



BIOFILMS IN HEALTH AND DISEASE

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INTRODUCTION



Biofilms

Microorganism identification

In vitro biofilm assembly

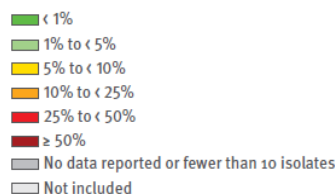
Biofilm carachterization

Biofilm phenotype / bacteria genome

Healthcare-associated infections (HAI) are a major public health problem.






2.6 million cases in long-term care facilities; 4.1 million cases in acute-care hospitals, which result in 37,000 annual deaths in Europe per year.

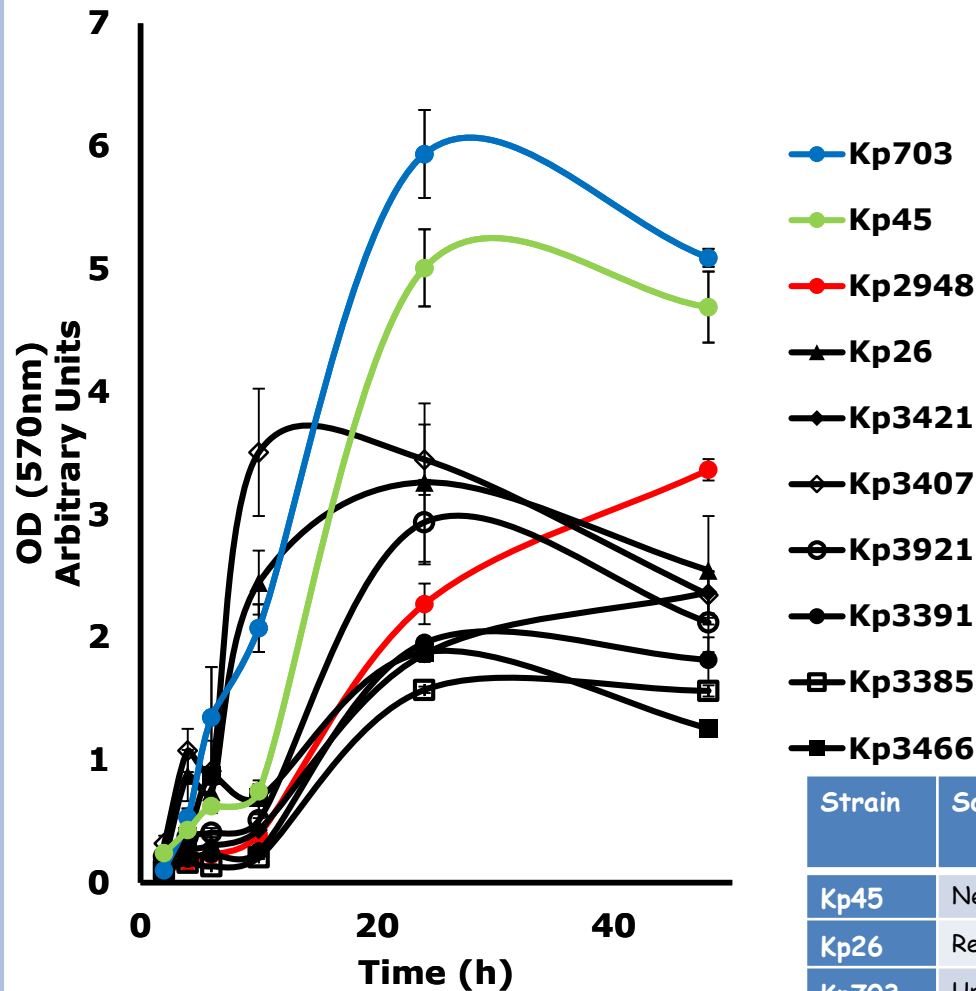
Klebsiella pneumoniae is a leading ethiologic agent.



25 to < 50%

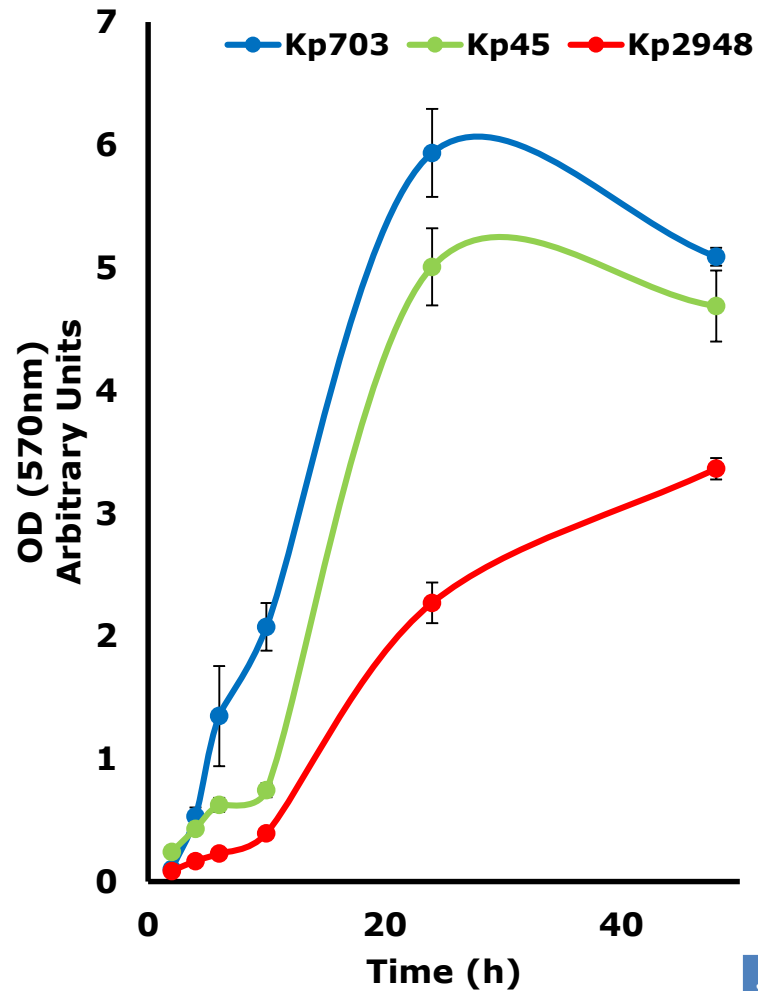
Non-visible countries
Liechtenstein
Luxembourg
Malta

Antibiotic	2012			2013			2014			2015			Trend 2012-2015
	N	%R	(95%CI)	N	%R	(95%CI)	N	%R	(95%CI)	N	%R	(95%CI)	
Fluorquinolones (F)	777	35,8	(32-39)	911	35,7	(33-39)	1712	36,5	(34-39)	2094	38,6	(36-41)	
3rd generation cephalosporins (C)	781	38,7	(35-42)	911	37,0	(34-40)	1712	40,9	(39-43)	2094	40,4	(38-43)	
Aminoglycosides (A)	780	31,8	(29-35)	912	29,4	(26-32)	1706	30,5	(28-33)	2090	32,6	(31-35)	
Carbapenems	749	0,7	(0-2)	904	1,8	(1-3)	1701	1,8	(1-3)	2085	3-4	(3-4)	
Combined resistance to FCA	776	25,1	(22-28)	909	21,7	(19-24)	1705	22,8	(21-25)	2084	25,0	(23-27)	



Strain	Source	Year	Serologic group	Fimbriae		β -lactamases
Kp45	Neck swab	1980	K:2	fimH	mrkD	TEM-1
Kp26	Rectal swab		K:2	n.a.	n.a.	TEM-1
Kp703	Urine		O:1	n.a.	mrkD	TEM-1
Kp3921		2010	n.a.	fimH	mrkD	CTX-M-15
Kp2948	Wound		K:2	fimH	mrkD	KPC-3; TEM-1
Kp3421	Urine	2011	n.a.	fimH	mrkD	CTX-M-15
Kp3407			n.a.	fimH	mrkD	KPC-3
Kp3466			n.a.	n.a.	n.a.	TEM-163
Kp3385			n.a.	fimH	mrkD	KPC-3

Klebsiella pneumoniae biofilms



4h

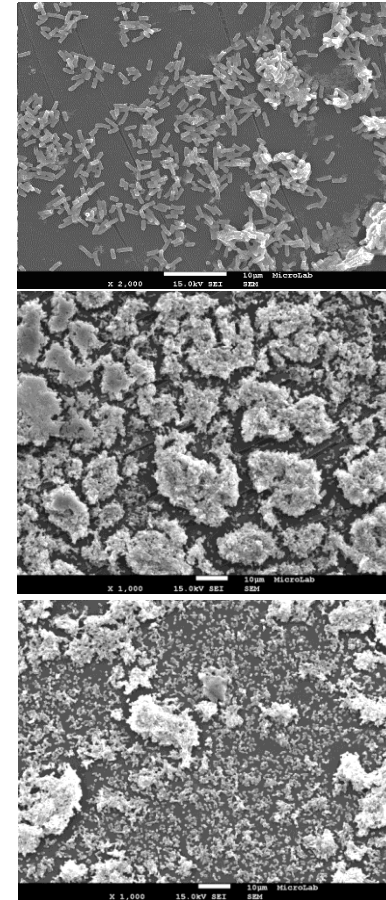
12h

24h

Attachment

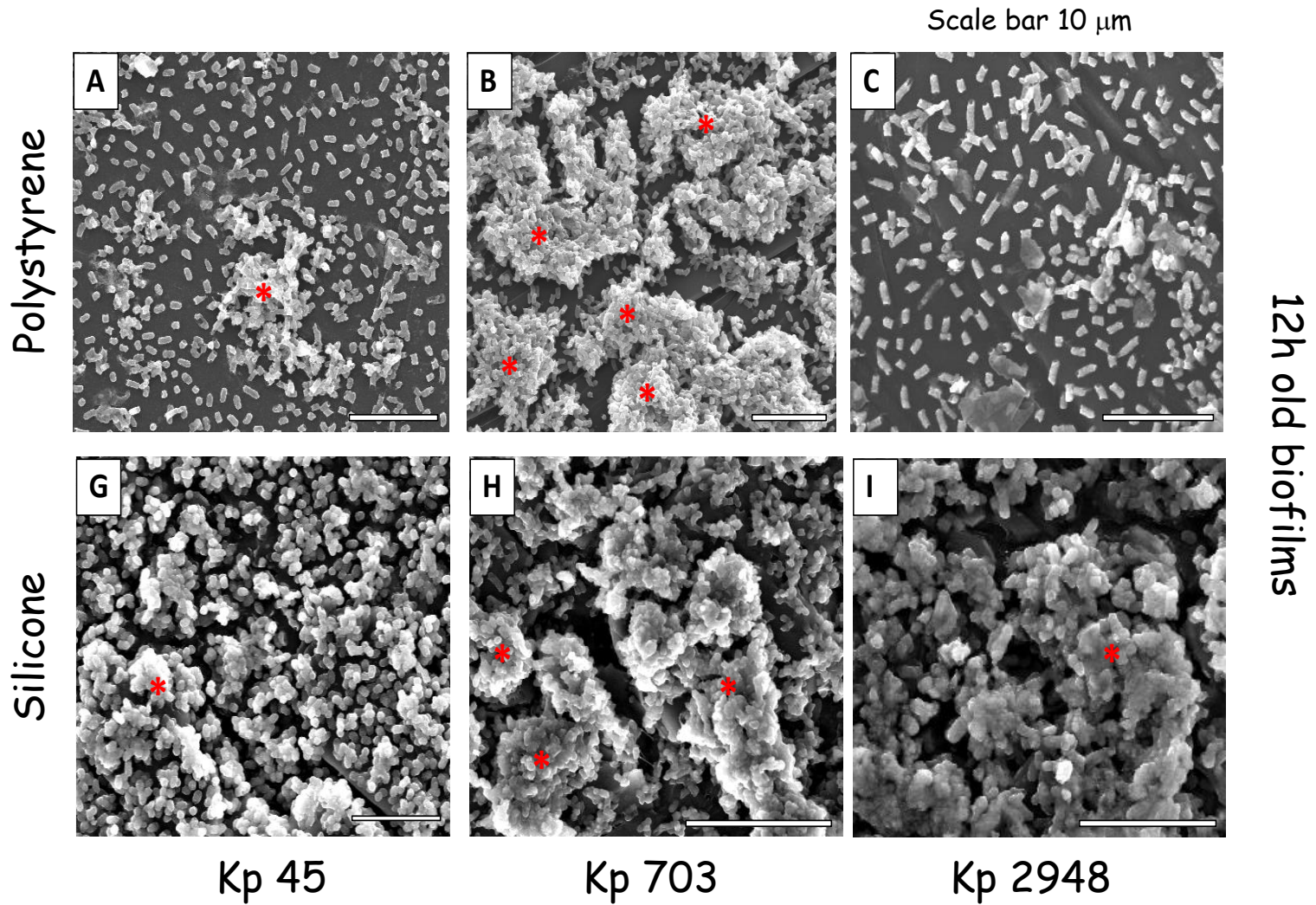
Maturation

Dispersion

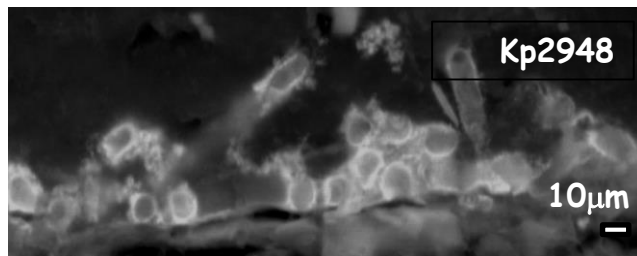
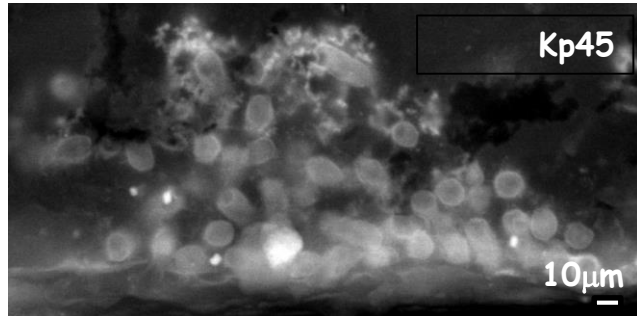
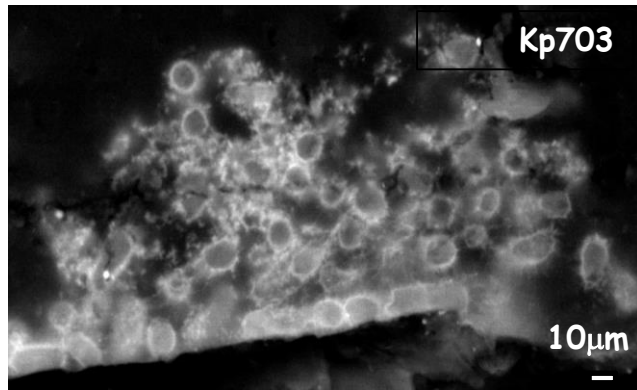


Strain	Source	Year	Serologic group	Fimbriae		β -lactamases
Kp45	Neck swab	1980	K:2	fimH	mrkD	TEM-1
Kp703	Urine		O:1	n.a.	mrkD	TEM-1
Kp2948	Wound	2010	K:2	fimH	mrkD	KPC-3; TEM-1

Klebsiella pneumoniae biofilms

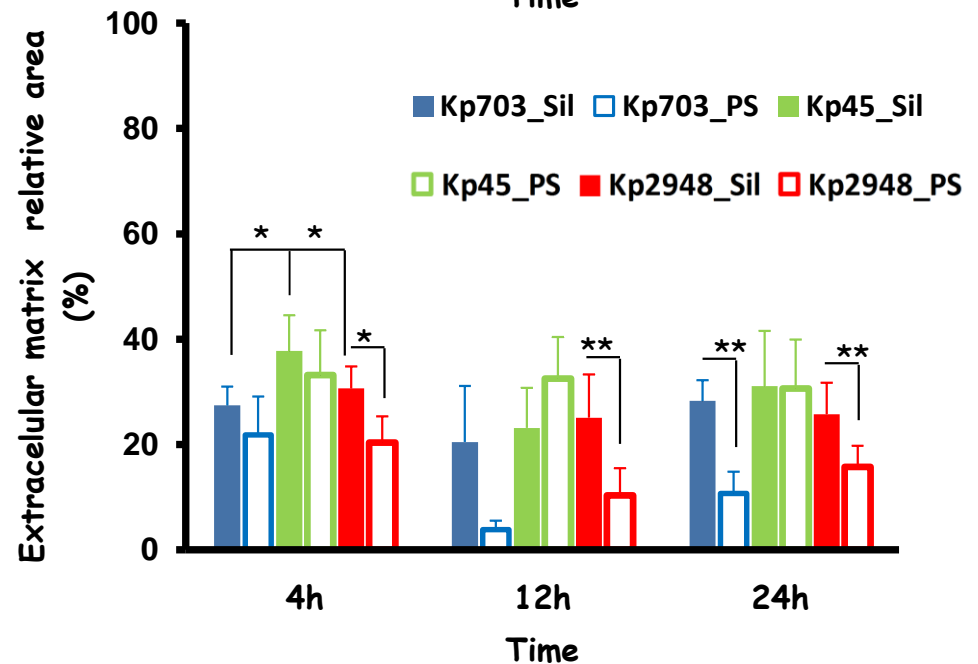
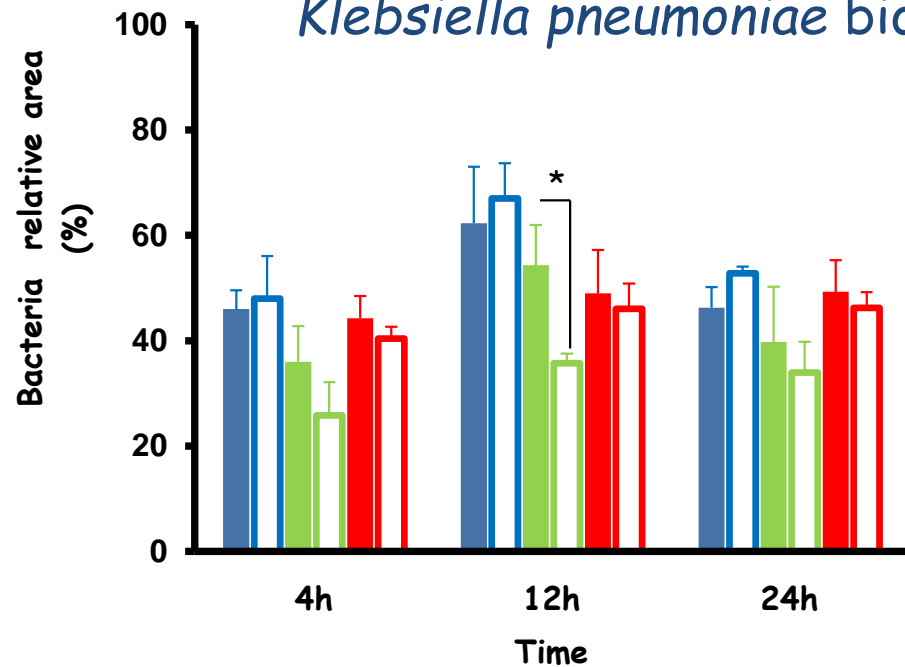


4h old silicone biofilms

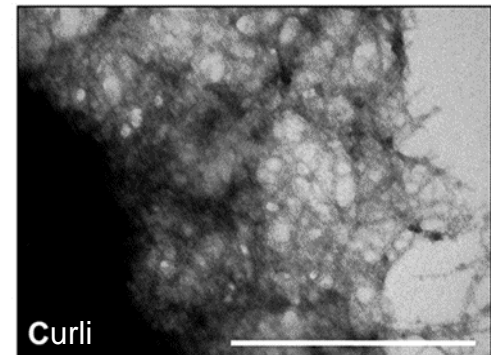
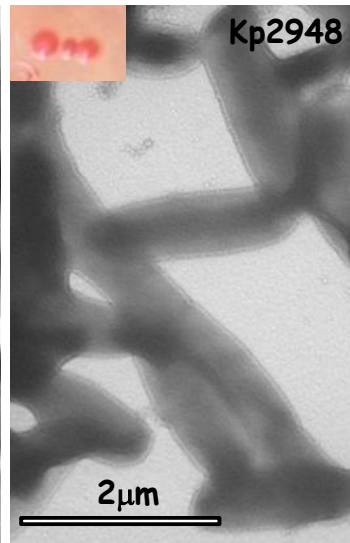
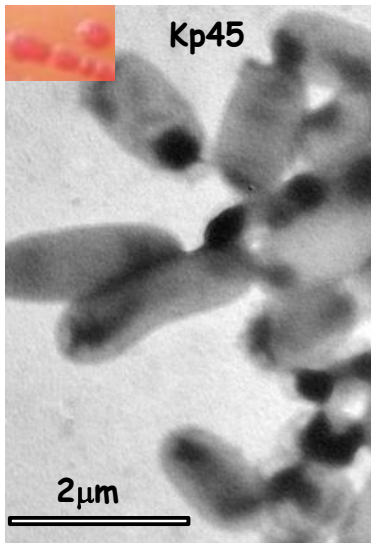
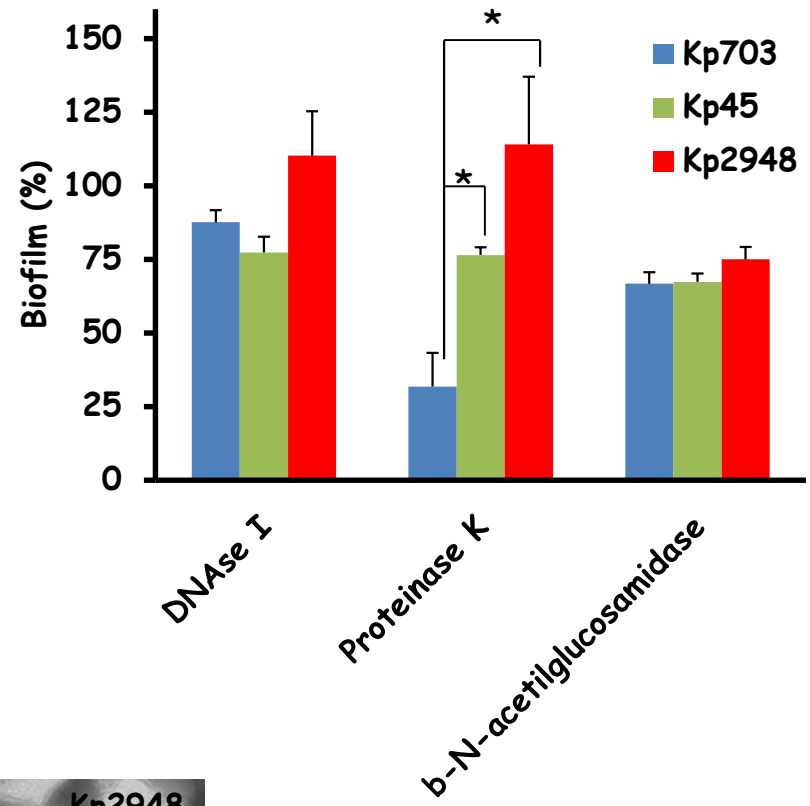


Scale bar 10 μm

Klebsiella pneumoniae biofilms



Extracellular polymeric matrix



Biscola *et al.* *Appl. Environ. Microbiol.* 2011;77:2201-2208

Klebsiella pneumoniae biofilms vs antibiotic resistance

Bacteria		MIC (µg/ml)			
		Amoxicillin	Fosfomycin	Gentamicin	Vancomycin
Planktonic	<i>K. pneumoniae</i> 45	250	0,781	3.05	500
	<i>K.pneumoniae</i> 703O:1	250	< 0.488	0,76	500
	<i>K.pneumoniae</i> 2948	> 500	0.781	1,52	1000
Biofilm	<i>K. pneumoniae</i> 45	10	=	8	ND
	<i>K.pneumoniae</i> 703O:1	10	1000	257	ND
	<i>K.pneumoniae</i> 2948	5	=	2	ND

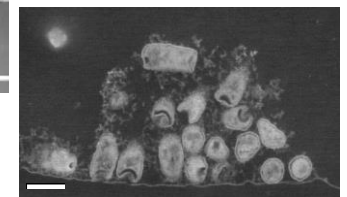
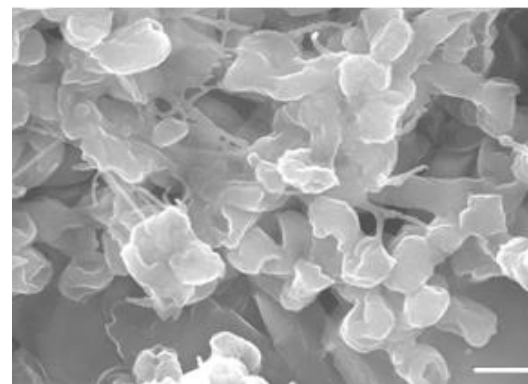
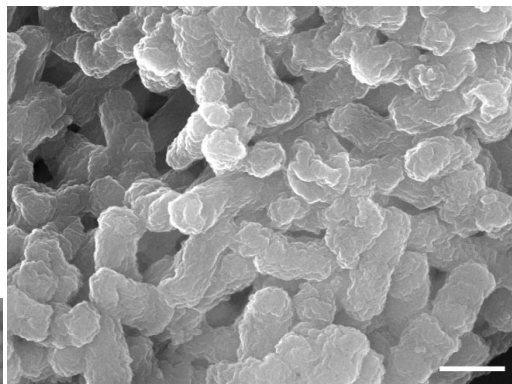
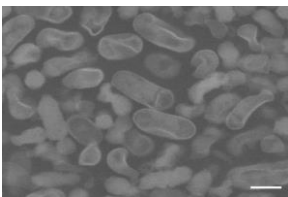
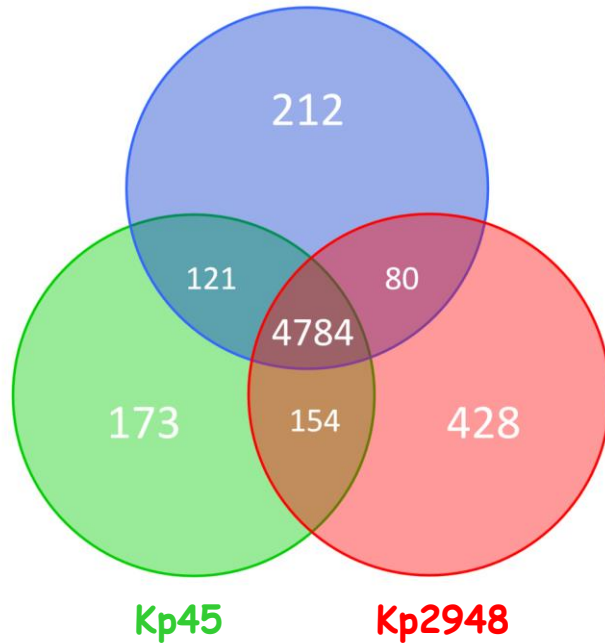


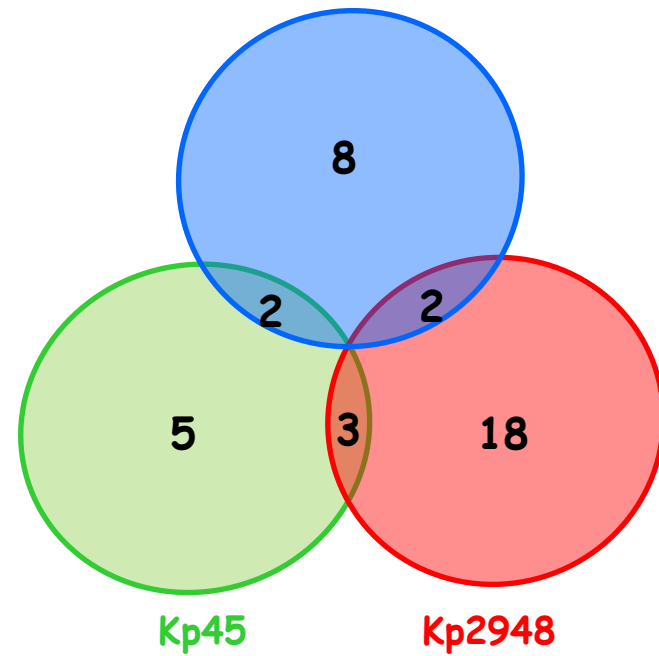
Table 1. Genetic differences in the accessory genome of the *K. pneumoniae* isolates.

Genomic region ¹	Approximate length (Kbp)	Isolate			Relevant information ^{2,3}
		Kp703	Kp45	Kp2948	
1	45	v	---	---	Intact prophage (predicted by PHAST)
2	41	v	---	---	Intact prophage (predicted by PHAST)
3	4	v	---	---	Includes predicted AMR genes : tetracycline resistance genes <i>tet(A/G)</i> and <i>tetR</i>
4	6	v	---	---	Includes Ribose ABC-transporter system proteins (permease RbsC, ATP-binding RbsA)
5	11	v	---	---	Includes genes encoding a Filamentous hemagglutinin and a Hemolysin transporter protein.
6	57	v	---	---	Includes genes encoding a Haemolysin expression modulating protein , the YdeA protein and a Programmed cell death antitoxin MazEF
7	12	v	---	---	Putative prophage elements : predicted annotation includes several hypothetical proteins, a purine NTPase and a prophage CP4-57 integrase
8	2	v	v	---	Aerotaxis sensor receptor protein
9	13	v	v	---	Includes an additional Urea ABC transport system (another similar gene cluster is carried by all three isolates)
10 ⁴	36	v	---	---	Incomplete prophage (predicted by PHAST) carrying a strain-specific Glycosaminoglycan attachment site encoding gene
11 ⁴	36	---	v	---	Incomplete prophage (predicted by PHAST) carrying a strain-specific Transcription activator <i>gutR</i> gene
12	12	---	v	v	Putative plasmid fragments carrying the virulence-associated proteins VagC and VagD.
13	37	---	v	---	Intact prophage (predicted by PHAST)
14	43	---	v	---	Intact prophage (predicted by PHAST)
15	57	v	---	v	Includes genes encoding Type IV pili-related proteins , a TonB-dependent protein, Iron acquisition yersiniabactin synthesis enzymes ; and a predicted AMR gene likely associated with resistance to tetracycline and aminoglycosides (<i>fyuA</i>)
16	2	v	---	v	Includes a predicted AMR gene : blaTEM-1A - class A beta-lactamase
17	13	---	v	v	Includes genes encoding Type I secretion related proteins (LapC, ATPase LapB, LapE, agglutinin RTX).
18	7	---	v	v	Includes predicted AMR genes : Aminoglycoside 3'-phosphotransferase strA and strB [or APH(3')-Ib and APH(6)-Id, respectively] likely associated with resistance to aminoglycosides.
19	11	---	v	---	Predicted annotation includes several hypothetical proteins and putative phage-like elements.
20	39	---	v	---	Intact prophage (predicted by PHAST)
21	17	---	---	v	Questionable prophage (predicted by PHAST)
22	11	---	---	v	Incomplete prophage (predicted by PHAST)
23	40	---	---	v	Intact prophage (predicted by PHAST)
24	25	---	---	v	Questionable prophage (predicted by PHAST)
25	37	---	---	v	Questionable prophage (predicted by PHAST)
26	12	---	---	v	Incomplete prophage (predicted by PHAST)
27	5	---	---	v	Includes genes encoding a putative serine protease (from the Peptidase S8 Subtilisin superfamily) and a cell division protein FtsH.
28	27	---	---	v	Putative fragment of a conjugative plasmid including genes encoding Type IV pili-related proteins , a Programmed cell death antitoxin MazE and a zinc metalloproteinase Mpr protein.
29	1	---	---	v	Includes a predicted AMR gene : trimetoprim resistance gene <i>dhfrA14</i> coding for a Dihydrofolate reductase
30	40	---	---	v	Intact prophage (predicted by PHAST)
31	4	---	---	v	Includes predicted AMR genes : tetracycline resistance genes <i>tet(A/G)</i> and <i>tetR</i> ; and proteins from the Glutathione-dependent pathway of formaldehyde detoxification
32	17	---	---	v	Includes a predicted AMR gene (KPC-3 beta-lactam resistance gene) and gene encoding a Chromate resistance protein ChrB
33	22	---	---	v	Includes genes coding for a arsenic resistance operon and a outer membrane protein or related peptidoglycan-associated (lipo)protein
34	5	---	---	v	Includes genes coding for a phosphonate ABC transport system and the RuBisCO operon transcriptional regulator CbbR
35	1	---	---	v	Includes a predicted AMR gene : SHV-161 beta-lactam resistance gene
36	3	---	---	v	Includes a predicted AMR gene : AC(6')-Ib putative fluoroquinolone resistance gene.
37	2	---	---	v	Includes a predicted AMR gene : putative Sulphonamide resistance gene <i>sul1</i> coding for a Dihydropteroate synthase
38	1	---	---	v	Includes a predicted AMR gene : putative Sulphonamide resistance gene <i>sul2</i> coding for a Dihydropteroate synthase

Pan-genome
Kp703



Accessory -genome
Kp703



FINAL REMARKS

Biofilm transcriptome and metabolome

Biofilms on medical devices

Materials : Biofilm interaction

ACKNOWLEDGMENTS



Department of Infectious Diseases
Microbiology: JC Rodrigues; L Reis
Bioinformatics: JP Gomes; V Vorges

Department of Human Genetics
L Vieira; C Carneiro

Department of Environmental Health
M Bandeira, S Sousa, P Morgado
S Paulino



A. Duarte



PA Carvalho

