ASX Announcement

Recce Pharmaceuticals
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ACN 124 849 065

RECCE® 327 Demonstrates World First Multiple Mechanisms of Action against *E. coli* bacteria

Highlights:

- **RECCE® 327 (R327) Mechanism of Action '*unlike that of any antibiotic seen before*' with multiple mechanisms identified in independent study - to be presented at upcoming World Microbe Forum (20-24 June)**
- **R327 rapidly and irreversibly bactericidal against Gram-negative *Escherichia coli* (*E. coli*) bacteria, in both active and stationary phase cells - outperforming best in class commercial antibiotics**

Sydney Australia, 27 May 2021: Recce Pharmaceuticals Ltd (**ASX:RCE**) (**FSE:R9Q**) (**Company**), the Company developing New Classes of Synthetic Anti-infectives, is pleased to announce positive results from a pre-clinical study investigating the Mechanism of Action (MoA) of its lead compound RECCE® 327 (R327). R327 is a broad-spectrum synthetic anti-infective that has potential to address the urgent global health threat posed by antibiotic resistant superbugs and emerging viral pathogens.

The study was performed by independent, world leaders in bacterial Mechanism of Action analysis and antibiotic profiling.

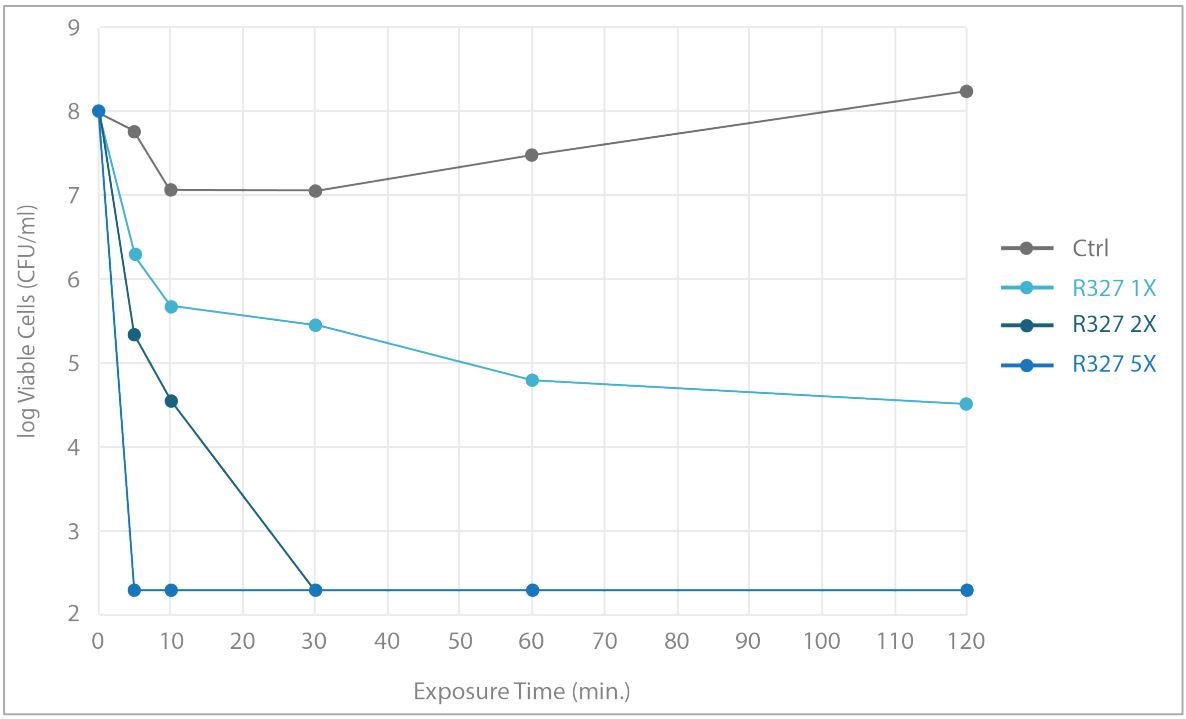
Key takeaways from this study are as follows:

- **R327 rapidly and irreversibly shuts down cellar energetics** (ATP production) – primary MoA
- **R327 affects the assembly of bacterial cell division** complex, components that require cellular energy to remain assembled, confirming its ability to disrupt cellular bioenergetics
- R327 results in the decreased formation of the bacterial cell division complex into ring-like structures (Z-rings) in a concentration dependent manner
- **R327 does not permeabilize the cell membrane or alter the integrity of the outer membrane** of *E. coli* cells – intended activity without toxicity
- At higher concentrations **and subsequent to ATP shut down cell lysis (bacterial bursting due to their uniquely high internal pressures) can occur as a further mechanism of action**
- **R327 rapidly and irreversibly bactericidal to slow-growing**, quiescent or stationary phase *E. coli* cells in addition to actively dividing *E. coli* cells
- Within a minute, the highest concentration of R327 used, 5x minimum inhibitory concentration (MIC), was observed to reduce viable cell counts reported as cell forming units per milliliter of culture (CFU/ml) 100-fold (>$1x10^7$ to $1x10^5$ at timepoint 0)

- **Current antibiotics rarely retain bactericidal activities against nondividing or stationary-phase bacterial cells**; however, R327 showed **remarkable activity against slow-growing bacteria thereby indicating potential antibacterial activity in biofilms**
- In comparison to ampicillin and ciprofloxacin, **R327 is able to outperform both of these antibiotics** in bactericidal activity (as measured by viable cell counts) against stationary cells

Study 1 – Determination of Minimum Inhibitory Concentration

Initial studies were undertaken to determine the MIC of R327 against various test strains of Gram-negative *Escherichia coli* (*E. coli*) bacteria in their actively growing (dividing) form to ultimately elucidate R327's MoA. All conditions started with $1x10^8$ CFU/ml *E. coli* cells which were then treated with R327 for the indicated period of time.



Viable Cell Counts for E.coli ATCC 25922 treated with B0063 for the indicated time before washout, initial inoculum of ~108 CFU/ml.

The bactericidal effect was evident immediately in all tested concentrations. In 5x MIC of R327 'crashing' the ATP viability of bacterial cells in minutes, reportedly faster then any antibiotic tested previously.

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Study 2 – Efficacy of R327 against actively growing or dividing *E. coli*

Second study determined whether R327 affects the assembly of the bacterial cell division complex. Cell division in bacteria is mediated by the tubulin-like protein FtsZ – a protein essential for cell division (bacteria reproduction) that requires cellular energy.

The *E. coli* clinical isolates were treated to 1x and 5x MIC for 30 minutes and showed 22% (1x MIC) and 17% (5x MIC) of the cells contained FtsZ rings, with the remaining cells containing small FtsZ-GFP filaments, suggesting **mid-cell FtsZ rings had disassembled**.

By 60 minutes, only 17% (1x MIC) and 8% (5x MIC) contained FtsZ rings. The remaining cells displayed small amount of **FtsZ-GFP and was completely diffused throughout the cell**, indicating a **disassembled FtsZ-GFP**.

These results highlight a decreased occurrence of FtsZ-GFP rings in a concentration dependent manner, concluding that **treatment with R327 leads to disassembly of the FtsZ-GFP rings (not allowing bacterial cells to divide, multiply and spread infection).**

- Images on next page -

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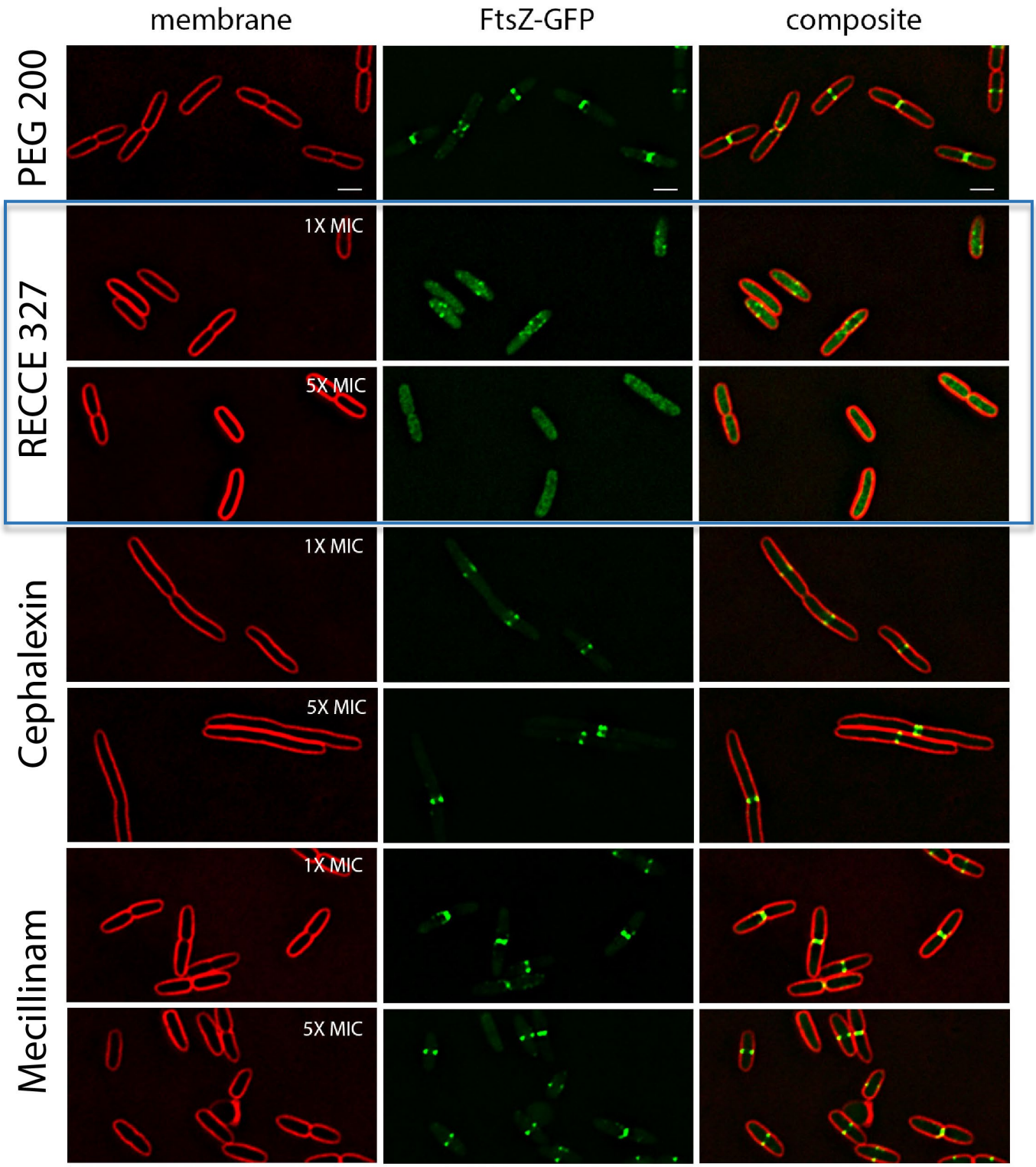
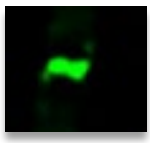
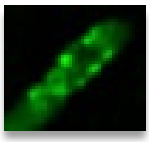


E. coli, expressing FtsZ-GFP, treated with R327, PEG200 and Control Antibiotics after 30 minutes of treatment

Key:



FtsZ rings – a protein essential for cell division (bacteria reproduction)



Dissassembled FtsZ rings - indicating the loss of ability for bacteria to reproduce

The image shows treatment of R327 against *E. coli* at 1x and 5x MIC leading to disassembly of the FtsZ-GFP rings, supporting initial studies which indicated R327 inactivates cellular bioenergetics and is rapidly and irreversibly bactericidal.

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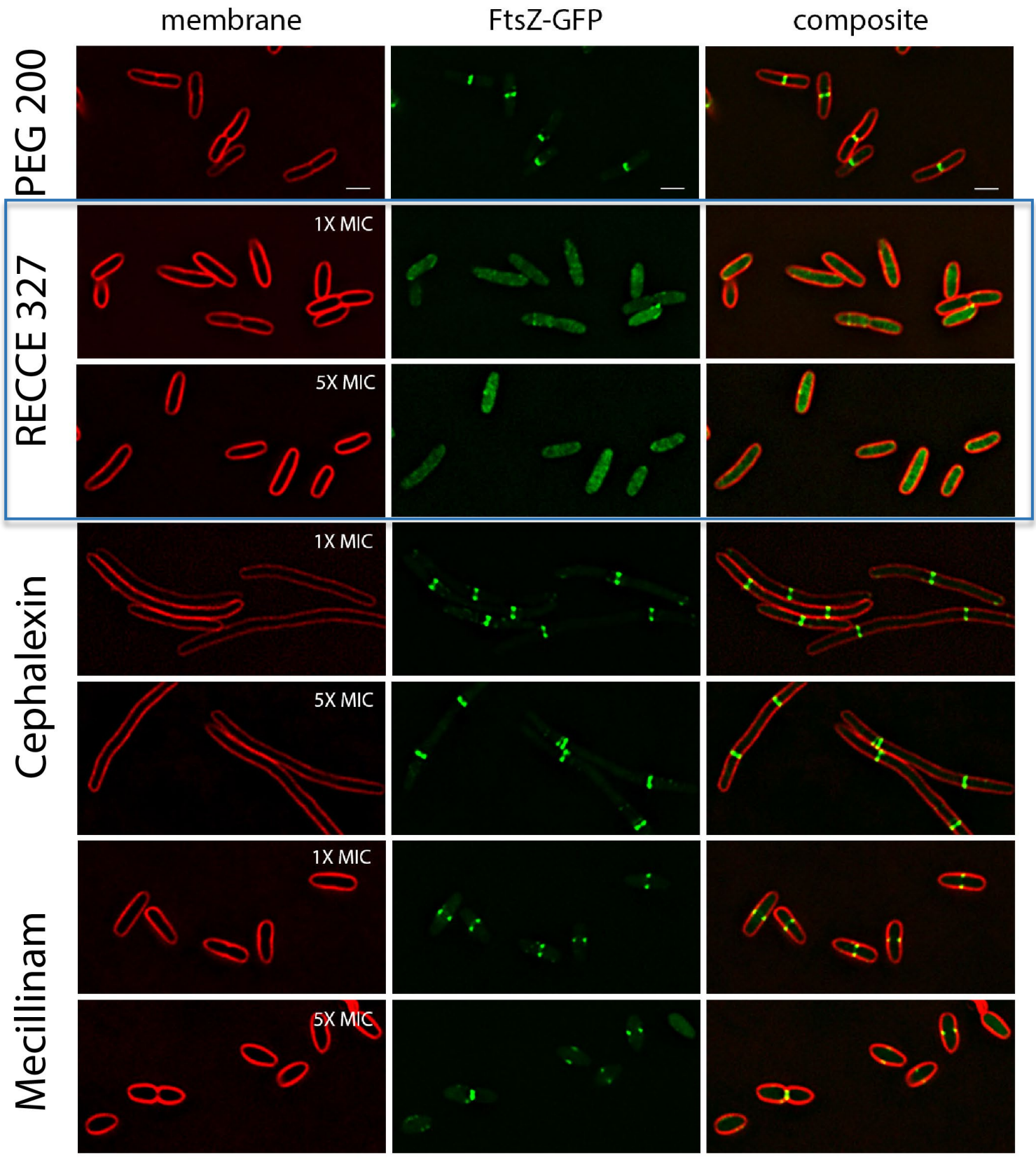

E. coli, expressing FtsZ-GFP, treated with R327, PEG200 and Control Antibiotics after 60 minutes of treatment

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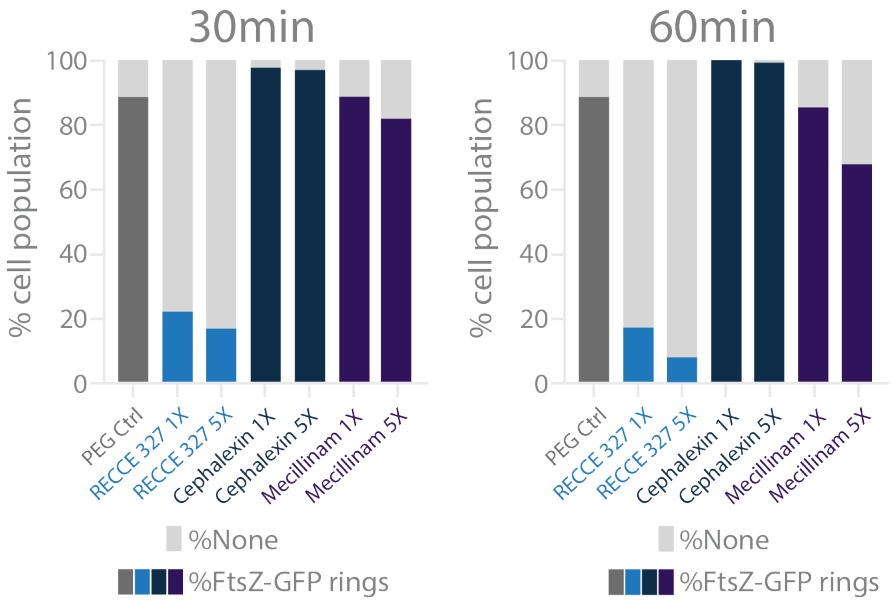
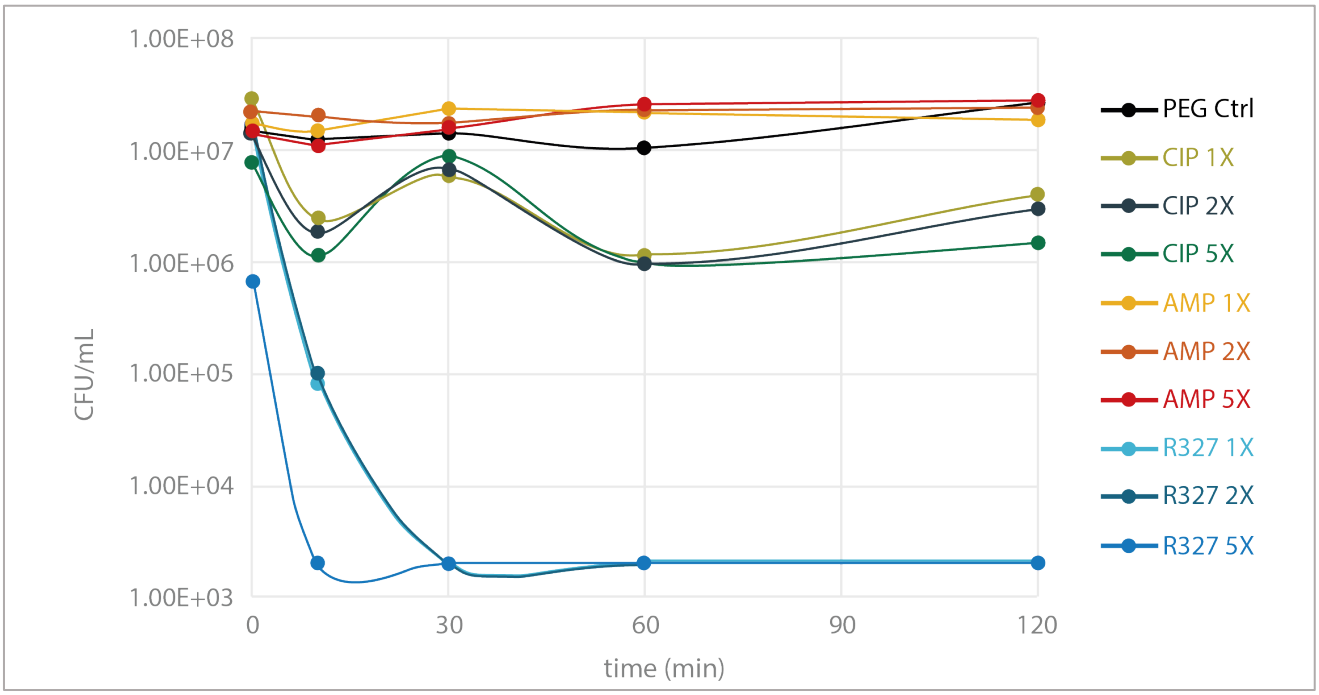


Quantification of FtsZ-GFP rings in *E. coli* treated with R327 and other control antibiotics Cephalexin and Mecillinam at 30 minutes and/or 60 minutes

Study 3 – Efficacy of R327 against nondividing or stationary-phase *E. coli*

A separate, but related study undertaken was to determine whether R327 is active against nondividing or stationary-phase *E. coli* using clinical isolate cells.



Viable cell counts for *E. coli* (ATCC 25922) stationary cells treated with RECCE® 327 and other control antibiotics ampicillin (AMP) and ciprofloxacin (CIP) for indicated time. Cell counts are shown as colony forming units (CFU) per milliliter on a logarithmic scale.

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Current antibiotics rarely retain bactericidal activities against nondividing bacterial cells; however, R327 shows significant activity against both slow-growing bacteria and actively dividing *cells,* thereby enabling continuous treatment of infections throughout the bacterial cell life cycle.

After only 10 minutes of R327 at 1x and 2x MIC, the viable cell counts decreased 100 fold, and after another 30 minutes, cell count decreased further to 10,000 fold (approximately). When exposed to the highest concentration, 5x MIC of R327, viable cell counts decreased 10,000 fold within 10 minutes. In comparison to the positive and negative controls, the report found ampicillin to be nearly completely resistant and ciprofloxacin only having mild activity against the cells.

These findings are aimed at elucidating R327's mechanism of action and will be presented at the World Microbe Forum taking place virtually 20-24 June 2021. Similarly, studies are underway to explore the effect and MoA of R327 on Gram-positive bacteria.

Recce Executive Director & Chief Scientific Officer Michele Dilizia says: "The data from this study further supports the potential of RECCE® 327 to provide a novel and universal way to treat harmful infections. We are excited about these highly encouraging results as they provide important insights regarding how RECCE® 327 is able to work repeatedly against the same strain of bacteria and their superbug forms.

This announcement has been approved for release by Recce Pharmaceuticals Board

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About Recce Pharmaceuticals Ltd

Recce Pharmaceuticals Ltd (ASX: RCE) is pioneering the development and commercialisation of New Classes of Synthetic Anti-Infectives designed to address the urgent global health problems of antibiotic resistant superbugs and emerging viral pathogens.

Recce's anti-infective pipeline is unique and comprised of broad-spectrum synthetic polymer antibiotics RECCE® 327, RECCE® 435, and RECCE® 529 for viral infections with unique mechanisms of action against hyper-mutation on bacteria and viruses, respectively.

Patented lead candidate RECCE® 327 has been developed for the treatment of blood infections and sepsis derived from *E. coli* and *S. aureus* bacteria – including their superbug forms. Recce's new antibiotic compound, RECCE® 435, has been formulated for oral use.

The FDA has awarded RECCE® 327 *Qualified Infectious Disease Product* designation under the *Generating Antibiotic Initiatives Now* (GAIN) Act – labelling it for Fast Track Designation, plus 10 years of market exclusivity post approval. Further to this designation, RECCE® 327 has been included on The Pew Charitable Trusts *Global New Antibiotics in Development Pipeline* as the only synthetic polymer and sepsis drug candidate in development.

Recce wholly owns its automated manufacturing, ready to support first-in-human clinical trials. Recce's anti-infective pipeline seeks to exploit the unique capabilities of RECCE® technologies targeting synergistic, unmet medical needs.

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