

## Cemetery soil as a potential factor in the spread of antibiotic resistance

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### INTRODUCTION & AIM

#### Graveyard environment

- one of the dominant forms of burial
- during decomposition - release up to 0.6 l of leachate/kg body weight into the soil
- environment pollution with heavy metals, toxic substances, and potentially AMR
- improper establishment – on the hills, in nearby water reservoirs, lack of sewage monitoring and treatment systems

#### Graveyard and AMR

- antimicrobial resistance – increasing cause of death globally
- during decomposition – bacteria proliferation, including potentially resistant ones, with a higher probability of transmitting resistance
- genetic material exchange with bacteria present in the surrounding environment
- so far - known as a source of contamination, also with AMR, but research is very limited

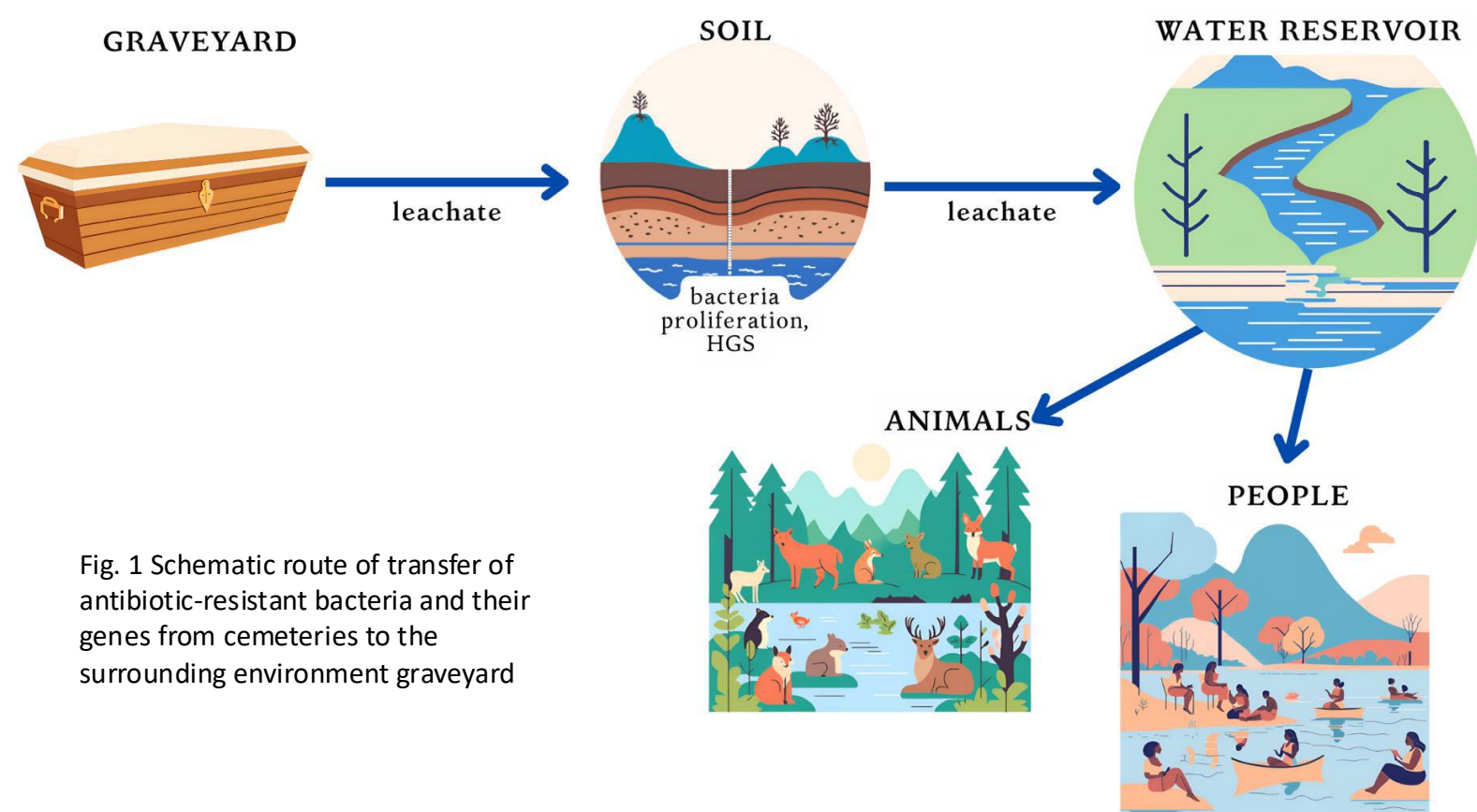


Fig. 1 Schematic route of transfer of antibiotic-resistant bacteria and their genes from cemeteries to the surrounding environment graveyard

### METHOD

Research material - soil samples from 5 cemetery sites located in the Kuyavian-Pomeranian voivodeship

- 5 samples of each, surface soil (up to 20 cm deep) and from under the coffin (up to 1.5-2 m), collected during exhumation
- Locations: Chodecz and Wąbrzeźno (Fig. 2)

Microbiological part:

- Determining the number of bacteria against selected antibiotics:
  - amoxicilline – 16 µg/ml
  - cefuroxime - 16 µg/ml
  - doxycycline - 8 µg/ml
  - tetracycline - 8 µg/ml
- Isolation of selected resistant strains - identification (MALDI-TOF)

Molecular part:

- DNA extraction from soil
- Multiplex PCR for resistance genes
- Electrophoresis and visualization

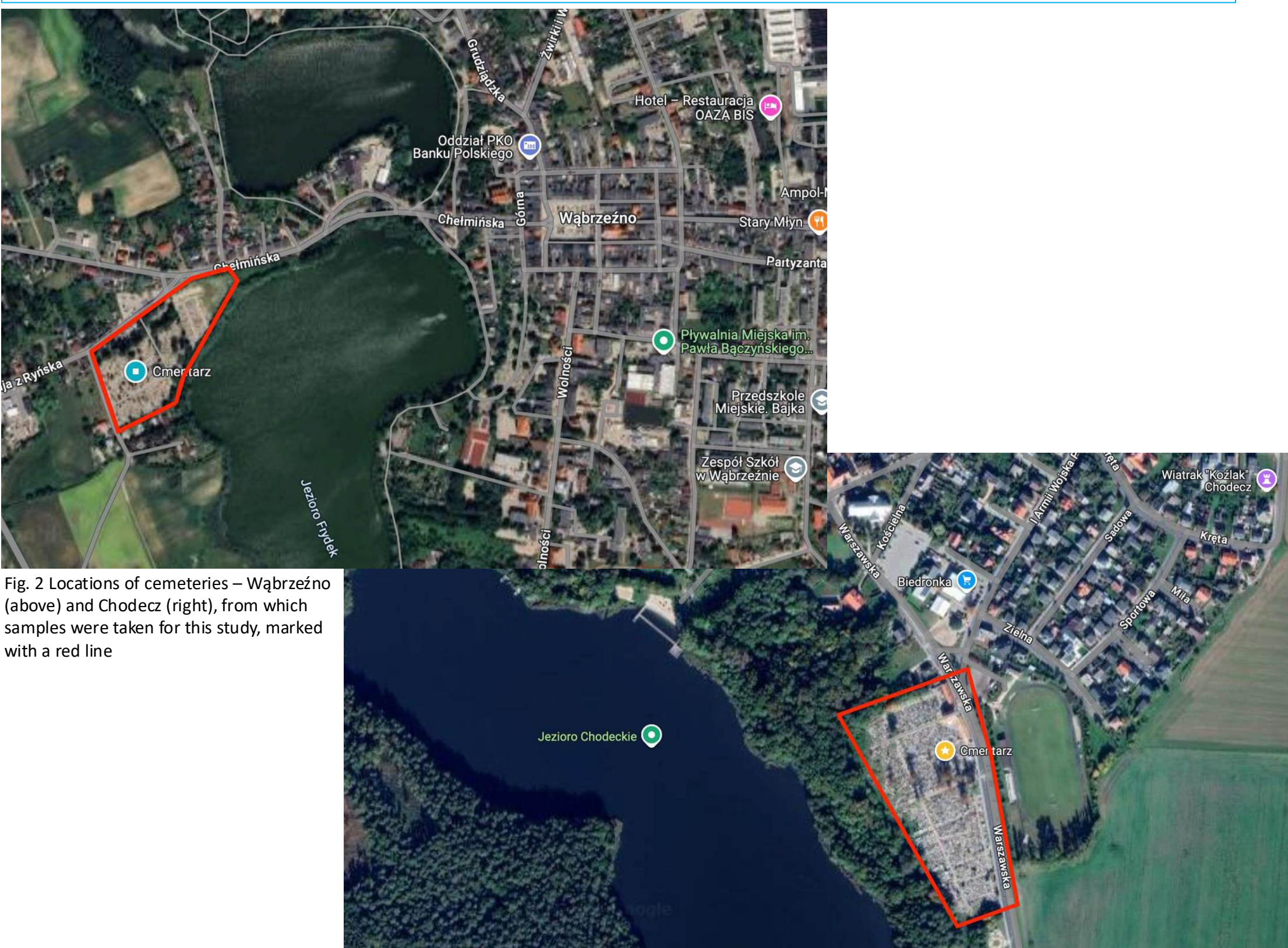


Fig. 2 Locations of cemeteries – Wąbrzeźno (above) and Chodecz (right), from which samples were taken for this study, marked with a red line

### RESULTS & DISCUSSION

- The highest number of bacterial colonies was recorded for cefuroxime, followed by amoxicillin and tetracycline, the rarest were resistant to doxycycline
- Antibiotic-resistance genes frequency (Tab.1)

DNA extraction from soil, followed by multiplex PCR for resistance genes towards beta-lactam antibiotics, tetracyclines, sulfonamides, and Integron 1

- The most frequent resistance gene was Int1, then Sull, blaSHV, blaVIM and blaTEM.
- blaCTXM, TetA, and TetB were not recorded. A slightly higher number of resistance genes was recorded for samples under the coffin

MALDI-TOF identification:

In total – 72 bacterial isolates

- 37 – unidentified
- 35 – identified -> Including pathogens
  - *Stenotrophomonas maltophilia*
  - *Serratia odorifera*
  - *Mycobacterium mucogenicum*
  - *Staphylococcus aureus* – water sample
  - *Pseudomonas oryzae* – water sample

The average number of antibiotic-resistant bacteria in cemetery soil in different locations

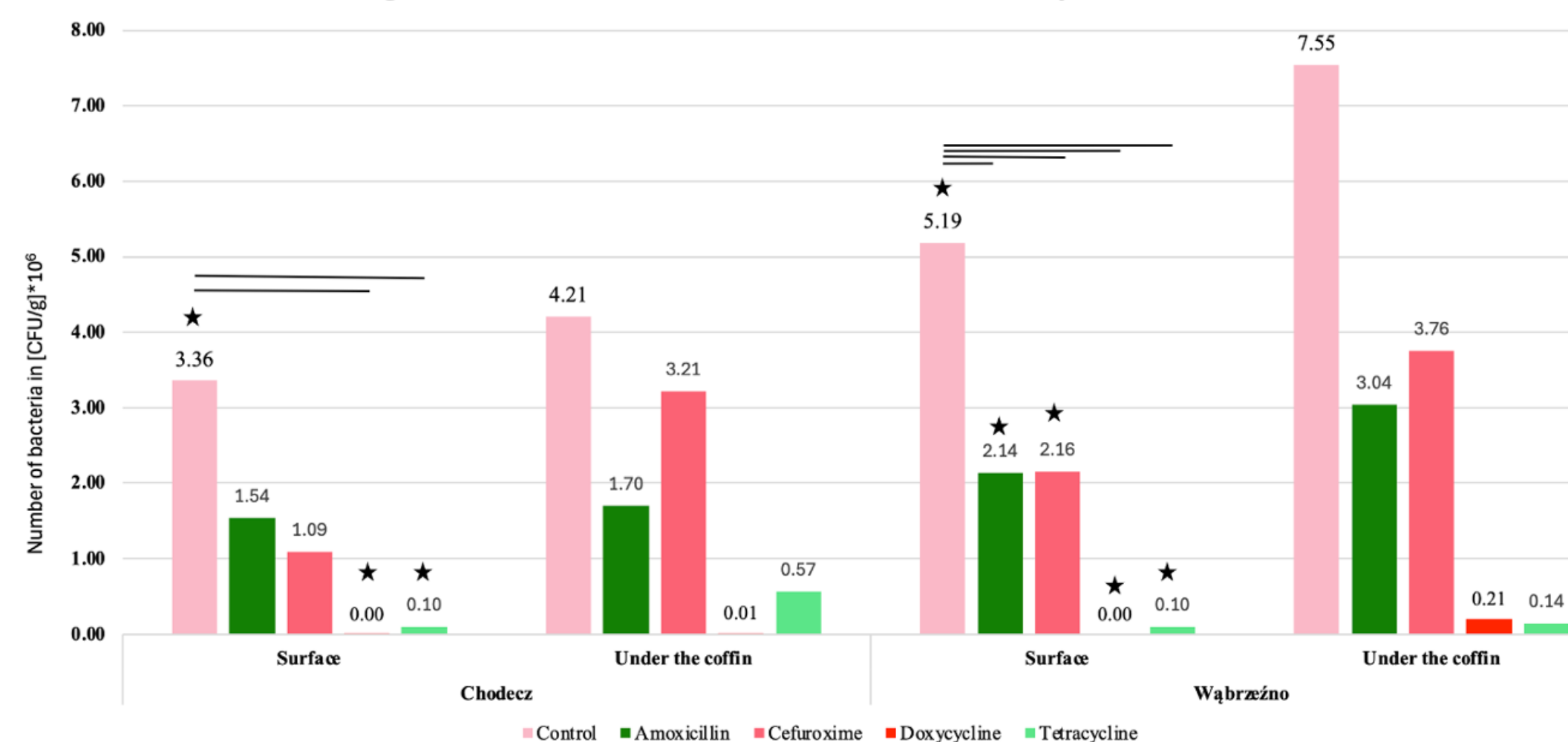


Fig. 3 The average number of antibiotic-resistant bacteria

| Gene            | Chodecz  |                  | Wąbrzeźno |                  |
|-----------------|----------|------------------|-----------|------------------|
|                 | Surface  | Under the coffin | Surface   | Under the coffin |
| blaSHV          | 1        | 3                | 3         | 2                |
| blaOXA          | 0        | 0                | 0         | 1                |
| blaCTX-M        | 0        | 0                | 0         | 0                |
| blaTEM          | 0        | 1                | 1         | 4                |
| blaVIM          | 0        | 1                | 3         | 2                |
| TetA            | 0        | 0                | 0         | 0                |
| TetB            | 0        | 0                | 0         | 0                |
| TetC            | 0        | 0                | 0         | 1                |
| TetD            | 1        | 1                | 1         | 0                |
| TetE            | 0        | 0                | 0         | 1                |
| TetM            | 0        | 1                | 0         | 0                |
| Sull            | 2        | 4                | 3         | 3                |
| Int1            | 3        | 5                | 3         | 4                |
| <b>In total</b> | <b>7</b> | <b>16</b>        | <b>14</b> | <b>18</b>        |

Tab. 1 Selected antibiotic resistance genes frequency in analyzed samples

### CONCLUSION

- So far - the occurrence of antibiotic-resistant bacteria and their genes in cemetery soil samples has been confirmed
- More common - resistance towards beta-lactam antibiotics than tetracycline
- A slightly higher occurrence of AMR occurred in under the coffin soil samples, with significant difference recorded only for a few surface samples - important - very limited number of samples

### FUTURE WORK / REFERENCES

- Water samples analysis – as described above, collected from each cemetery – water inflow and outflow – in the process
- Metagenomic sequencing of soil samples, particularly regarding resistome – in the process
- So far – published review paper dedicated to the presented topic [Tarnawska et al., 2023](#)