

# Evaluating the Versatility of [BMIM]Cl on the Inhibition of Bacterial Growth

## Summary

Ionic liquids are organic salts with melting points below 100°C. They have a low volatility, low flammability, high electrical conductivity and low viscosity. This research evaluated the antimicrobial properties of bleach, typically employed for this purpose, and 1-Butyl-3-methylimidazolium chloride (shown in figure 1). The effectiveness of inhibiting bacterial growth using a range of concentrations is evaluated herein. We were interested in testing out the versatility of [BMIM]Cl on the inhibition of E.coli k-12 at the lowest possible concentration to see if it would have the same, if not better, results than using the same concentration of a household bleach.

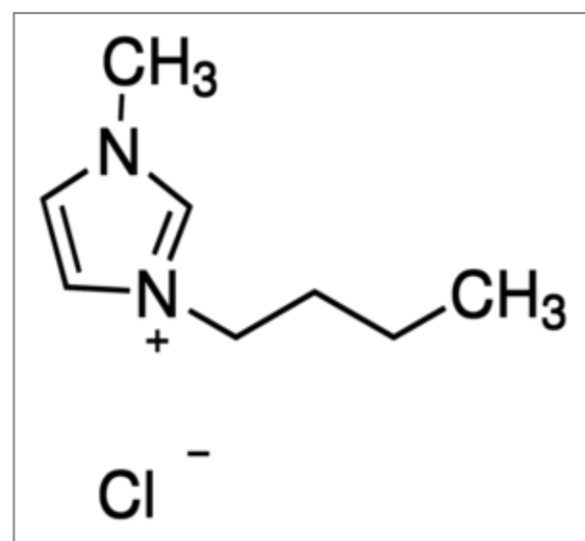


Figure 2 showing the structure of 1-Butyl-3-methylimidazolium chloride also known as [BMIM]Cl

## Research aims

This research aims to see the correlation between the use of [BMIM]Cl and household bleach to kill E.coli k-12 at the lowest concentration possible. E.coli k-12 is a gram-negative type of bacteria meaning that it has thinner cell walls and is less tolerant towards Ionic Liquid toxicity meaning that it will be killed easily compared to gram positive bacteria as it has thinner cells walls so allows substances to penetrate through easily. Figure 3 and Figure 4 shows some of the petri dishes containing the zones of inhibition that have been formed as a result of the antibacterial properties of the ionic liquid and bleach. Even though there was one round of testing, these results are inconclusive, and the process would need to be repeated to obtain results to show how effective [BMIM]Cl is against bacteria.

		Zones of Inhibition (mm)			Mean (mm)	% Uncertainty
		Plate 1	Plate 2	Plate 3		
Bleach	10%	6.0	10.5	11.0	9.2	5.40
	15%	13.0	7.5	7.0	9.2	5.40
	20%	14.5	10.5	8.5	11.2	0.04
Ionic Liquid [EMIM]Br	10%	5.5	N/A (Mould developed)	6.0	5.8	0.09
	15%	6.5	N/A (Mould developed)	8.0	7.3	0.07
	20%	6.5	N/A (Mould developed)	6.5	6.5	0.08

Figure 2 showing the results collected comparing the zones of inhibition using bleach and [BMIM]Cl

## Experimental method

We assumed that the bleach and the ionic liquid have a concentration of 100% and diluted them to compare the effects of both at different concentrations as shown below:

- Diluted household bleach with water in the ratio of:
  - 2ml:18ml (10%)
  - 3ml:17ml (15%)
  - 4ml:16ml (20%)
- Diluted [BMIM]Cl with water in the ratio of:
  - 2ml:18ml (10%)
  - 3ml:17ml(15%)
  - 4ml:18ml(20%)

Equipment used:

- Used single-use inoculation loops to spread the E.coli k12 onto the LB agar plates
- Placed standard hole punched sized filter paper disks into each beaker containing different concentrations of [BMIM]Cl and bleach for 1 minute
- Placed the soaked paper disks from the different concentrations of bleach onto the agar plate using tweezers
- Repeated with soaked paper disks from different concentrations of [BMIM]Cl
- Incubated the petri dishes at 37°C (optimum temperature for E.coli k12)
- Measured the diameter of the zone of inhibition

## Results

It can be concluded that after the tests were carried out, the ionic liquid produced results that were very similar despite the differences in concentrations. With the bleach, however as the concentration increased the size of the zone of inhibition also increased. However, these results are inconclusive as this test was only carried out once; if more tests were done, it would ensure a conclusive result as plate 2 of the ionic liquid had developed mould on the petri dish (shown in figure 3 and figure 4), covering the zone of inhibition thus making it invalid.

The plates with bleach showed on average, the higher the concentration of bleach, the greater the zone of inhibition. The use of bleach at a 10% is typically in line with the concentration of a hypochlorite solution of household bleach.

The results for the ionic liquid are inconclusive due to the development of mould on plate 2 but it could perhaps be seen that the optimum concentration for the ionic liquid to be most effective would be to use a 15% concentration. This would need to be experimented further to support this claim. See figure 2 for more details on the data collected from the practical.

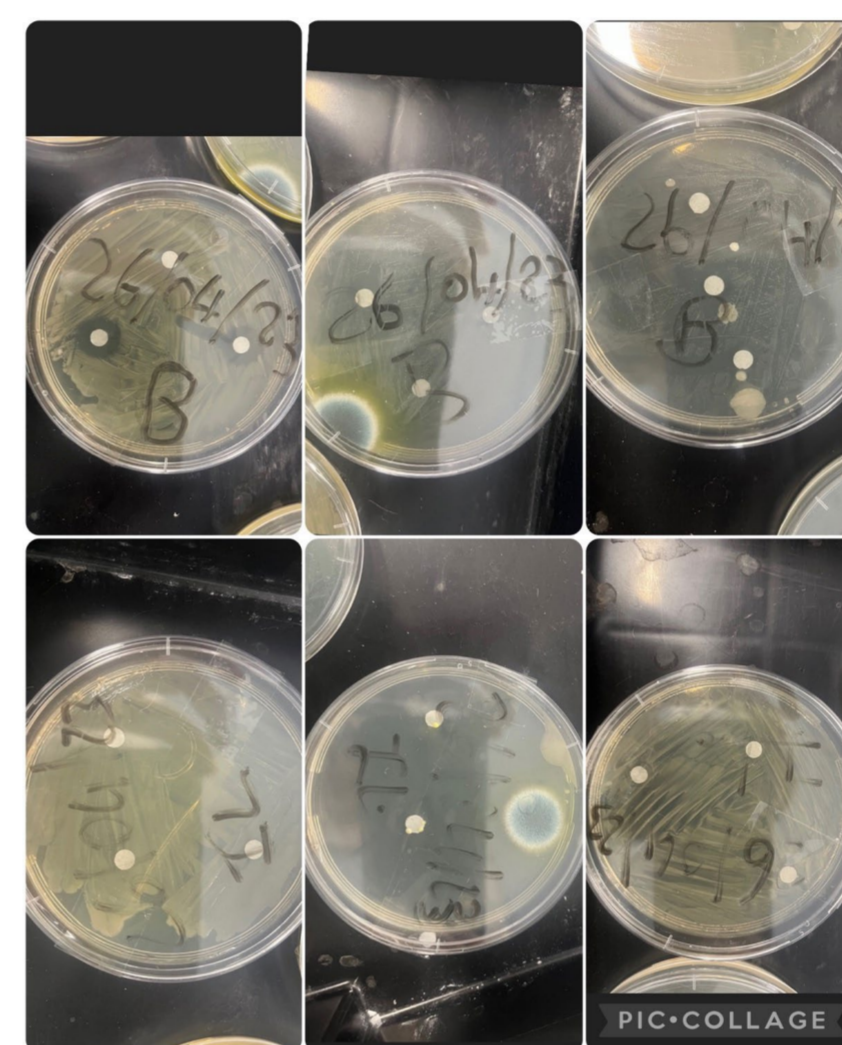


Figure 3 showing the growth of mould and E.coli and the zones of inhibition

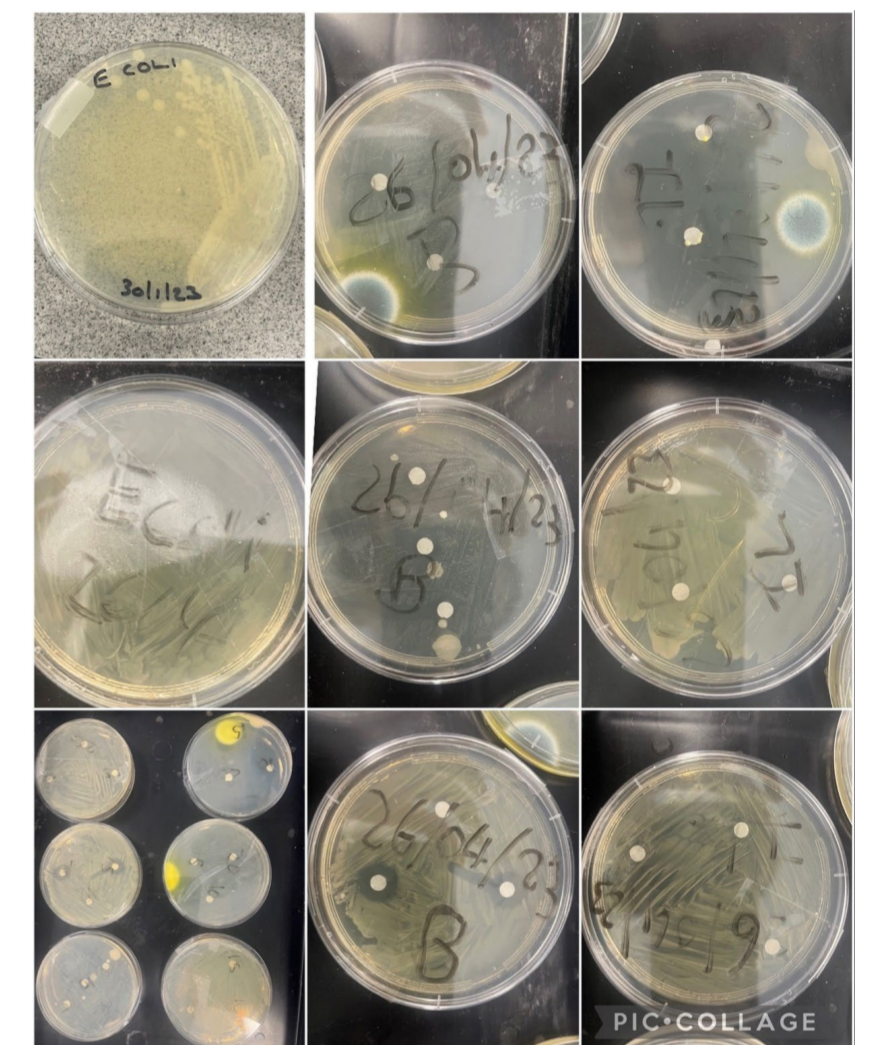
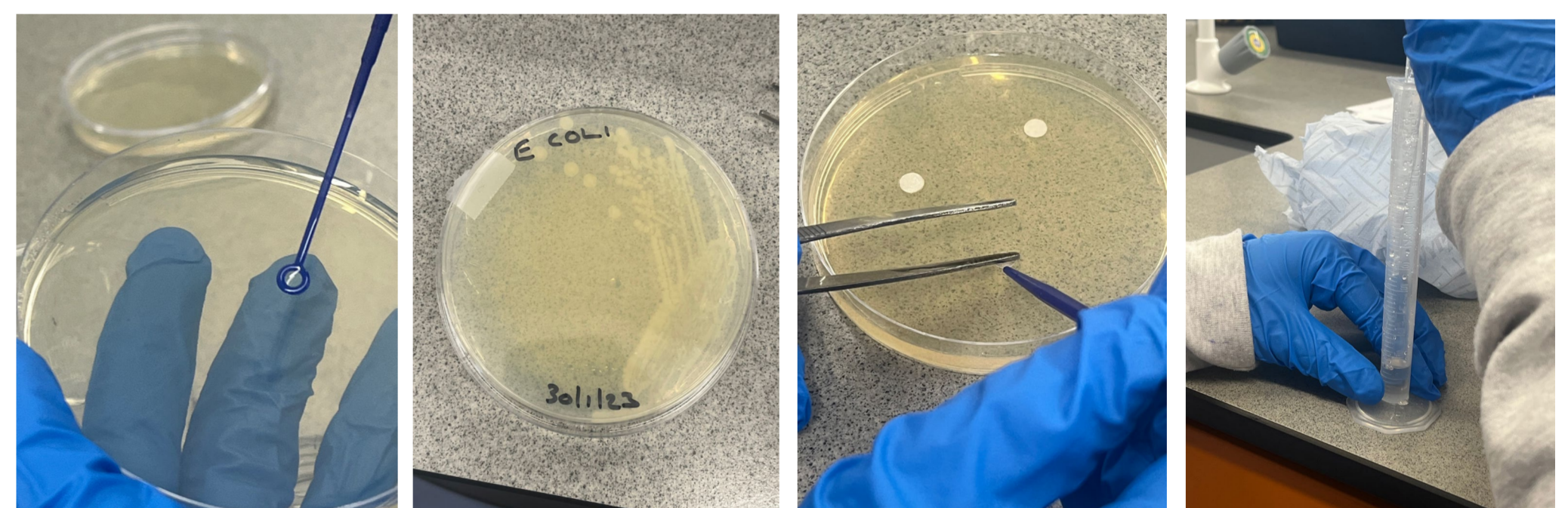


Figure 4 Showing petri dishes and the zones of inhibition around the paper discs



## Analysis & conclusions

In one of our previous experiments, the results showed that ionic liquid has weaker antibacterial properties compared to household bleach at lower concentrations: 5%, 10% and 15%. At 5% and 10%, the zone of inhibition were not visible, however the 15% plate of ionic liquids had a visible zone of inhibition. After the results of the experiment, we decided to increase the concentrations of the ionic liquid and the household bleach for further comparison.

While [BMIM]Cl did show some antibacterial properties, the household bleach was able to kill more bacteria in the same concentration of the ionic liquid. The ionic liquid has shown some results in creating a zone of inhibition; however, it is too early to say whether it is a better antibacterial agent than bleach at killing E.coli k-12.

The next step would be to repeat our experiment to see whether the mould developing on plate 2 was due to an error in our aseptic technique or caused by the [BMIM]Cl. If no mould develops when this experiment is repeated, we can conclude that either our aseptic technique was incorrect, or contamination occurred in our previous experiment. However, if no mould develops, we can remeasure the zone of inhibition and see which concentration from each [BMIM]Cl and Bleach were most effective

## References

- Ferraz, R. et al. (2020) Synthesis and antibacterial activity of ionic liquids and organic salts based on penicillin G and amoxicillin hydrolysate derivatives against resistant bacteria. *Pharmaceutics*. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7150922/>
- Gundolf, T., Kalb, R., Rossmann, P. and Mester, P. (2022). Bacterial Resistance Toward Antimicrobial Ionic Liquids Mediated by Multidrug Efflux Pumps. *Frontiers in Microbiology*, 13. doi:<https://doi.org/10.3389/fmicb.2022.883931>.
- Flieger, J. and Flieger, M. (2020). Ionic Liquids Toxicity—Benefits and Threats. *International Journal of Molecular Sciences*, 21(17), p.6267. doi:<https://doi.org/10.3390/ijms21176267>.
- Caister.com. (2020). Gram-negative Bacteria. [online] Available at: <https://www.caister.com/highveld/microbiology/gram-negative-bacteria.html>.
- Liwarska-Bizukojc, E., Maton, C. and Stevens, C.V. (2015). Biodegradation of imidazolium ionic liquids by activated sludge microorganisms. *Biodegradation*, [online] 26(6), pp.453–463. doi:<https://doi.org/10.1007/s10532-015-9747-0>.