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IMMEM XI

NEXT GENERATION SEQUENCING: THE KEY TO UNDERSTAND *Klebsiella pneumoniae* BIOFILMS?

P35

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Introduction

The incidence of healthcare-associated infections (HAI) is determined by underlying disease conditions and exposure to high risk medical interventions. In Portugal since 1980s *K. pneumoniae* is a recognized etiological agent of epidemic and endemic HAIs. An increasing rate of *K. pneumoniae* strains resistant either to extended cephalosporins or carbapenems has been observed and one of the mechanisms responsible for the emergence of drug resistance could be the biofilm assembly. The capacity of *K. pneumoniae* to form biofilm was first described in the 1980s for abiotic surfaces and ten years later on biotic surfaces. The antibiotic failure to penetrate through the biofilm layers, the emergence of mutations which might be easily transferred horizontally, and quorum sensing have been pointed as responsible for the increased antibiotic resistance.

In the present study we aim to study the connection between biofilm structure, biofilm kinetic assembly and antibiotic resistance profile found into *K. pneumoniae* strains with and without capsule. The identification of the genes involved in biofilm assembly using full genome sequencing of strains was also a goal.

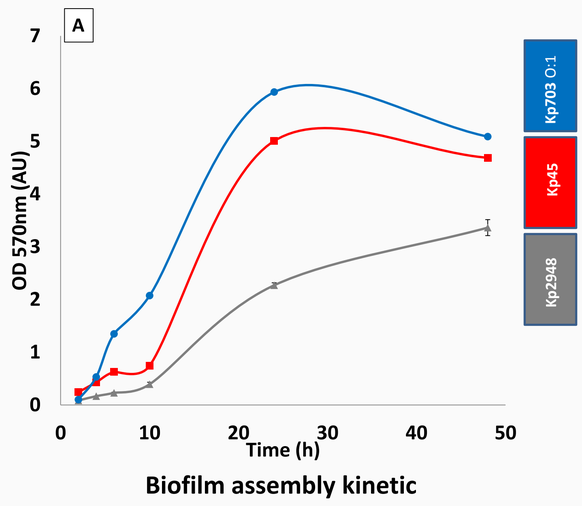
Material and Methods

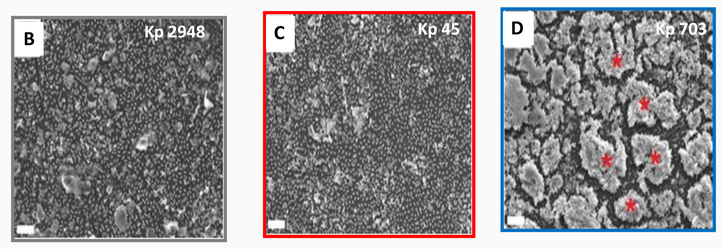
Three *K. pneumoniae* isolates were studied the encapsulated Kp703 with cell wall serotype O:1 and capsulated isolates Kp45 and Kp2948 both with capsular serotype K2. The bacterial ability to assemble biofilms was evaluated on cell culture plates, as well as on stain steel and silicon surfaces. For SEM analysis, biofilms were allowed to form on six wells cell culture plates for 12h at 37ºC. DNA was extracted using QiAamp DNA mini kit following the manufactures instructions. Full genome sequence was performed using NGS platform MiSeq (Illumina Inc., San Diego, CA, USA) according to the manufacturer's instructions. The RAST platform was used for annotation and MAUVE platform for multiple alignments.

Results

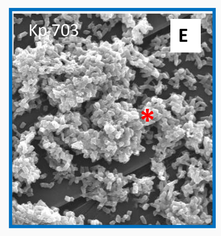
1. Biofilms

All strains showed different biofilm assembly kinetics. The encapsulated *K. pneumoniae* Kp703 with wall serotype O:1 was the best biofilm assembler followed by the isolates Kp45 and Kp2948 both with capsule serotype K2. This result was reproducible for biofilm assembly on 3 different surfaces.



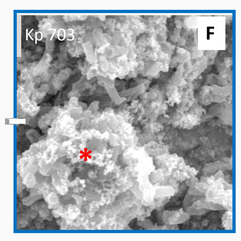


Images of 12h old biofilms assembled on cell culture plates by Kp 2948 (B), Kp45 K:2 (C) and Kp703 O:1 (D) For Kp 703 O:1



Kp 703 O:1 showed the best biofilms with the same age on stain steel (E) and silicon (F).

(*) Mature biofilm structures



2. Biofilms and antibiotic resistance

Higher MIC values were obtained for amoxicillin, fosfomycin and gentamicin at biofilm form (2) when compared to planktonic form (1), exception for fosfomycin against Kp 45 and Kp 2948.

The biofilm assembler from Kp703 strain registered dramatic increases in MIC values ranging from 10 fold for amoxicillin to 1000 fold for fosfomycin).

1

Bacteria	MIC (µg/mL) Amoxicillin	Fosfomycin	Gentamicin
Kp45	250	0.781	3.05
Kp703	250	<0.488	0.76
Kp2948	>500	0.781	1.52

2

Biofilm	MIC (µg/mL) Amoxicillin	Fosfomycin	Gentamicin
Kp45	>2500	0.781	24.4
Kp703	>2500	500	195
Kp2948	2500	0.781	3.05

3. Genomic analysis

3.1. Multilocus Sequence Typing (MLST)

Kp45 and Kp2948 strains had the same ST14

ST	gapA	infB	mdh	pgi	phoE	rpoB	tonB
14	1	6	1	1	1	1	1
15	1	1	1	1	1	1	1

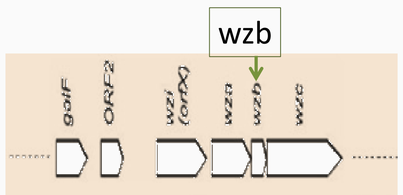
Kp703 had a different sequence type ST15

These sequence types differ only in Locus *infb*: *infb-6* and *infb-1*

3.2. Sequence analysis of Capsular and Biofilms genes

The encapsulated Kp 703 have specific characteristics of the genetic regions not found against others strains namely:

- formation of capsule - the absence of the gene wzb. Genes *wza, wzc* from capsular cluster showed 100% homology among three strains;
- biofilms assembly could be facility by filamentous hemagglutinin, a putative exoprotein involved in adhesion and biofilm formation



Conclusion

- The structure and composition of the biofilms are distinct and independent of the antibiotic resistance profile of each strain;
- The encapsulated *K. pneumoniae* 703 considered less virulent, have a better performance as biofilm assembler and exhibited the highest increase in antibiotic resistance when organized within biofilms;
- The results are in support of the role of filamentous hemagglutinin adherence and biofilm formation. The findings may be applied in active and passive immunization strategies against *Klebsiella pneumoniae.*