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Comparison of the sensitivity of *Pseudomonas aeruginosa* to disinfectants according to the growth conditions

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Summary

During the evaluation of the bactericidal effect of some disinfectants, the authors observed major differences of sensitivity in the case of *Pseudomonas aeruginosa* depending on the culture medium used for the preparation of the inoculum. In particular for chloroxylenol the results were much more favourable when the culture was made in brain heart infusion (Difco) than in tryptic soy broth (Difco).

The evaluation of the antimicrobial effectiveness of a disinfectant always requires in a first phase an in vitro experimentation. Some of us (Devleeschouwer and Dony, 1981) described in a previous publication a method which meets some fundamental criteria such as the standardization of the test conditions.

Reybrouck (1977), based on the same principles, proposed a very similar technique but different also by the choice of the culture media used for the preparation of the inoculum. Following the observations of some divergences between the conclusions obtained by both techniques, in particular for the bactericidal effect of some disinfectants on *Pseudomonas aeruginosa* ATCC 15442, it seemed interesting to investigate more specifically the influence of the culture medium on the sensitivity of this species.

All the other conditions beeing equal and corresponding to those described previously by us (Dony and Devleeschouwer, 1978) we compared the results

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obtained by using for the preparation of the inoculum either the tryptic soy broth (Difco) recommended by Reybrouck or the brain heart infusion (Difco) that we preferred.

The detailed method has been published previously (Devleeschouwer and Dony, 1981). Briefly, the method is related to the classical suspension tests (organisms suspended in the investigated solution) with repeated additions of inoculum (capacity tests). The inoculum prepared either in medium A (tryptic soy broth; Difco) or medium B (Brain heart infusion; Difco) is standardized in order to contain between 2×10^7 and 2×10^8 bacteria/ml disinfectant solution. The surviving bacteria in the solution are counted after fixed times. The trial is performed in water of known hardness with (P +) or without (P −) organic material. Results are expressed in terms of: satisfactory (S), when the germicidal effect after 10 min of contact is currently greater or equal to 5 log cycles; non-satisfactory (NS), when the reduction of the bacterial population is below 5 log cycles; and limit (L), when in a series of experiments some of the results give a bacterial reduction ranging between 4 and 5 log cycles.

Culture media

Medium A (tryptic soy broth; Difco):

Tryptone	17 g
Soytone	3 g
Dextrose	2.5 g
Sodium chloride	5 g
Dipotassium phosphate	2.5 g
Distilled water	1000 ml

autoclaved 15 min at 121°C.

Medium B (brain heart infusion; Difco):

Calf brains, infusion from	200 g
Beef heart, infusion from	200 g
Proteose peptone	10 g
Dextrose	2 g
Sodium chloride	5 g
Disodium phosphate	2.5 g

37 g are dissolved in 1 litre of distilled water and autoclaved 15 min at 121°C.

Bacterial strain

The strain used is *Pseudomonas aeruginosa* ATCC 15442

Table 1 gives the results obtained for several products using both media for the preparation of the inoculum. For the majority of the products no difference has been recorded (products C, D, G, H, I, J). In the case of the association of cetrimide and chlorhexidine (E and F), the results were less favourable when the organisms were cultured in tryptic soy broth. Lastly for chloroxylenol (A and B) the strain when cultured in brain heart infusion appears susceptible whereas it is resistant when

TABLE 1

COMPARISON OF THE MEDIA USED FOR THE PREPARATION OF THE INOCULUM

Active compounds	Concentration of active compounds (%)	Brain heart infusion		Tryptic soy broth	
		P−	P+	P−	P+
A Chloroxylenol	0.245	S	S	R	R
B Chloroxylenol	0.147	S	S	R	R
C Chloroxylenol	0.06	S	S	S	S
EDTA	0.082				
D Alkyls and aryls phenols	0.5	S	S	S	S
E Chlorhexidine gluconate	0.015				
Cetrimide	0.15	I	I	I	R
F Chlorhexidine gluconate	0.0075				
Cetrimide	0.075	S	R	I	R
G *n*-Alkyl dimethyl benzyl am.Chl.	0.15				
EDTA	0.02	S	R	S	R
H Formaldehyde	0.085	R	R	R	R
I Formaldehyde	0.51	R	R	R	R
J Formaldehyde	0.85	S	S	S	S

incubated in tryptic soy broth. This difference of sensitivity according to the growth medium was not recorded for the association of chloroxylenol and EDTA (0.082%) (product C).

Our observations point out the need for perfect standardization of the test conditions of an in vitro trial in order to obtain reproducible results between various laboratories. Beyond this preoccupation for standardization these observations raise more fundamental problems concerning the mode of action of some disinfectants on *Pseudomonas aeruginosa*.

As a matter of fact, the difference of sensitivity of this organism to chloroxylenol according to the growth medium could result from a permeability difference linked to the growth conditions. Dankert and Schut (1975) investigated the influence of some factors such as growth and environmental conditions which can affect the sensitivity of *Pseudomonas aeruginosa* to chloroxylenol. In particular, they emphasized the importance of the growth duration on the resistance and the influence of bivalent cations, especially magnesium, effect not recorded for other Gram-negative species. They described a potentiation of the action of chloroxylenol by EDTA less pronounced if the growth medium contained low amounts of magnesium. According to these authors, this observation can be explained on basis of the reaction between EDTA and the external wall structures. EDTA acts on these structures at the level of the bounds assumed by the divalent cations with a consecutive liberation of lipopolysaccharide (endotoxin) and exposure of the basal layer of peptidoglycans (Gray and Wilkinson, 1965a and b; Eagon and Carson, 1965; Brown and Richards, 1965; Brown and Melling, 1969).

According to Hamilton (1971) the role of the cell wall as an impermeable barrier could explain the ineffectiveness not only of some phenols but also of quaternary ammonium compounds and antibiotics such as polymyxin against *Pseudomonas aeruginosa*.

The analysis of data of the literature shows that the problem remains unsolved (Brown 1975; Holloway et al., 1979). Stated on the obtained results, this 'impermeability' could occur by the bacteria when grown in tryptic soy broth but not when cultured in brain heart infusion. So we think that we propose an experimental model which allows to investigate more extensively the mechanisms involved in the effectiveness of disinfectants and antibiotics on an organism feared in nosocomial infections.

References

Brown, M.R.W., Resistance of *Pseudomonas aeruginosa*, J. Wiley London, 1975.

Brown, M.R.W. and Melling, J., Role of divalent cations in the action of polymyxin B and EDTA on *Pseudomonas aeruginosa*. J. Gen. Microbiol., 59 (1969) 263–274.

Brown, M.R.W. and Richards, R.M.E., Effect of ethylene diamine tetracetate on the resistance of *Pseudomonas aeruginosa* to antibacterial agents. Nature (Lond.), 207 (1965) 1391–1393.

Dankert, J. and Schut, I.K., The antibacterial activity of chloroxylenol in combination with ethylenediamine tetra-acetic acid. J. Hyg., 76 (1975) 11–22.

Dony, J. and Devleeschouwer, M.J., Evaluation de l'efficacité des désinfectants: épreuves préliminaires in vitro. J. Pharm. Belg., 33 (1978) 120–125.

Devleeschouwer, M.J. and Dony, J., An in vitro test for the evaluation of the efficacy of disinfectants. Int. J. Pharm., 9 (1981) 49–58.

Eagon, R.G. and Carson, K.J., Lysis of cell walls and intact cells of *Pseudomonas aeruginosa* by ethylenediamine tetraacetic acid and lysozyme. Canad. J. and Microbiol., 11 (1965) 193–201.

Gray, G.W. and Wilkinson, S.G., The action of ethylenediaminetetra-acetic acid on *Pseudomonas aeruginosa*. J. Appl. Bacteriol., 28 (1965a) 153–164.

Gray, G.W. and Wilkinson, S.G., The effect of ethylenediaminetetra-acetic acid on the cell walls of some Gram-negative bacteria. J. Gen. Microbiol., 39 (1965b) 385–399.

Hamilton, W.A., Membrane active antibacterial compounds. In Hugo, W.B. (Ed.), Inhibition and Destruction of the Microbial Cell, Academic Press, London, 1971, pp. 77–106.

Holloway, B.W., Krishnapillai, V. and Morgan, A.F., Chromosomal genetics of *Pseudomonas*. Microbiol. Rev., 43 (1979) 73–102.

Reybrouck, G., Een nieuwe 'in vitro' test voor de bepaling der kiemdodende eigenschappen van chemische ontsmettingsmiddelen. Proefschrift ter verkrijging van de graad van geagregeerde van het Hoger Onderwijs. Acco, Leuven, 1977, pp. 220–226.