1. Introduction

Acinetobacter baumannii is a frequent cause of infections in hospitals around the world, which are difficult to treat due to the organisms inherent antibiotic resistance. It is particularly prevalent in intensive care units (ICUs) with around 18% of A. baumannii infections in the UK resulting in bacteremia (mostly in infants and the elderly) in 2018. Due to the challenge of treating infections caused by this bacteria there is considerable interest in exploring natural products for novel antimicrobial activity. Honey has been used for many centuries in traditional medicines to treat infections. It has antibacterial properties as well as anti-oxidant and anti-inflammatory activities.

The aim of this project is to screen our collection of 270 honey samples for the presence of natural compounds, which may demonstrate antibacterial activity against clinical isolates of A. baumannii.

Hypothesis: Honey samples from geographically distinct locations may contain antibacterial compounds with activity against A. baumannii.

Objectives
1. To confirm the identity of clinical isolates of A. baumannii provided by Public Health Wales using phenotypic and genotypic methods.
2. To determine sensitivity to antibiotics commonly used to treat this pathogen.
3. To determine sensitivity of isolates to honey.
4. To identify honey samples worthy of future study as a potential source of antimicrobial compounds with activity against A. baumannii.

2. Methods

A total of 45 clinical isolates (T1-T45 strains) collected from wounds and the respiratory tract of infected patients were provided by Public Health Wales (PHW). The following tests were performed:

A. Phenotypic characterization: Gram stain, Oxidase test, Catalase test, MacConkey agar, Haemolysis test, Citrate test, Motility test, Incubation at 44°C and in CO2.

B. Genotypic characterization:

1. Polymerase Chain Reaction (PCR) BlaOXA-1 and OXA-23 gene primers—species specific ID
2. BlaOXA-23 (marker of antibiotic resistance) and Class 1 integrase (Int1) genes.

C. Antibiotics susceptibility: Disk diffusion assay, using EUCAST standard test for Imipenem, Meropenem, Ciprofloxacin, Levofloxacin, Amikacin, Gentamicin, Netilmicin, Tobramycin.

D. Honey susceptibility: Well diffusion assay

3. Results

Table 1 below shows typical results for A. baumannii. Using these phenotypic tests we provisionally identified 40 isolates as A. baumannii.

4. Discussion and Conclusion

The controls used in this study A. baumannii ATCC 19568, OXA-23 clone1 and C2 were confirmed the presence of all three genetic markers including BlaOXA-1, BlaOXA-23, and Int1. Using a combination of phenotypic and genotypic markers only 33 of the 40 isolates provided by PHW were identified as A. baumannii. These isolates were subdivided into 4 distinct genetic groups. Nine of the 11 (82%) isolates that had both BlaOXA-1 and BlaOXA-23 genes were resistant to all tested antibiotics. Upon preliminary testing of the in-house honey sample collection the A. baumannii OXA-23 Clone 1 and C2 were found to be significantly more resistant to honey than ATCC 19568. Using these isolates the 15 most antibacterial honey samples were identified and will be used to determine the honey sensitivity of the five clinical isolates of A. baumannii identified based on their genetic and antibiotics susceptibility profiles.

5. Future work

6. References