**Supplementary Material**

**Comprehensive Analysis of *Acinetobacter baumannii* in Aquatic Environments and Fish Microbiota: Integrating Culture-Dependent, 16S Metagenomics, and Antibiotic Resistance Profiling**

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**Supplementary Tables**

**Supplementary Table 1.** *A. baumannii* isolates from Bucharest wastewater, surface water and intra-hospital infection samples.

|  |  |  |
| --- | --- | --- |
| **Strain code** | **Isolation source** | **Culture media /Isolation source** |
| 22012 ENE 6 | surface water – downstream river | CHROMagar ESBL |
| 22012 ENE 3 | surface water – downstream river | CHROMagar ESBL |
| 22012 ENE 5 | surface water – downstream river | CHROMagar ESBL |
| 22012 CA 6 | surface water – downstream river | CHROMagar Acinetobacter |
| 22012 CA 1 | surface water – downstream river | CHROMagar Acinetobacter |
| 22012 CA 4 | surface water – downstream river | CHROMagar Acinetobacter |
| 22012 CA 5 | surface water – downstream river | CHROMagar Acinetobacter |
| 22013 ENE 4 | WWTP - influent | CHROMagar ESBL |
| 22013 ENE 1 | WWTP - influent | CHROMagar ESBL |
| 22013 CA4 | WWTP - influent | CHROMagar Acinetobacter |
| 22013 CA5 | WWTP - influent | CHROMagar Acinetobacter |
| 22013 CA1 | WWTP - influent | CHROMagar Acinetobacter |
| 22013 CA2 | WWTP - influent | CHROMagar Acinetobacter |
| 22013 CA3 | WWTP - influent | CHROMagar Acinetobacter |
| 22014 ENE 4 | WWTP - effluent | CHROMagar ESBL |
| 22014 ENE 5 | WWTP - effluent | CHROMagar ESBL |
| 22014 ENE 2 | WWTP - effluent | CHROMagar ESBL |
| 22014 ENE 3 | WWTP - effluent | CHROMagar ESBL |
| 22014 CA 4 | WWTP - effluent | CHROMagar Acinetobacter |
| 22014 CA 5 | WWTP - effluent | CHROMagar Acinetobacter |
| 22014 CA 6 | WWTP - effluent | CHROMagar Acinetobacter |
| 22014 CA 1 | WWTP - effluent | CHROMagar Acinetobacter |
| 22014 CA 2 | WWTP - effluent | CHROMagar Acinetobacter |
| 22014 CA 3 | WWTP - effluent | CHROMagar Acinetobacter |
| 22014 COL N 5 | WWTP - effluent | CHROMagar Colistin |
| 22015 COL N 2 | WWTP – active sludge | CHROMagar Colistin |
| 22015 ENE 6 | WWTP – active sludge | CHROMagar ESBL |
| 22015 ENE 1 | WWTP – active sludge | CHROMagar ESBL |
| 22015 ENE 4 | WWTP – active sludge | CHROMagar ESBL |
| 22015 CA 4 | WWTP – active sludge | CHROMagar Acinetobacter |
| 22015 CA 1 | WWTP – active sludge | CHROMagar Acinetobacter |
| 22015 CA 3 | WWTP – active sludge | CHROMagar Acinetobacter |
| 22015 CA 2 | WWTP – active sludge | CHROMagar Acinetobacter |
| Intra-hospital infections Fundeni Hospital | 1 Abc | rectal swab |
|  | 2 Abc | rectal swab |
|  | 3 Abc | bronchial secretion |
|  | 4 Abc | bronchial secretion |
|  | 5 Abc | plague secretion |
|  | 6 Abc | bronchial secretion |
|  | 7 Abc | bronchial secretion |
|  | 8 Abc | plague secretion |
|  | 9 Abc | plague secretion |
|  | 10 Abc | bronchial secretion |
|  | 11 Abc | bronchial secretion |
|  | 12 Abc | Rectal swab |
|  | 13 Abc | bronchial secretion |
|  | 14 Abc | peritoneal fluid |
|  | 24 Fundeni | rectal swab |
|  | 3 Fundeni | rectal swab |
|  | 49 Fundeni | rectal swab |

**Supplementary Table 2.** *A. baumannii* isolates from Targoviste wastewater and surface water samples.

|  |  |  |
| --- | --- | --- |
| Strain code | Isolation source | Culture media |
|  |  |  |
| 22016 COL N6 | surface water-upstream | CHROMagar Colistin |
| 22016 CNE 1 | surface water-upstream | CHROMagar CARBA |
| 22016 CNE 2 | surface water-upstream | CHROMagar CARBA |
| 22016 CNE 3 | surface water-upstream | CHROMagar CARBA |
| 22016 CNE 4 | surface water-upstream | CHROMagar CARBA |
| 22016 ENE 4 | surface water-upstream | CHROMagar ESBL |
| 22016 CA 1 | surface water-upstream | CHROMagar Acinetobacter |
| 22016 CA 2 | surface water-upstream | CHROMagar Acinetobacter |
| 22016 CA 3 | surface water-upstream | CHROMagar Acinetobacter |
| 22016 CA 4 | surface water-upstream | CHROMagar Acinetobacter |
| 22016 CA 5 | surface water-upstream | CHROMagar Acinetobacter |
| 22016 CA 6 | surface water-upstream | CHROMagar Acinetobacter |
| 22017 CNE 1 | surface water-downstream | CHROMagar CARBA |
| 22017 CNE 2 | surface water-downstream | CHROMagar CARBA |
| 22017 CNE 3 | surface water-downstream | CHROMagar CARBA |
| 22017 CA 1 | surface water-downstream | CHROMagar Acinetobacter |
| 22017 CA 2 | surface water-downstream | CHROMagar Acinetobacter |
| 22017 CA 3 | surface water-downstream | CHROMagar Acinetobacter |
| 22017 CA 4 | surface water-downstream | CHROMagar Acinetobacter |
| 22017 CA 5 | surface water-downstream | CHROMagar Acinetobacter |
| 22018 CA 1 | WWTP-influent | CHROMagar Acinetobacter |
| 22018 CA 2 | WWTP-influent | CHROMagar Acinetobacter |
| 22018 CA 3 | WWTP-influent | CHROMagar Acinetobacter |
| 22018 CA 4 | WWTP-influent | CHROMagar Acinetobacter |
| 22018 CA 5 | WWTP-influent | CHROMagar Acinetobacter |
| 22018 CA 6 | WWTP-influent | CHROMagar Acinetobacter |
| 22019 CA 4 Targov | WWTP-effluent | CHROMagar Acinetobacter |
| 22019 CNE 4 | WWTP-effluent | CHROMagar CARBA |
| 22019 CNE 5 | WWTP-effluent | CHROMagar CARBA |
| 22019 CNE 6 | WWTP-effluent | CHROMagar CARBA |
| 22019 CNE 1 | WWTP-effluent | CHROMagar CARBA |
| 22019 CNE 2 | WWTP-effluent | CHROMagar CARBA |
| 22019 CNE 3 | WWTP-effluent | CHROMagar CARBA |

**Supplementary Table 3.** **CFU/100 mL** in surface water samples.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Isolation source | Acinetobacter phenotype | ESBL phenotype | CARBA phenotype | Colistin phenotype |
| **CFU /100 mL** | **CFU/100 mL** | **CFU/100 mL** | **CFU/100 mL** |
| Upstream WWTP Bucharest- Dâmbovița river | 127.95 | 395.33 | 302.66 | 400 |
| Upstream WWTP Targoviste - Ialomiṭa river | 334.88 | 400 | 400 | 30.69 |
| Downstream WWTP Bucharest- Dâmbovița river | 1580.17 | 23063.06 | 1853.65 | 12341.4 |
| Downstream WWTP Targoviste - Ialomiṭa river | 469.38 | 568.18 | 174 | 66451.6 |

**Supplementary Table 4.** **CFU/100 mL** in wastewater samples.

|  |  |  |  |
| --- | --- | --- | --- |
| Isolation source | Acinetobacter phenotype | ESBL phenotype | CARBA phenotype |
| **CFU /100 mL** | **CFU/100 mL** | **CFU/100 mL** |
| Bucharest WWTP-influent | 55000 | 71153.84 | 8125 |
| Targoviste WWTP-influent | 12954.54 | 4848.48 | 1162.79 |
| Active sludge WWTP Bucharest | 5000 | 2790.69 | 670.58 |
| WWTP Bucharest-effluent | 1863.36 | 118571.4 | 13846.15 |
| WWTP Targoviste-effluent | 109.09 | 678.57 | 235.5 |

**Supplementary table 5**. Antimicrobial resistance profiles of *A. baumannii* isolated from Bucharest WWTP and Fundeni Hospital in 2022.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Isolation source/antibiotics** | **β-lactams (%)** | **Fluoroquinolones (%)** | **Aminoglycosides (%)** | **Tetracyclines (%)** |
| **Intra-hospital Infections** | 98 | 100 | 94 | 94 |
| **WWTP Influent** | 25 | 14 | 43 | 0 |
| **WWTP Active sludge** | 31 | 29 | 57 | 14 |
| **WWTP Effluent** | 21 | 9 | 36 | 18 |
| **WWTP Downstream** | 31 | 14 | 43 | 0 |

**Supplementary Table 6.** Antimicrobial resistance profiles of *A. baumannii* isolated from Targoviște WWTP in 2022.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Isolation source/antibiotics** | **β-lactams (%)** | **Fluoroquinolones (%)** | **Aminoglycosides (%)** | **Tetracyclines (%)** |
| **WWTP Upstream** | 42 | 17 | 54 | 0 |
| **WWTP Influent** | 33 | 33 | 50 | 13 |
| **WWTP Effluent** | 3 | 0 | 36 | 14 |
| **WWTP Downstream** | 29 | 0 | 50 | 0 |

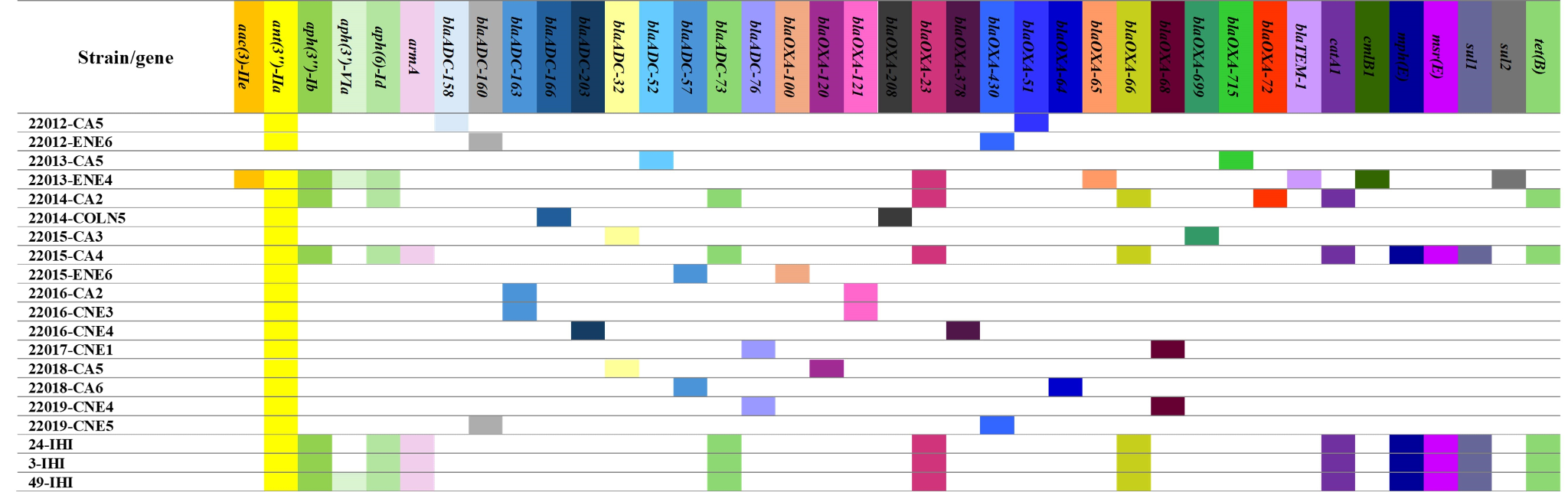
**Supplementary Table 7**. Genetic support for AR of *A. baumannii* isolated from Bucharest WWTP and Fundeni Hospital in 2022.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Isolation source/gene** | ***bla*OXA-23**  **(%)** | ***bla*OXA-24**  **(%)** | ***bla*OXA-51**  **(%)** | ***bla*TEM**  **(%)** |
| **Intra-hospital Infections** | 62 | 46 | 62 | 15 |
| **WWTP Influent** | 14 | 0 | 100 | 14 |
| **WWTP Active sludge** | 28 | 0 | 100 | 0 |
| **WWTP Effluent** | 19 | 9 | 100 | 0 |
| **WWTP Downstream** | 14 | 14 | 100 | 0 |

**Supplementary Table 8**. Genetic support for the AR of *A. baumanii* isolated from Targoviște WWTP in 2022.

|  |  |  |
| --- | --- | --- |
| **Isolation source/gene** | ***bla*OXA-51 (%)** | ***bla*CTX-M (%)** |
| **WWTP Upstream** | 100 | 0 |
| **WWTP Influent** | 100 | 0 |
| **WWTP Effluent** | 100 | 0 |
| **WWTP Downstream** | 100 | 13 |

**Supplementary Table 9**. ARGs profiles for *A. baumannii* isolated from WWTPs and Fundeni Hospital in Romania



**Supplementary Table 10**. Virulence factors profiles for *A. baumannii* isolated intra-hospital infections and WWTPs in Romania.

