability influencing their persistence in the sewage system (45).

The wastewater environment serves as a significant reservoir for antibiotic-resistant bacteria. The current study reveals that ***Klebsiella pneumoniae*** bacterial strain, exhibits resistance to commonly used antibiotics. The same finding reported in previous studies that confirm the resistant activity of *Klebsiella ssp* (46,47 and 48). This resistance, attributed to chromosomal Ab lactamase, poses a serious public health concern, aligning with global recommendation by The WHO (49). The current study emphasizes the risk of antibiotic resistance dissemination through hospital sewage discharge into environmental wastewater, particularly in setting lacking sewage treatment plants (37). Uncontrolled antibiotic use and self-medication contribute to the selection pressure favoring resistant organisms, compounding the issue. ***Klebsilla pneumoniae,*** a significant pathogen capable of extended- spectrum b-lactamase (ESBL) production, exhibited both sensitivity and resistance to various antibiotic in the current study. Both ***Staphylococcus aureus*** and ***Staphylococcus hominis,*** present resistance to most tested antibiotics (18). While isolates sensitive to antibiotics pose no threat. The rising antibiotic resistance in ***Klebsilla pneumoniae***, ***Staphylococcus aureus*** and ***Staphylococcus hominis,*** especially in residential wastewaters, emerges as a critical public health concern (46 and 47). The discharge of hospital sewage into environmental wastewaters increases the risk of spreading antibiotic resistance, further compounded by ineffective or lacking sewage treatment in many hospitals, notably in developed countries (50, 51). The uncontrolled use of antibiotics and self-medication contributes to a selection pressure favoring organisms with antibiotic resistance genes, adding to the complexity of this issue.

**Conclusion:** Before releasing wastewater into the Tigris River, it is crucial to pre-treat it to eliminate chemical compounds, particularly antibiotics, which might lead to bacterial antibiotic resistance. Pathogenic bacteria developing resistance to antibiotics results in more challenging infectious diseases making them harder for specialists to treat compared to non-resistant counterparts. Antibiotic resistance entails simultaneous health and economic consequences. Extended hospitalization periods for patients with bacterial infections become necessary, potentially leading to increased death rates if bacterial cause is not eliminated. From an economic perspective, higher medical costs are incurred when resorting to alternative, more expensive antibiotics. Adherence to antibiotic prescription guidelines by licensed medical professionals is essential. Despite the development of new antibiotics through global commercial competition, antibiotic resistance remains a significant threat unless the general public changes its behavior regarding antibiotic use. This change must involve measures to reduce the spread of bacterial infections, including vaccinations and promoting general hygiene practices such as handwashing and ensuring food cleanliness. Oversight of the pharmacies and their employees is crucial, along with continuous guidance from governmental medical organizations on the dangers of indiscriminate antibiotic use. A thorough inquiry would be required, taking into account hospital procedures, patient demographics, waste management procedures, and the overall hospital surroundings environment.

**Recommendation:** Based on our research findings, we strongly advocate performing comprehensive whole-genome sequencing for the three isolates ***Klebsiella pneumoniae, Staphylococcus aureus,*** and ***Staphylococcus hominis***- exhibiting resistance to the majority of the assessed antibiotics. Specifically, we urge the identification of resistance genes inherent in each bacterial strain, notably focusing on the B-Lactamase gene prevalent in *Klebsiella* bacteria. This approach would significantly enrich the study by providing a detailed understanding of their genetic makeup. However, it's important to note that the investigation of other genes associated with pathogenicity and reproductive mechanisms is beyond the scope of our study.

**References:**


1. Teixeira, F.J. and Gomes, C.S., 2021. Mineral water: essential to life, health, and wellness. In *Minerals latu sensu and Human Health: Benefits, Toxicity and Pathologies* (pp. 517-556). Cham: Springer International Publishing.

2. Caruso, G., 2013. Microbes and their use as indicators of pollution. *J Pollut Eff Cont*, *1*, p.e102.

3. Saxena, G., Bharagava, R.N., Kaithwas, G. and Raj, A., 2015. Microbial indicators, pathogens and methods for their monitoring in water environment. *Journal of water and health*, *13*(2), pp.319-339.

4. Maizel, A.C. and Remucal, C.K., 2017. The effect of advanced secondary municipal wastewater treatment on the molecular composition of dissolved organic matter. *Water research*, *122*, pp.42-52.

5. Warqa'a, N.M. and Muhammed, N.A., 2014. Pollutionary effect of the Medical city waste water on the Tigris river bacterial indicators on Baghdad city. *Iraqi Journal of Science*, *55*(1), pp.106-112.

6. AK Al-Hiyaly, S., Ma'alah, W.N. and N AL-Azzawi, M., 2016. Evaluating the effects of medical city wastewater on water quality of Tigris River. *Engineering and Technology Journal*, *34*(3), pp.405-417.

7. Al Sultan, A.A., Kadhem, R.J. and Alabdraba, W.M., 2017. Evaluation of the quality of disposed wastewater in selected hospitals in Baghdad City. *Journal of Environmental Studies*, *17*(1), pp.35-41.

8. Abd Al Satar, N.H. and Sachit, D.E., 2021. ASSESSMENT OF HOSPITAL WASTEWATER QUALITY AND MANAGEMENT IN BAB-AL MUADHAM REGION AT BAGHDAD. *Journal of Engineering and Sustainable Development*, *25*(3), pp.44-50.

9. Abed, A.N., Ahmed, O., Abdulmajeed, M. and Khazaal, Y.M., 2021. Evaluate Multi-Drug Resistance Pattern of Microbial Flora from Hospital Premises of Baghdad Hospitals. *Journal of Teikyo medical* https://www.teikyomedicaljournal.com. ISSN: 03875547 Volume 44, Issue 06, December.

10. Pacarynuk, L.A. and Danyk, H.C., 2004. Biochemical Tests. *Principles of Microbiology, Laboratory Manual, Spring, TX, USA*, pp.28-34.

11. Clarke, S.C., 2004. Collins and Lyne's Microbiological Methods. *British Journal of Biomedical Science*, *61*(2), p.113.

12. Sib, E., Lenz-Plet, F., Barabasch, V., Klanke, U., Savin, M., Hembach, N., Schallenberg, A., Kehl, K., Albert, C., Gajdiss, M. and Zacharias, N., 2020. Bacteria isolated from hospital, municipal and slaughterhouse wastewaters show characteristic, different resistance profiles. *Science of The Total Environment*, *746*, p.140894.

13. Zhang, Xiaolei, Song Yan, Jiaxin Chen, R. D. Tyagi, and Ji Li. 2020 "Physical, chemical, and biological impact (hazard) of hospital wastewater on environment: presence of pharmaceuticals, pathogens, and antibiotic-resistance genes." In *Current Developments in Biotechnology and Bioengineering*, pp. 79-102. Elsevier, 2020.

14. Kümmerer, K. and Henninger, A., 2003. Promoting resistance by the emission of antibiotics from hospitals and households into effluent. *Clinical microbiology and infection*, *9*(12), pp.1203-1214.

15. Kumar, A. and Pal, D., 2018. Antibiotic resistance and wastewater: Correlation, impact and critical human health challenges. *Journal of environmental chemical engineering*, *6*(1), pp.52-58.

16. Myo Clinic website ., 2023. Flu shot: Your best bet for avoiding influenza. *1998-2024 Mayo Foundation for Medical Education and Research (MFMER). All rights reserved.* https://www.mayoclinic.org/diseases-conditions/flu/in-depth/flu-shots/art-20048000

17. Seguni, N.Z., Kimera, Z.I., Msafiri, F., Mgaya, F.X., Joachim, A., Mwingwa, A. and Matee, M.I., 2023. Multidrug-resistant *Escherichia coli and Klebsiella pneumoniae* isolated from hospital sewage flowing through community sewage system and discharging into the Indian Ocean. *Bulletin of the National Research Centre*, *47*(1), p.66.

18. Thompson, J.M., Gündoğdu, A., Stratton, H.M. and Katouli, M., 2013. Antibiotic resistant *Staphylococcus aureus* in hospital wastewaters and sewage treatment plants with special reference to methicillin-resistant *Staphylococcus aureus* (MRSA). *Journal of applied microbiology*, *114*(1), pp.44-54.

19. Abd Al Satar, N.H. and Sachit, D.E., 2021. ASSESSMENT OF HOSPITAL WASTEWATER QUALITY AND MANAGEMENT IN BAB-AL MUADHAM REGION AT BAGHDAD. *Journal of Engineering and Sustainable Development*, *25*(3), pp.44-50.

20. Nakayama, M., Nakajima-Kambe, T., Katayama, H., Higuchi, K., Kawasaki, Y. and Fuji, R., 2008. High catalase production by *Rhizobium radiobacter* strain 2-1. *Journal of bioscience and bioengineering*, *106*(6), pp.554-558.

21. Nasir, N., Sayeed, M.A. and Jamil, B., 2019. *Ralstonia pickettii* bacteremia: an emerging infection in a tertiary care hospital setting. *Cureus*, *11*(7).

22. Singh, P., Montano, A. and Bostick, A., 2021. Rapid severe sepsis from *Pseudomonas fluorescens/putida* bacteremia due to skin and soft tissue infection–a case report. *Annals of Medicine and Surgery*, *70*, p.102845.

23. Worthington, T., Lambert, P.A. and Elliott, T.S.J., 2000. Is hospital-acquired intravascular catheter-related sepsis associated with outbreak strains of coagulase-negative staphylococci?. *Journal of Hospital Infection*, *46*(2), pp.130-134.

24. Macía-Heras, M., Donate-Correa, J., Alcoba-Flórez, J., Pulido-Reyes, G., Martín-Nú, E., Martín-Padrón, J.J. and Méndez, S., 2013. Daptomycin, Methicillin Resistant *Staphylococcus hominis Catheter*-Related Bacteraemia in a Hemodialysis Patient.

25. Fida, M., Li, P., Wang, Y., Alam, S.K. and Nsabimana, A., 2023. Water contamination and human health risks in Pakistan: a review. *Exposure and Health*, *15*(3), pp.619-639.

26. Zhou, X., Zhou, X., Wang, C. and Zhou, H., 2023. Environmental and human health impacts of volatile organic compounds: A perspective review. *Chemosphere*, *313*, p.137489.

27. Jones, E.R., Bierkens, M.F., Wanders, N., Sutanudjaja, E.H., van Beek, L.P. and van Vliet, M.T., 2022. Current wastewater treatment targets are insufficient to protect surface water quality. *Communications Earth & Environment*, *3*(1), p.221.

28. De Neeling, A.J., Van den Broek, M.J.M., Spalburg, E.C., van Santen-Verheuvel, M.G., Dam-Deisz, W.D.C., Boshuizen, H.C., Van De Giessen, A.W., Van Duijkeren, E. and Huijsdens, X.W., 2007. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Veterinary microbiology*, *122*(3-4), pp.366-372.

29. Giacometti, A., Cirioni, O., Schimizzi, A.M., Del Prete, M.S., Barchiesi, F., D'Errico, M.M., Petrelli, E. and Scalise, G., 2000. Epidemiology and microbiology of surgical wound infections. *Journal of clinical microbiology*, *38*(2), pp.918-922.

30. Rostkowska, O.M., Kuthan, R., Burban, A., Salińska, J., Ciebiera, M., Młynarczyk, G. and Durlik, M., 2020. Analysis of susceptibility to selected antibiotics in *Klebsiella pneumoniae*, Escherichia coli, Enterococcus faecalis and Enterococcus faecium causing urinary tract infections in kidney transplant recipients over 8 years: single-center study. *Antibiotics*, *9*(6), p.284.

31. Ghasemnejad, A., Doudi, M. and Amirmozafari, N., 2019. The role of the blaKPC gene in antimicrobial resistance of Klebsiella pneumoniae. *Iranian journal of microbiology*, *11*(4), p.288.

32. Fernández-Natal, I., Saez-Nieto, J.A., Medina-Pascual, M.J., Albersmeier, A., Valdezate, S., Guerra-Laso, J.M., Rodríguez, H., Marrodán, T., Parras, T., Tauch, A. and Soriano, F., 2013. Dermabacter hominis: a usually daptomycin-resistant gram-positive organism infrequently isolated from human clinical samples. *New microbes and new infections*, *1*(3), pp.35-40.

33. Aniba, R., Barguigua, A., Dihmane, A., Momen, G., Nayme, K. and Timinouni, M., 2023. Prevalence and antibacterial resistance patterns of uropathogenic staphylococci in Casablanca, Morocco. *The Journal of Infection in Developing Countries*, *17*(10), pp.1436-1445.

34. Villacís, J.E., Castelán-Sánchez, H.G., Rojas-Vargas, J., Rodríguez-Cruz, U.E., Albán, V., Reyes, J.A., Meza-Rodríguez, P.M., Dávila-Ramos, S., Villavicencio, F., Galarza, M. and Gestal, M.C., 2023. Emergence of Raoultella ornithinolytica in human infections from different hospitals in Ecuador with OXA-48-producing resistance. *Frontiers in Microbiology*, *14*.

35. Batarilo, I., Maravic-Vlahovicek, G., Bedenic, B., Kazazic, S., Bingulac-Popovic, J., Slade-Vitkovic, M., Katić, S. and Jukic, I., 2022. Oxacillinases and antimicrobial susceptibility of *Ralstonia pickettii* from pharmaceutical water systems in Croatia. *Letters in Applied Microbiology*, *75*(1), pp.103-113.

36. Vitenko, T., Marynenko, N. and Kramar, I., 2023. Characteristics of sewage sludge composition for agricultural use.

37. Tomas, M.V.G., Tuliao, N.R.A.S., Tubban, J.K., Tumbaga, D.J.B. and Urmanita, F.F.S.,2021. Isolation and Characterization of Pathogenic Bacteria in Sewage Wastewaters in Tuguegarao City Cagayan.

38. Yuan, T. and Pian, Y., 2023. Hospital wastewater as hotspots for pathogenic microorganisms spread into aquatic environment: A review. *Frontiers in Environmental Science*, *10*.

39. Neidhöfer, C., Sib, E., Neuenhoff, M., Schwengers, O., Dummin, T., Buechler, C., Klein, N., Balks, J., Axtmann, K., Schwab, K. and Holderried, T.A., 2023. Hospital sanitary facilities on wards with high antibiotic exposure play an important role in maintaining a reservoir of resistant pathogens, even over many years. *Antimicrobial Resistance & Infection Control*, *12*(1), pp.1-17

40. Silva, V., Pereira, J.E., Maltez, L., Poeta, P. and Igrejas, G., 2022. Influence of Environmental Factors on Biofilm Formation of Staphylococci Isolated from Wastewater and Surface Water. *Pathogens*, *11*(10), p.1069.

41. Sarizadeh, G., Geravandi, S., Takdastan, A., Javanmaerdi, P. and Mohammadi, M.J., 2022. Efficiency of hospital wastewater treatment system in removal of level of toxic, microbial, and organic pollutant. *Toxin Reviews*, *41*(3), pp.721-730.

42. Rocha, J., Henriques, I., Gomila, M. and Manaia, C.M., 2022. Common and distinctive genomic features of Klebsiella pneumoniae thriving in the natural environment or in clinical settings. Scientific Reports, 12(1), p.10441.

43. Azuma, T., Katagiri, M., Sekizuka, T., Kuroda, M. and Watanabe, M., 2022. Inactivation of bacteria and residual antimicrobials in hospital wastewater by ozone treatment. Antibiotics, 11(7), p.862.(Pseudomonas putida)

44. Khleifat, K.M., Khalil, M.M., Al-kafaween, M.A., Alqaraleh, M., Al-limoun, M.O., Al-Qaisi, T.S., Farah, H.S., Nijris, O.N., Al-Tarawneh, A., Qaralleh, H. and Hajleh, M.N.A., 2022. Studying the relationship between women hormonal activity and the distribution of bacterial vaginosis and bacteria's antibiotics susceptibility. Journal of Applied Pharmaceutical Science, 12(12), pp.105-116. (Rhizobium radiobacter)

45. Becerra-Castro, C., Machado, R.A., Vaz-Moreira, I. and Manaia, C.M., 2015. Assessment of copper and zinc salts as selectors of antibiotic resistance in Gram-negative bacteria. Science of the Total Environment, 530, pp.367-372. (Ralstonia pickettii)

46. Amin, A., Ghumro, P.B., Hussain, S. and Hameed, A., 2009. Prevalence of antibiotic resistance among clinical isolates of Klebsiella pneumoniae isolated from a Tertiary Care Hospital in Pakistan. *Malaysian Journal of Microbiology*, *5*(2), pp.81-86.

47. Yolbas, I., Tekin, R., Kelekci, S., Tekin, A., Okur, M.H., Ece, A., Gunes, A. and Sen, V., 2013. Community-acquired urinary tract infections in children: pathogens, antibiotic susceptibility and seasonal changes. *Eur Rev Med Pharmacol Sci*, *17*(7), pp.971-6.

48. Sidjabat, H., Nimmo, G.R., Walsh, T.R., Binotto, E., Htin, A., Hayashi, Y., Li, J., Nation, R.L., George, N. and Paterson, D.L., 2011. Carbapenem resistance in Klebsiella pneumoniae due to the New Delhi metallo-β-lactamase. *Clinical infectious diseases: an official publication of the Infectious Diseases Society of America*, *52*(4), p.481.

49. Navon-Venezia, S., Kondratyeva, K. and Carattoli, A., 2017. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. *FEMS microbiology reviews*, *41*(3), pp.252-275.

50. Loudermilk, E.M., Kotay, S.M., Barry, K.E., Parikh, H.I., Colosi, L.M. and Mathers, A.J., 2022. Tracking Klebsiella pneumoniae carbapenemase gene as an indicator of antimicrobial resistance

dissemination from a hospital to surface water via a municipal wastewater treatment plant. *Water Research*, *213*, p.118151.

51. Chaoui, L., Mhand, R., Mellouki, F. and Rhallabi, N., 2019. Contamination of the surfaces of a health care environment by multidrug-resistant (MDR) bacteria. *International journal of microbiology*, *2019*.
52. Gajic, I., Kabic, J., Kekic, D., Jovicevic, M., Milenkovic, M., Mitic Culafic, D., Trudic, A., Ranin, L. and Opavski, N., 2022. Antimicrobial susceptibility testing: A comprehensive review of currently used methods. *Antibiotics*, *11*(4), p.427.
53. Najar, I.N., Das, S., Kumar, S., Sharma, P., Mondal, K., Sherpa, M.T. and Thakur, N., 2022. Coexistence of heavy metal tolerance and antibiotic resistance in thermophilic Bacteria belonging to Genus Geobacillus. *Frontiers in microbiology*, *13*, p.914037.
54. Truong, W.R., Hidayat, L., Bolaris, M.A., Nguyen, L. and Yamaki, J., 2021. The antibiogram: key considerations for its development and utilization. *JAC-antimicrobial resistance*, *3*(2), p.dlab060.
55. Köck, R., Schuler, F., Idelevich, E.A. and Schaumburg, F., 2021. Variability of antibiograms: how often do changes in the antimicrobial susceptibility pattern occur in isolates from one patient?. *Clinical Microbiology and Infection*, *27*(11), pp.1638-1643.
56. Melvin P. Weinstein *et al.,* 2020. Performance Standards for Antimicrobial Susceptibility Testing. *Clinical and Laboratory Standards institute*, pp. 1-332.
57. Akualing, J.S. and Rejeki, I.P.S., 2016. ANTIBIOGRAM (Antibiogram). *Indonesian Journal Of Clinical Pathology And Medical Laboratory*, *23*(1), pp.90-95.
58. Gajic, I., Kabic, J., Kekic, D., Jovicevic, M., Milenkovic, M., Mitic Culafic, D., Trudic, A., Ranin, L. and Opavski, N., 2022. Antimicrobial susceptibility testing: A comprehensive review of currently used methods. *Antibiotics*, *11*(4), p.427.
59. Fernández-Natal, I., Saez-Nieto, J.A., Medina-Pascual, M.J., Albersmeier, A., Valdezate, S., Guerra-Laso, J.M., Rodríguez, H., Marrodán, T., Parras, T., Tauch, A. and Soriano, F., 2013. Dermabacter hominis: a usually daptomycin-resistant gram-positive organism infrequently isolated from human clinical samples. *New microbes and new infections*, *1*(3), pp.35-40.
60. Meriyani, H., Sanjaya, D.A., Juanita, R.A. and Ernawati, D.K., 2023. A Narrative Review of Staphylococcus hominis Resistance Pattern: Multidrug-and Possible Extensively Drug-Resistance. *Indonesian Journal of Pharmacy/Majalah Farmasi Indonesia*, *34*(3).
61. Farinha, A., Vaz, Á., Assunção, J. and Vinhas, J., 2013. Unusual bacteria causing peritonitis in peritoneal dialysis–A single centre experience. *Port J Nephrol Hypert*, *27*(3), pp.187-195.
62. Massip, C., Mathieu, C., Gaudru, C., Miaut, V., Floch, P., Oswald, E., Segonds, C. and Guet-Revillet, H., 2019. In vitro activity of seven β-lactams including ceftolozane/tazobactam and ceftazidime/avibactam against Burkholderia cepacia complex, Burkholderia gladioli and other non-fermentative Gram-negative bacilli isolated from cystic fibrosis patients. *Journal of Antimicrobial Chemotherapy*, *74*(2), pp.525-528.