Colistin resistance and biofilm formation in international *Klebsiella pneumoniae* clones

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Introduction

Today, *Klebsiella pneumoniae* (KPN) is becoming one of the major causes of hospital-acquired infections due to its increasing resistance levels(1). Colistin is one of the last line antibiotics to treat these infections with multidrug resistant KPN. However, colistin resistance (CR) is being reported more frequently during the past years(2). Besides high resistance levels, KPN also is able to form biofilms, which enables the bacteria to escape form host immune reaction and contributes to the persistence of infection(3).

Here, we report our study on biofilm formation associated with CR phenotype in clinical isogenic KPN isolates. These strains belong to three of the most pathogenic sequence types that are currently predominant in Europe, ST258, ST383 and ST147.

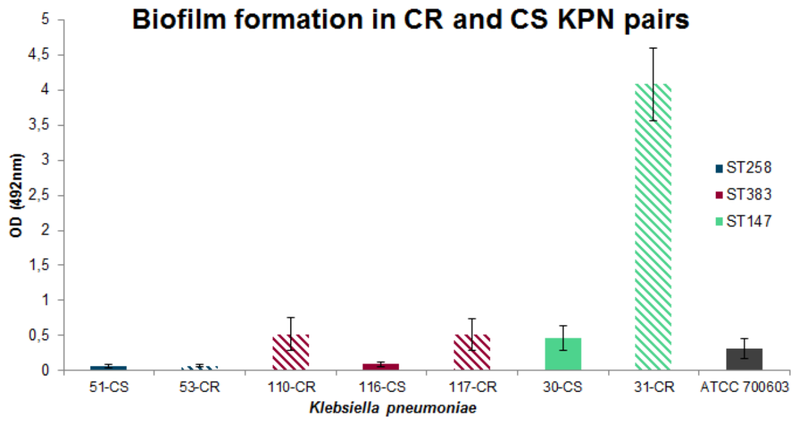

Figure 1. **Biofilm formation in CR and CS KPN pairs:** Increased biofilm formation for both ST383 and ST147 CR (hatched bars) phenotype. Weak biofilm formation for both ST258 CR and CS (full bars) phenotype. ATCC 700603 (grey) was used as a control strain. Error bars predict 95% CI. (OD = optical density)

Material and Methods

Analysis was performed on three clinical isogenic KPN pairs. A pair was defined as 2-3 strains isolated from the same patient at different time points. Strains were isolated from patients which were admitted to the intensive care unit at Tzaneio General Hospital of Piraeus, Greece or at the University General Hospital of Larissa, Greece (**Table 1**). The ST258 (51-CS, 53-CR) and ST147 pairs (30-CS, 31-CS) consisted of one CR strain and one colistin sensitive (CS) strain. The ST383 pair consisted of two CR strains (110-CR, 117-CR) and one CS (116-CS), where 110-CR was isolated prior to 116-CS and 116-CS prior to 117-CR. Antibiotic susceptibility testing was performed according to the 2014 CLSI guidelines(4). Disc diffusion was used for most conventional antibiotics and colistin MICs were performed by the macrobroth dilution.

Whole genome mapping (WGM) (ArgusTM system, Opgen, Gaithersburg, USA) was introduced to study the genomic structure of the ST383 and ST258 pairs. Isolated DNA was restricted with *AflII*. Assembly of DNA molecules, visualization and editing was performed using MapManager (Opgen) and Bionumerics software (Applied Maths, Belgium).

Biofilm formation was evaluated in an *in vitro* static biofilm assay where optical densities (OD492) were measured and compared with a simultaneously run KPN reference strain, ATCC 700603 (**Fig 1**).

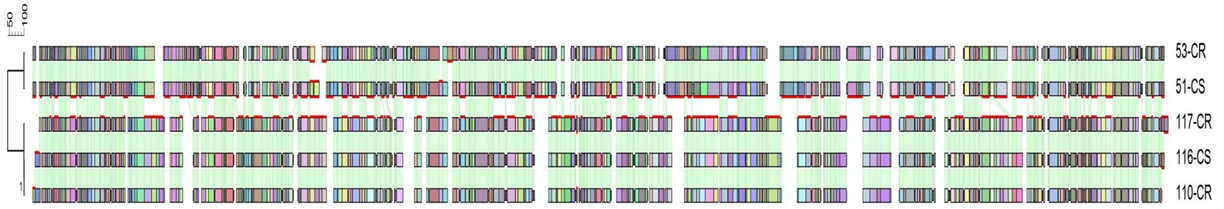

Figure 2. **Whole genome maps:** WGM of ST258 pair (51-CS and 53-CR) and ST383 pair (110-CR, 116-CS and 117-CR). Green lines indicate identity of restriction pattern among the maps and red horizontal marks represent the variations.

Results and Conclusion

Antibiotic susceptibility patterns showed that all strains were highly resistant to most conventional antibiotics, except for gentamicin and fosfomycin. All CS strains showed MICs ranging from 1-2µg/ml, CR strains showed MIC of 32µg/ml.

A significant increase ($p<0.001$) in biofilm formation was observed for both ST147 and ST383 CR strains, in comparison to their CS counterparts where biofilm formation was found to be weak. In contrast, both CR and CS ST258 strains showed very weak biofilm formation, confirming recent observations by Naparstek et al. (5) (**Figure 1**).

WGM showed a high similarity of 98.98% and 99.37%, within the ST258 pair and the ST383 pair respectively. Besides a prophage insertion of ~50kb in ST258 strain 53-CR, no other deletions, insertions or inversions >5kb were observed between the strains of each pair. Overall similarity between the ST383 and the ST258 clusters only was 30.60% (**Figure 2**).

Within the scope of this study we found enhanced biofilm formation associated with the CR phenotype of the ST383 and ST147 strains. While underlying genotypic mechanisms still need to be elucidated, the commonality of this phenomenon could have major implications in the clinical setting.

Strain ID	MLST Type	Site of isolation	Time of isolation	Colistin E-Test (µg/ml)	Col Macrobroth (µg/ml)
51-CS	258	Blood	04/2009	0.125	2
53-CR	258	Urine	05/2009	6	32
110-CR	383	Blood	01/2010	32	32
116-CS	383	CVC	04/2010	0.125	2
117-CR	383	Blood	04/2010	24	32
30-CS	147	Urine	12/2007	0.19	1
31-CR	147	Urine	01/2008	12	32

Table 1. Strain characteristics: clinical data, MLST types and colistin MICs (CVC = central venous catheter)

References

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