INTRODUCTION

Increased multidrug resistance became emerging medical problem, which has arisen mainly due to excessive and insufficient use of antimicrobials. It is especially dangerous when it comes to Urinary Tract Infections (UTI), which affects annually around 150 million people worldwide (Buscha, Ayed and Graa, 2013). Recently, high prevalence of Extended Spectrum β-Lactamases (ESBL) was observed within commonly isolated UTI pathogens - Escherichia coli and Klebsiella pneumoniae. E. coli is reported to cause 70-90% of the community and nosocomial acquired UTIs (Sugimoto et al., 2011), whereas K. pneumoniae is considered significant threat mainly for hospitalized patients due its ability to attach to plastic medical devices (Arens et al., 2017). ESBL produces β-lactamases, encoded by TEM and SHV genes in E. coli and K. pneumoniae, respectively (Mazzaroli, 2017). This enzyme easily hydrolyze β-Lactam ring commonly shared by 3rd generation antibiotics such as penicillin, carbapenems and cephalosporins (Bouacha et al., 2019), which are the first line treatment for UTIs. Furthermore, ESBL shows greater ability of biofilm formation (Neu et al., 2016). Biofilms can be simply defined as architectural structures of aggregated micro-organisms enclosed under complex matrix, which is mainly composed of Extracellular Polymeric Substance (EPS). ESP accounts for almost 90% of total biofilm biomass and helps to evade immune response of organism (Sato, 2014). The microcolonies in the biofilm are substrate for enzyme production, which influence horizontal gene transfer within bacteria. This can increase antimicrobial resistance up to 1000-fold (Oliveira et al., 2017). Moreover, planktonic bacteria can detach from fully matured biofilms and become biofilm-invasive areas in proximity, causing relapse of infections (Sato, 2014). Biofilm formation process is presented in Figure 1.

If ESBL infections are not treated properly, severe complications such as pyelonephritis and septicemia can occur. Furthermore, there is an increased risk of an ascending urinary tract infection that often results in renal failure and increasing mortality rate of patients. Such a difficult infection requires extended hospitalization process and prolonged treatment, increasing healthcare service expenses (Buscha, Ayed and Graa, 2013).

Development of biofilm among ESBL pathogens of UTI, issues urgent need for alternative treatment methods. The product of New Zealand bees, Manuka Honey, has been reported to exhibit antibacterial property against both Gram-positive and negative bacteria (Buscha, Ayed and Graa, 2013) and can be potential treatment for biofilms. Those properties are assigned to high concentration of sugar, acidity, leptinoid and a major component methylglyoxal (MGO). In addition, it has been noted that Manuka Honey is able to interact with macromolecules such as DNA, RNA and proteins (Carter et al., 2016).

The aim of the experiment was to evaluate the anti-biofilm efficacy of Manuka Honey on biofilm producing Uropathogenic strains in-vitro.

RESULTS

Table 1. Phenotypic identification via Double Disk Synergy has confirmed that E. coli TEM 5 and K. pneumoniae SHV18 are ESBL, thus both strains shown sensitivity on Cefetoxime and Cefazolin combined with Clavulanic Acid. Zone of inhibition in sets D52C and D64A differs 9mm for TEM3 and 7mm for SHV18. CPO – Cephalosporin; CTX – Cefotaxime; CAZ – Cefazolin; CV – Clavulanic Acid.

Table 2. Minimum Inhibitory Concentration (MIC) established for ESBL strains. No turbidity was visually observed in the wells containing mixture of biofilm with 50% (w/v) honey dilution and 100% (w/v) of honey dilutions. Calculations have demonstrated that biofilm production by all strains was achieved in 50% of honey for both TEM3 and SHV18. Positive values indicate inhibition of planktonic bacteria in, whereas negative ones indicate growth of planktonic bacteria in K. pneumoniae SHV18 in lower concentration of Manuka Honey. Standard deviation (SD) was calculated to evaluate distribution of obtained strains. Manuka honey concentration (%): 50%, 25% and 12.5%.

Table 3. Minimum Inhibitory Concentration (MIC) established for ESBL strains. No turbidity was visually observed in the wells containing mixture of biofilm with 50% (w/v) honey dilution and 100% (w/v) of honey dilutions. Calculations have demonstrated that biofilm production by all strains was achieved in 50% of honey for both TEM3 and SHV18. Positive values indicate inhibition of planktonic bacteria in, whereas negative ones indicate growth of planktonic bacteria in K. pneumoniae SHV18 in lower concentration of Manuka Honey. Standard deviation (SD) was calculated to evaluate distribution of obtained strains. Manuka honey concentration (%): 50%, 25% and 12.5%.

Figure 1. Five step process of biofilm formation.

Figure 2. 24h biofilm formation and reduction in biofilm biomass after treatment with 50% (w/v) of Manuka Honey.

Table 4. Mean OD values of biofilm formed by E. coli TEM3 and K. pneumoniae SHV18 in presence of Manuka Honey concentration (%). OD – Optical Density; H2O – Water; B – Blank; T – Treated.

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Figure 3. Proposed mechanism of Manuka honey action.

DISCUSSION AND CONCLUSION

This study shows anti-biofilm efficacy of Manuka honey against ESBL biofilm-producing strains. Greater biofilm inhibition activity and reduced ESBL production were observed for E. coli TEM3 compared to K. pneumoniae SHV18. Lower reduction can be related to better biofilm formation abilities of K. pneumoniae. Recently, K. pneumoniae was found to have fadler kinetics, which influence biofilm formation (Sugimoto et al., 2019). In addition, susceptibility of ESBL E. coli to Manuka honey was previously confirmed (Osík and Afagba, 2017).

Notwithstanding, significant reductions were observed; 50% of Manuka honey was not able to eradicatively biofilm biomass. It is common knowledge, that MICs of biofilm growth is higher than planktonic growth (Alves et al., 2014). Interestingly, concentration below 12.5% of the honey increased planktonic growth of K. pneumoniae, suggesting that presence of sugar stimulates bacterial growth. It is worth to mentioned that sugars provided with honey do not enhance biofilm formation (Lu et al., 2013).

The component of Manuka honey is a major antagonist of developing biofilms. Thus, mechanisms of Manuka honey action should be further explored. Presumably, high osmotic pressure outside created by honey may destroy extracellular matrix allowing active components to penetrate and interact with bacteria on the molecular level (Figure 3).

Table 5. Results of biofilm formation inhibition by Manuka honey.

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