Creation of library of carbapenemase and extended spectrum beta-lactamase producing Escherichia coli and Klebsiella pneumoniae

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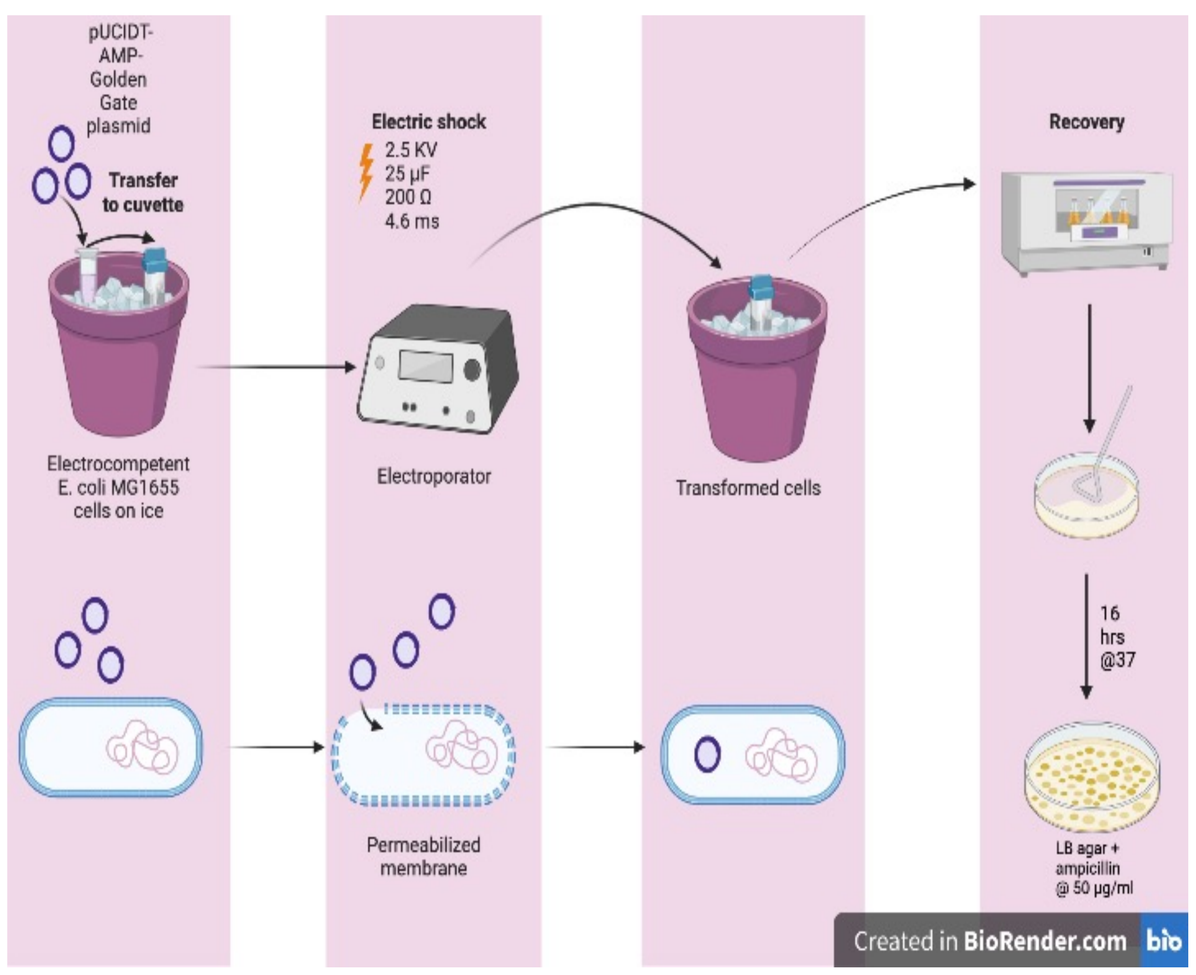
Background

- Antimicrobial resistant bacteria will kill more people than cancer by 2050 (Tagliabue and Rappuoli, 2018).
- The World Health Organisation published a catalogue of critical pathogens in 2017
- First priority on this list = carbapenem and ESBL producing Enterobacteriaceae e.g. *Klebsiella pneumoniae* and *Escherichia coli* (https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed).
- Plasmids are main mediator of AMR global dissemination (Yamashita *et al,* 2014).

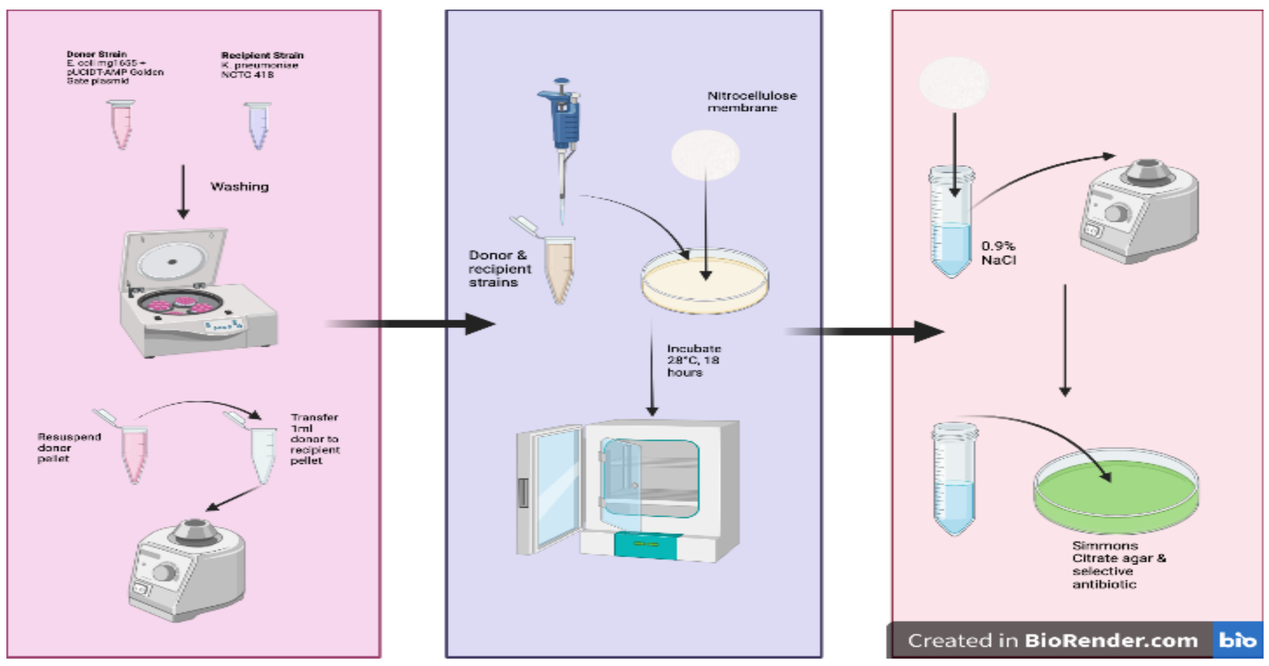
Methods

- x5 synthesized pucIDT-AMP Golden Gate plasmids were obtainedfrom IDT™, each only differing in 1 AMR gene; *bla*$_{KPC}$, *bla*$_{CTX\text{-}M\text{-}15}$, *aac(6')-Ib-cr, armA, and bla*$_{NDM}$.
- Plasmids were transformed into E. coli MG1655 electrocompetent cells through electroporation.
- Transformed E.coli was selected on LB plates containing ampicillin at 50ug/ml.
- Presence of plasmid was confirmed by PCR using primers specific to each gene
- In order to create a library of carbapenem and ESBL producing K. pneumoniae, plasmids were conjugated from E. coli into K. pneumoniae through filter mating; donor and recipient strains were incubated on a nitrocellulose membrane overnight at 37C. Growth on the nitrocellulose membrane was spread onto Simmons Citrate to select for K. pneumoniae.

Electroporation



Conjugation



Results

- Five different resistance genes of clinical importance (*bla*$_{KPC}$, *bla*$_{CTX\text{-}M\text{-}15}$, *aac(6')-Ib-cr, armA, and bla*$_{NDM}$) were individually transformed into *E.coli* MG1655 on Golden Gate PUCIDT plasmid backbone using electroporation
- These plasmids were then conjugated into K. pneumoniae NCTC 418 through filter mating.
- This created library of strains of E. coli and K. pneumoniae which all contain same plasmid backbone, but with different AMR gene.

Conclusions & Future Directions

- Generation of highly similar pathogens differing by only the AMR gene is a valuable resource to understand the impact of the AMR gene on different pathogens.
- This library of pathogens will be used for proteomic, metabolomic and bioinformatic analysis of the affect of the AMR on the bacterial cell.
- Library will be used to discover potential drug targets, to resurrect current antimicrobial therapies.

References

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- https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed
- Images created with BioRender.com

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