

# In vitro effect of ceftazidime-avibactam pressure on ceftazidime-avibactam resistance in KPC-producing *Klebsiella pneumoniae* clinical isolates.

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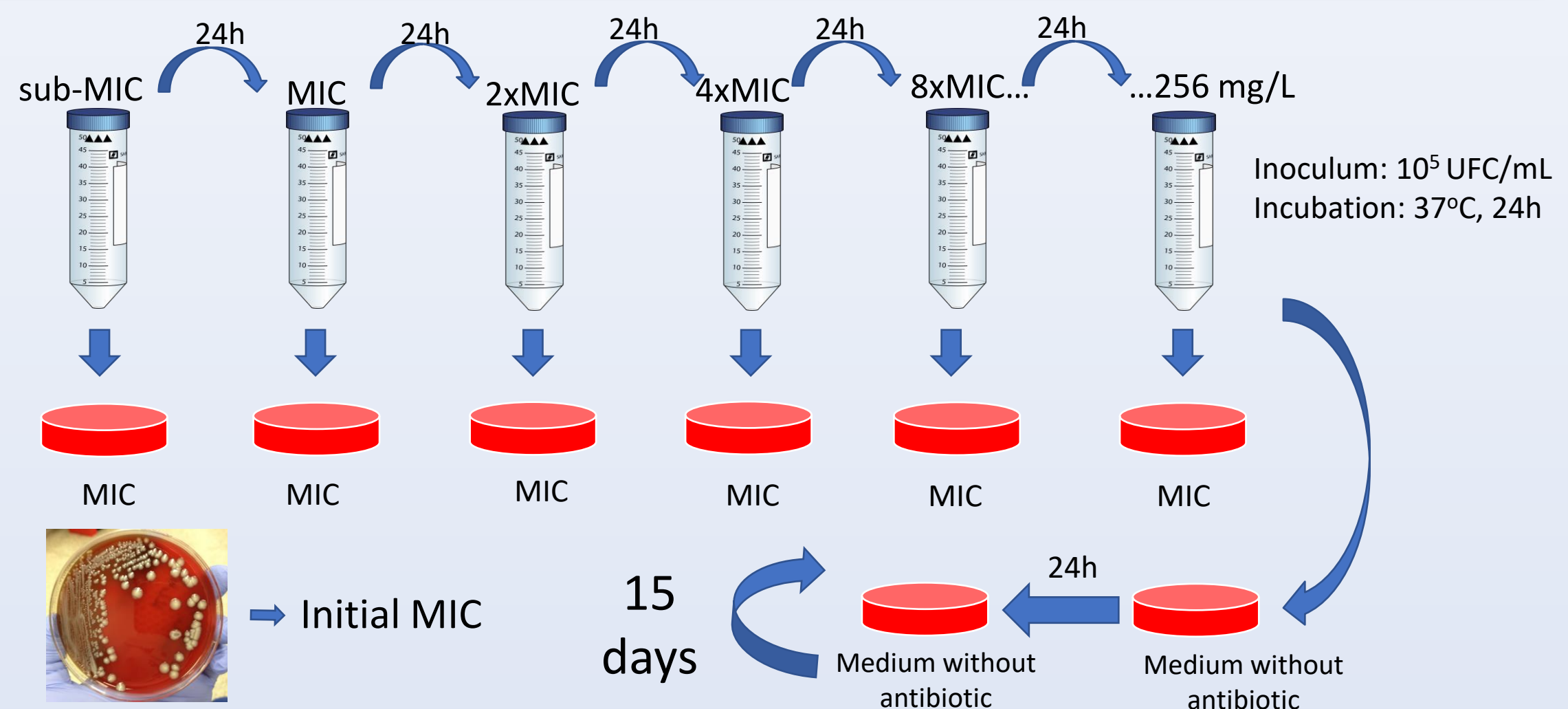
## INTRODUCTION AND PURPOSE

Infections caused by KPC-producing *Klebsiella pneumoniae* represent a challenge due to the limited available treatment choices. In this context, ceftazidime-avibactam (CAZ-AVI) is postulated as an alternative treatment effective against class A  $\beta$ -lactamases such as KPC [1]. But, recent data reported the failure of CAZ-AVI treatment of infections by KPC-producing *K. pneumoniae* due to the development of CAZ-AVI resistance [2]. However, little is known concerning the CAZ-AVI resistance development by CAZ-AVI selective pressure. Here, we aimed to determine *in vitro* whether the exposure of KPC-producing *K. pneumoniae* clinical isolates to CAZ-AVI subinhibitory concentrations could lead to the selection of CAZ-AVI resistant isolates.

## METHODS

Seventeen KPC-producing *K. pneumoniae* clinical isolates were analyzed. **Minimum inhibitory concentrations (MICs)** of CAZ-AVI were determined by broth microdilution assay using a fixed AVI concentration of 4 mg/L [3]. Moreover, these isolates were further **exposed to increasing concentrations of CAZ and fixed 4 mg/L of AVI**, from a sub-MIC up to 256/4 mg/L of CAZ-AVI (or the concentration able to kill the bacterial isolate) at 37°C with shaking during 24h. **New MICs to CAZ-AVI were determined in each condition and after 15 days without CAZ-AVI pressure.**

Therefore, in order to demonstrate that *bla*<sub>KPC</sub> gene is responsible for acquisition of CAZ-AVI resistance in KPC-producing *K. pneumoniae*, *bla*<sub>KPC-2</sub> and *bla*<sub>KPC-3</sub> were cloned into a reference *K. pneumoniae* CECT 997 strain. Resistance or susceptibility were determined according to EUCAST criteria [3].



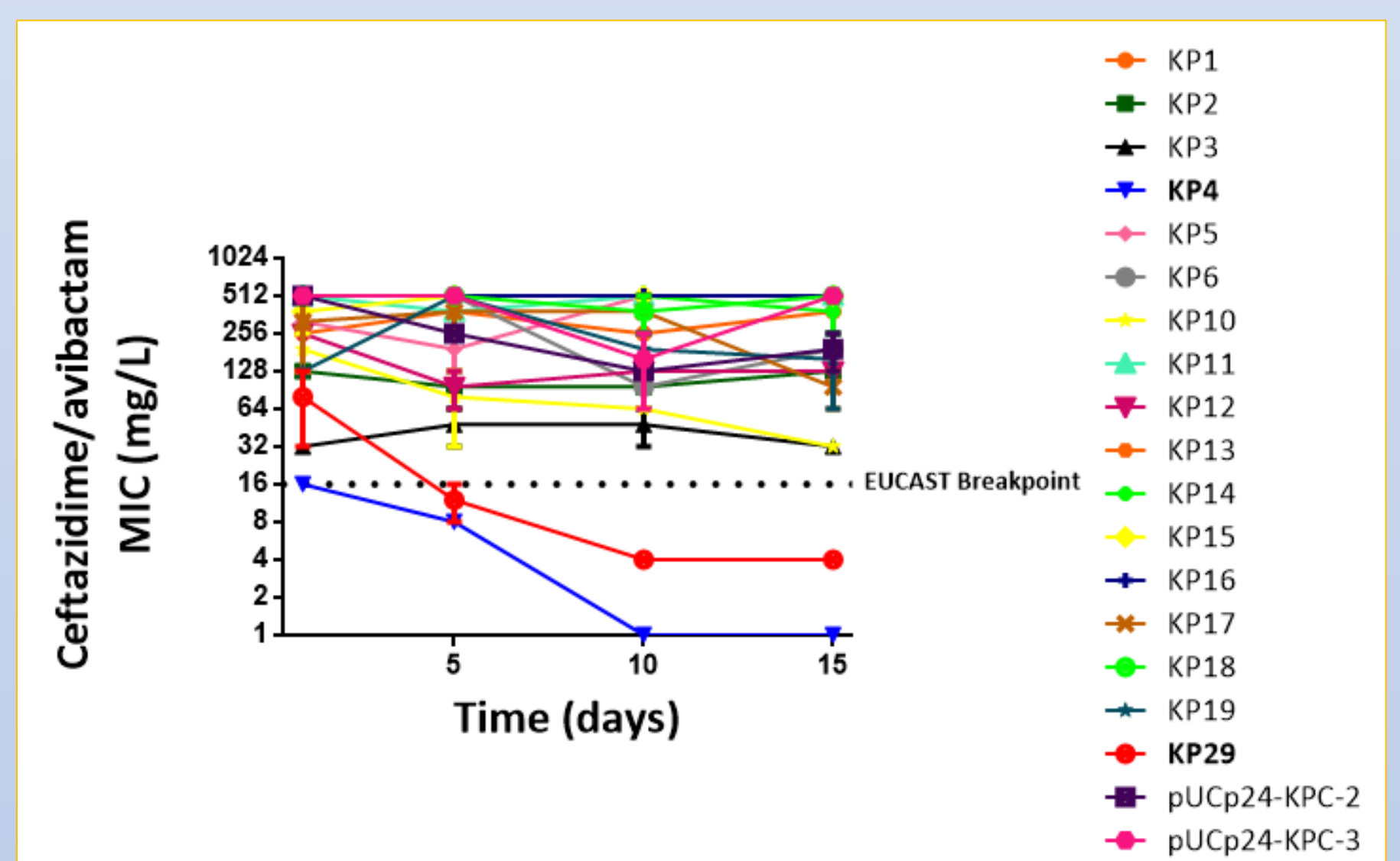
## RESULTS

- All (17/17, 100%) KPC-producing *K.pneumoniae* isolates were able to grow at high concentrations of CAZ-AVI ( $\geq 64/4$  mg/L), increasing their resistance to CAZ-AVI  $\geq 8$ -fold (Table 1).
- Fifteen of the 17 (88.2%) resistant isolates maintained the acquired CAZ-AVI resistance 15 days without CAZ-AVI pressure (Figure 1).
- The *K. pneumoniae* CECT 997 mutants with *bla*<sub>KPC-2</sub> or *bla*<sub>KPC-3</sub> were able to grow up to 256/4 mg/L of CAZ-AVI, displaying and maintaining CAZ-AVI MIC shift from  $<0.01/4$  mg/L (susceptible) to 512/4 mg/L (resistant) (Table 1) (Figure 1).

**Table 1.** Characteristics of the analyzed isolates and results of the CAZ-AVI selective pressure.

| Isolate                                    | Source                 | ST      | <i>bla</i> <sub>KPC</sub> | CAZ-AVI Pressure (mg/L) | MIC CAZ-AVI (mg/L)  | Fold change |
|--|------------------------|---------|---------------------------|-------------------------|---------------------|-------------|
| KP1  | Blood                  | 512     | KPC-3                     | 0.5 → 256               | 1 → 256 (S → R)     | 256         |
| KP2  | Rectal exudate         | Unknown | KPC-2                     | 2 → 256                 | 4 → 256 (S → R)     | 64          |
| KP3  | Intraabdominal abscess | 512     | KPC-3                     | 4 → 256                 | 8 → 128 (S → R)     | 16          |
| KP4  | Rectal exudate         | Unknown | KPC-2                     | 1 → 64                  | 2 → 16 (S → R)      | 8           |
| KP5  | Rectal exudate         | 745     | KPC-3                     | 1 → 256                 | 2 → 256 (S → R)     | 128         |
| KP6  | Unknown                | 353     | KPC-3                     | 0.25 → 256              | 0.5 → 512 (S → R)   | 1024        |
| KP10                                       | Unknown                | 784     | KPC-2                     | 0.5 → 256               | 1 → 512 (S → R)     | 512         |
| KP11                                       | Unknown                | 86      | KPC-2                     | 0.125 → 256             | 0.25 → >512 (S → R) | >2048       |
| KP12                                       | Unknown                | 258     | KPC-2                     | 1 → 256                 | 2 → 256 (S → R)     | 128         |
| KP13                                       | Unknown                | 376     | KPC-3                     | 2 → 256                 | 4 → >512 (S → R)    | >128        |
| KP14                                       | Unknown                | 392     | KPC-2                     | 0.125 → 256             | 0.25 → 256 (S → R)  | 1024        |
| KP15                                       | Unknown                | 258     | KPC-11                    | 0.25 → 256              | 0.5 → >512 (S → R)  | >1024       |
| KP16                                       | Unknown                | 340     | KPC-3                     | 1 → 256                 | 2 → 512 (S → R)     | 256         |
| KP17                                       | Unknown                | 231     | KPC-3                     | 0.25 → 256              | 0.5 → 128 (S → R)   | 256         |
| KP18                                       | Unknown                | 166     | KPC-2                     | 0.25 → 256              | 0.5 → 256 (S → R)   | 512         |
| KP19                                       | Unknown                | 307     | KPC-3                     | 1 → 256                 | 2 → 256 (S → R)     | 128         |
| KP29                                       | Unknown                | 512     | KPC-3                     | 1 → 64                  | 2 → 64 (S → R)      | 32          |
| <i>K. pneumoniae</i> CECT 997-pUCp24-KPC-2 | -                      | -       | KPC-2                     | 0.02 → 256              | <0.01 → 512 (S → R) | >51200      |
| <i>K. pneumoniae</i> CECT 997-pUCp24-KPC-3 | -                      | -       | KPC-3                     | 0.02 → 256              | <0.01 → 512 (S → R) | >51200      |

ST, Sequence Type; S, susceptible; R, resistant.



**Figure 1.** CAZ-AVI MIC Values for the clinical isolates and mutants analyzed over the 15 days without CAZ-AVI pressure. pUCp24-KPC-2 and pUCp24-KPC-3, the *K. pneumoniae* CECT 997 mutants.

## CONCLUSIONS

- These data suggest that exposure of KPC-producing *K. pneumoniae* to subinhibitory CAZ-AVI concentrations could lead to the selection of CAZ-AVI resistant isolates.
- The acquisition of CAZ-AVI resistance seems to be stable over the time, without CAZ-AVI pressure conditions.
- The *bla*<sub>KPC-2</sub> and *bla*<sub>KPC-3</sub> genes are involved in the acquisition of CAZ-AVI resistance.
- Programs for optimizing the use of antibiotics should consider these data to avoid the increase of CAZ-AVI resistance.

## REFERENCES

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