

## Exploring a link between pathogens structure's and resistance to disinfectant

I. João<sup>1</sup>, L. Reis<sup>1</sup>, P. Carvalho<sup>2</sup>, A. Duarte<sup>3</sup>, L. Jordão<sup>1</sup>

<sup>1</sup>Departamento de Doenças Infeciosas, Instituto Nacional de Saúde Dr Ricardo Jorge, Av. Padre Cruz, 1649-016 Lisboa, Portugal,

<sup>2</sup>CEMS, Departamento de Bioengenharia, Instituto Superior Técnico, Universidade Técnica de Lisboa, Av. Rovisco Pais, 1049-001 Lisboa, Portugal,

<sup>3</sup>Departamento de Microbiologia e Imunologia; iMed. UL; Faculdade de Farmácia; Universidade de Lisboa, Av. Prof. Gama Pinto; 1649-003 Lisboa; Portugal  
email: [mariajordao@insa.min-saude.pt](mailto:mariajordao@insa.min-saude.pt)

In recent years healthcare-associated infections (HAIs) infections have gained growing importance. Among their etiological agents are classical (e.g. *K. pneumoniae*, *S. aureus*) and emergent pathogens, previously neglected, such as nontuberculous mycobacteria (NTM). The ability to resist to antibacterial agents, such as antibiotics and disinfectants, is shared by all of them. Here we aim to establish a link between bacterial virulence, disinfectant resistance and structure.

Bacterial reference strains and clinical isolates were grown in adequate medium. NTM susceptibility test to antibiotics was evaluated by broth microdilution method and interpreted according to NCCLS guidelines in a previous work. The susceptibility to disinfectants (hydrogen peroxide, ammonium quaternary salts [AQS] and glutaraldehyde [GA]) was performed by two different methods: broth microdilution and diffusion in solid medium. In order to evaluate the effect of these agents in bacteria a scanning electron microscopy (SEM) study was performed.

Hydrogen peroxide was the less effective disinfectant. The efficiencies of QAS and GA were similar. Nevertheless, among NTM we identified one isolate resistant to all disinfectants tested (Table 1). The SEM analysis showed that different disinfectants caused different effects on bacteria suggesting different action mechanisms (Figure 1).

Bacterial resistance to antibiotics and disinfectants vary in the same manner. The mechanisms involved in the resistance are not fully elucidated and more studies are needed to provide effective conclusions. Biofilm formation can be part of the mechanism involved both in antibiotic/disinfectant resistance development and infection propagation.

The authors acknowledge Fundação para a Ciência e Tecnologia for the grant C2008-C2008\_P2.

1. ECDC Europe, [http://ec.europa.eu/health/patient\\_safety/docs/council\\_2009\\_en.pdf](http://ec.europa.eu/health/patient_safety/docs/council_2009_en.pdf)
2. ECDC Europe, 2012, Surveillance of surgical site infections in European hospitals - HAISSE protocol, [http://www.ecdc.europa.eu/en/publications/Publications/120215\\_TED\\_SSI\\_protocol.pdf](http://www.ecdc.europa.eu/en/publications/Publications/120215_TED_SSI_protocol.pdf)

Microorganism	Diffusion in solid medium						Broth Microdilution			
	Halo diameter (mm)						MIC (%)			
	H <sub>2</sub> O <sub>2</sub>		GA		QAS		H <sub>2</sub> O <sub>2</sub>	GA	QAS	
	30v	10v	purum	6%	purum	6%				
Slow NTM	60/2008	15.5	0	9	0	72	50	1.13	3.75	3.10
	247/08	19	23	13	0	110	80	0.56	0.39	3.10
	388/08	24	0	8	0	64	64	0.56	3.39	3.10
	552/08	43	26	44	140	>140	>140	0.56	0.39	3.10
	553/08	34.5	16.5	17	10	84	54	0.56	0.19	12.5
	935/08	43	0	10	9	84	84	1.13	0.19	3.10
Fast NTM	747/08	36	36	17	5	31	0	2.25	0.19	12.5
	549/08	0	0	0	0	0	0	2.25	0.19	12.5
	<i>M. smegmatis</i>	31	31	17.5	9	20	17	---	---	---
Bacteria	<i>P. aeruginosa</i>	15	15.5	12.5	8	14	10	---	---	---
	<i>Kp ID3407</i>	28.5	20	12	7	22	7.5	---	---	---
	<i>Kp ID3466</i>	24	18	13.5	0	12.5	8.5	---	---	---
	<i>Kp 703O:1</i>	23	20.3	14	0	14	11.3	0.01	0.78	0.39
	<i>Kp 2948</i>	19.8	15.5	12.8	0	28	10	0.04	0.78	1.56
	<i>E. coli BG2-3</i>	30	29	14	7	36	21	2.25	0.10	12.5

Table 1 – Evaluation of bacteria susceptibility to disinfectants glutaraldehyde (GA); ammonium quaternary salts (QAS) and Hydrogen peroxide(H<sub>2</sub>O<sub>2</sub>).

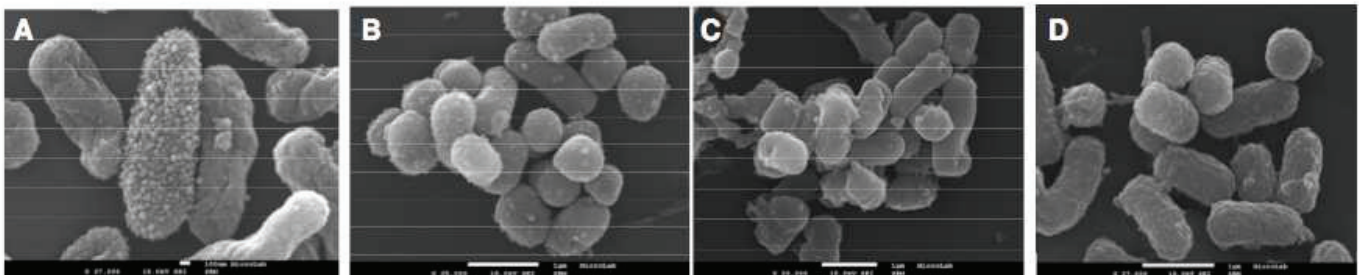


Fig. 1 – Effect of disinfectants on the structure of *Klebsiella pneumoniae* 3466 (SEM analysis).

The native structure of *K.pneumoniae* 3466 (A) and after treatment with 6% ammonium quaternary salts solution (B), undiluted ammonium quaternary salts (C) or 30volumes hydrogen peroxide solution acquired by SEM are shown.