

Animal Models in Early Evaluation of Antibacterial Agents

Niels Frimodt-Møller, MD Dr.Sc.
Dept. of Clinical Microbiology,
Statens Serum Institut,
Copenhagen, Denmark

Experimental animal experiments: History

- Optochin, *S. pneumoniae* - mice

Morgenroth, 1911

- Prontosil, *S. pyogenes* - mice

Domagk, 1935

- Penicillin, *S. aureus* - mice

Florey & Chain, 1939

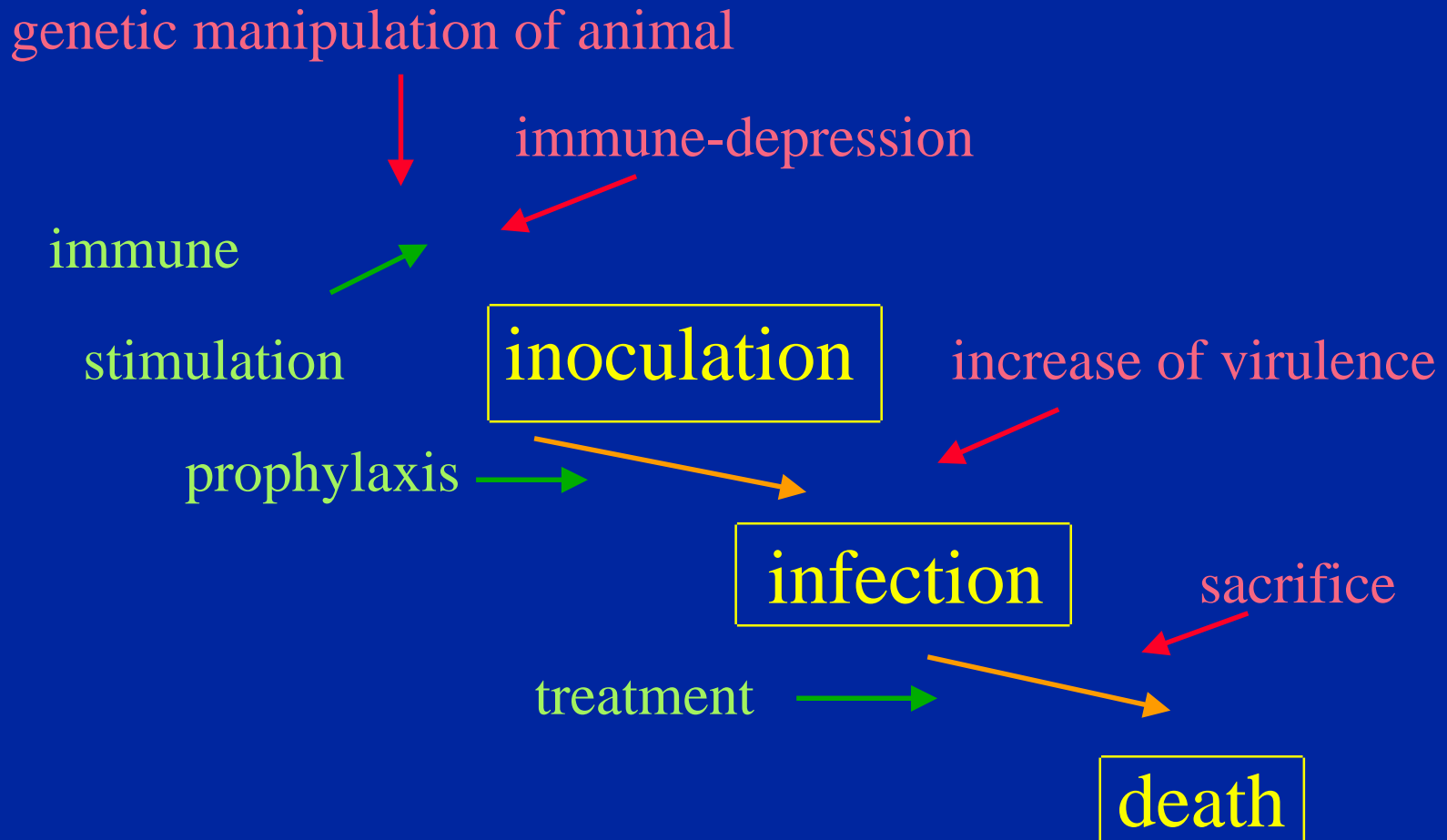
Experimental animal models: Definition of antibiotic effect in special situations

- Antibiotic effect in surgical prophylaxis - guinea pigs *Burke & Miles*
- Importance of bactericidal activity in treatment of endocarditis - rabbits *Wilson & others*
- Importance of bactericidal activity in treatment of meningitis - rabbits *Merle & Sande*

Why animal models ?

- avoid damage to humans/ethical reasons
- infection rare or acute and serious (e.g.meningitis)
- prove correlation between infection and microorganism under controlled conditions
- test treatment modalities/prophylaxis
- investigate for side effects
- measure antibiotic concentrations and determine bacterial counts *in situ*

Experimental animal models: Manipulation/modulation



Animal experimentation in antibiotic research: Ethical issues

- Is it ethical to use animals in experimental research, when we have in vitro models ?
- Is this experiment necessary and can it answer the proposed questions ? monkey \gg mice
- Is the welfare of the animals being used in this experiment given due consideration ?
- Are the data gained by such experiments being utilized to best advantage ?
- If used, does the protocol fulfill the issues concerning design, statistics etc. that we would demand from a clinical study ?

Screening of substances for antimicrobial activity in vivo: Considerations prior to animal experimentation

- Determination of in vitro antimicrobial properties. incl. development of resistance
- In vitro determination of toxicity ?
- Solubility and stability of substance & preparation of suitable formulation for administration.
- Determination of extent of protein binding
- Suitable measurement of fluid concentration available ?

Experimental animal model: Choice of animal

- Screening

- septicaemia
- peritonitis
- thigh myositis

- Animals

- mice
- rats

- Specialized model

- endocarditis
- meningitis
- pneumonia

- Animals

- rabbit
- guinea pig
- monkey

Factors influencing antimicrobial activity in in vivo tests

- Inoculum size
- Virulence
- Growth in vivo - generation time in vivo
- Timing of treatment
- Method of antibiotic administration
- Pharmacokinetics/pharmacodynamics of antibiotic
- Development of resistance in vivo

Experimental animal model: Choice of bacterium

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- Relation to clinical situation ?
- In vitro susceptibility ?
- Intra- or extracellular ?
- Virulence to animal ?
- Type of infection produced ?

Methods of immunosuppression for enhancement of virulence of bacteria for inoculation

- Target
 - bone marrow
 - neutrophils
 - macrophages
 - complement
 - tuftsin
 - immunoglobulin
 - T-lymfocytes
 - interleukins
- Method
 - = irradiation
 - = cytostatics
 - = mucin, bakers yeast
 - = cobra venom factor
 - = splenectomy
 - = anti-Ig
 - = thymctomy
 - = antibody, chemical

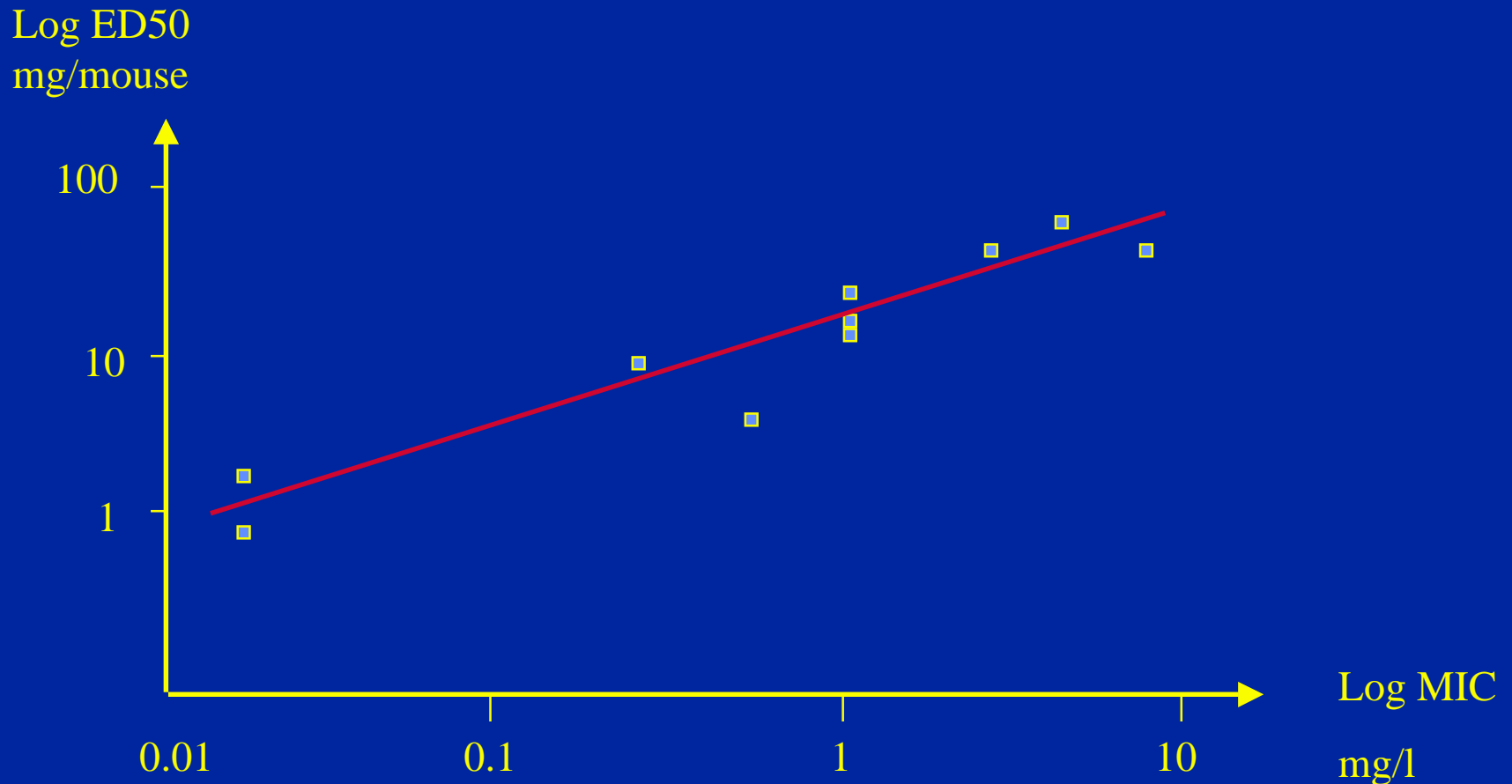
**Mouse peritonitis model: Vancomycin and teichoplanin
against ten PRP with Pen MIC's from 0.016 to 8 mg/l**

	Vancomycin	Teichoplanin
MIC (mg/l)	0.125-0.25	0.016-0.05
ED50 (mg/kg)	0.58	0.32
Protein- binding (%)	20-28%	90-94%

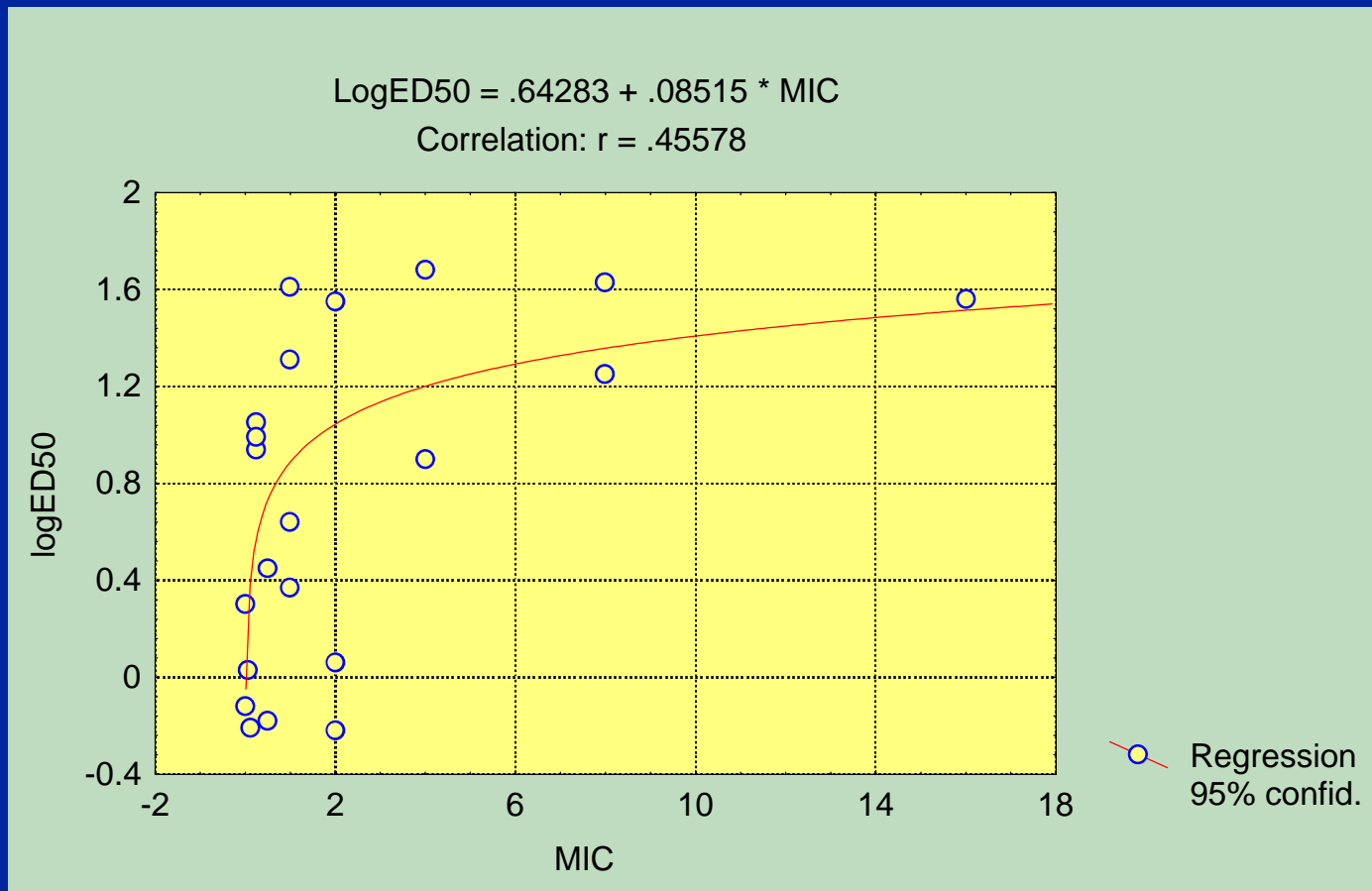
Effect of schedule of administration on therapeutic efficacy of penicillin on *S. pyogenes* infection in mice

	Inoculum = 100 cfu		Inoculum = 10.000 cfu		Inoculum = 1000.000 cfu	
	Single injection	4 x 3 h interval	Single injection	4 x 3 h interval	Single injection	4 x 3 h interval
CD50 mg/kg	0.35	0.26	22	0.43	50.7	0.53

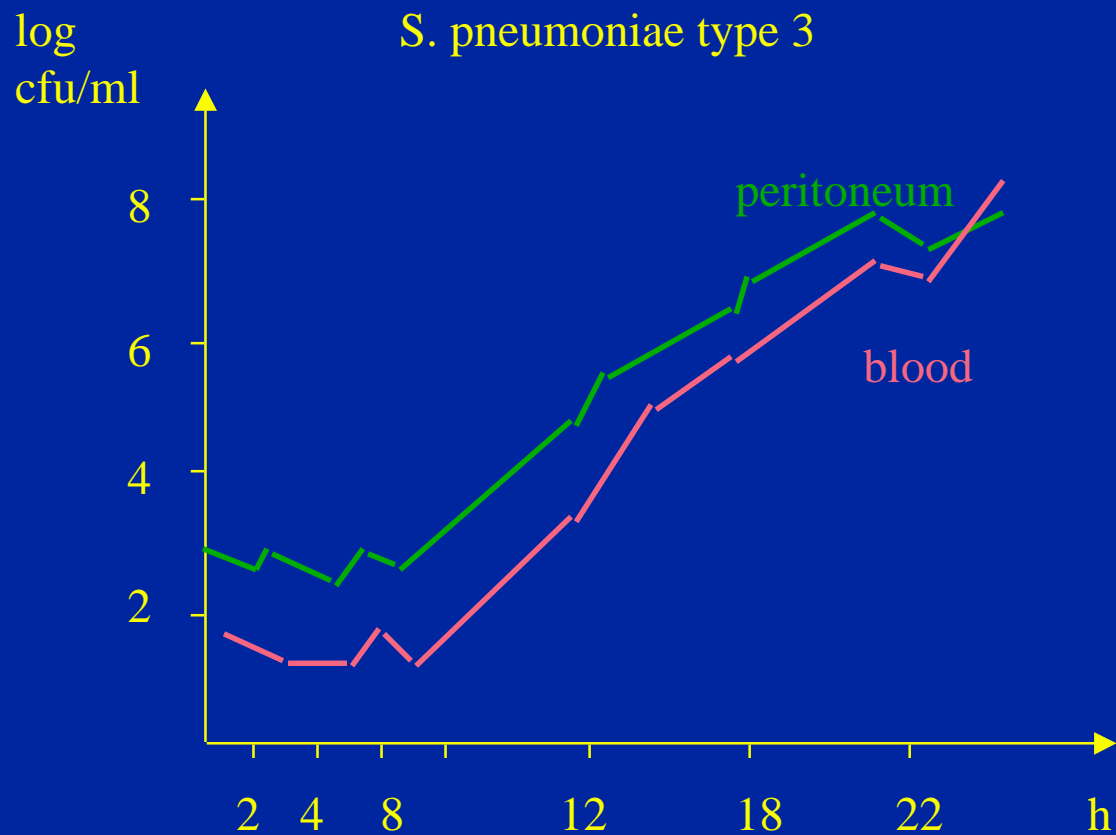
Mouse protection test: Correlation between MIC and ED50 for pneumococci with varying penicillin susceptibility



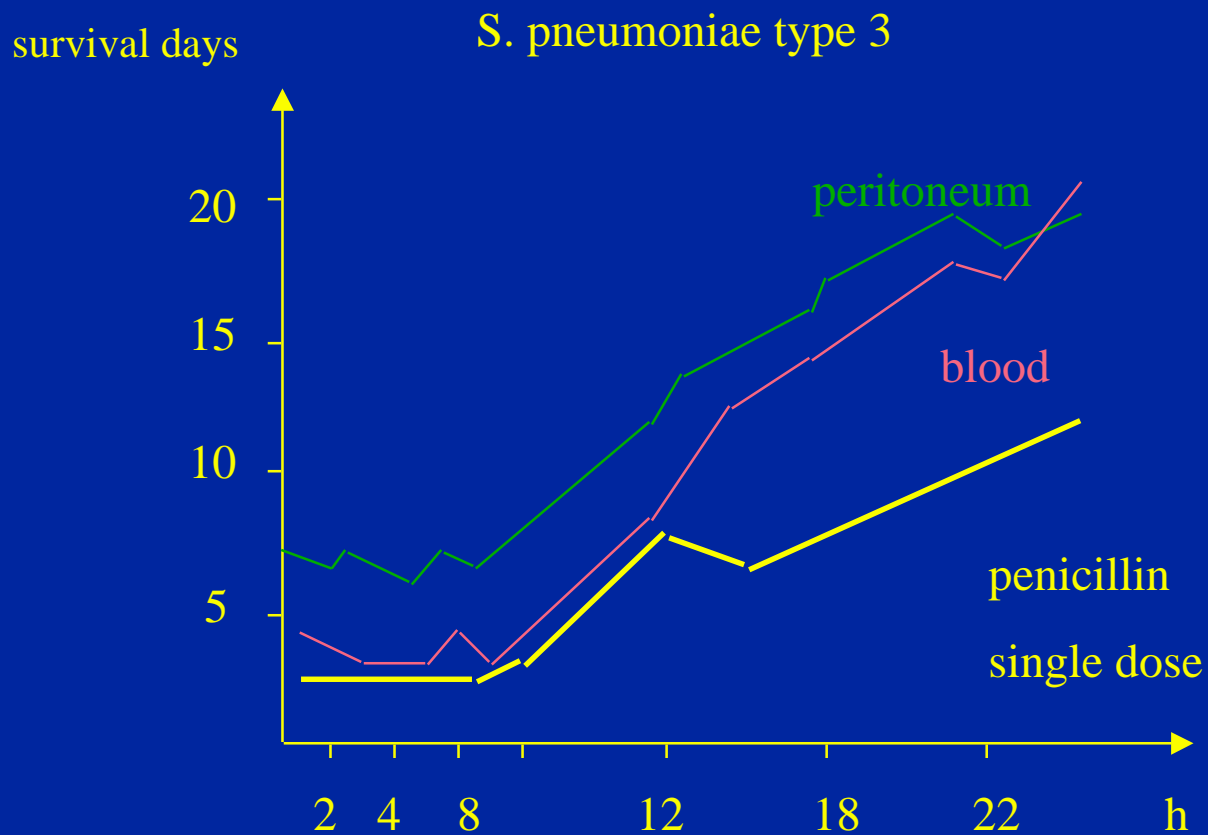
Beta-lactam antibiotics and the mouse-peritonitis model: Correlation MIC vs. effect for penicillins and cephalosporins



Experimental animal model for antibiotic effect: Importance of growth phase in vivo



Experimental animal model for antibiotic effect: Importance of growth phase in vivo



Comparison of cephalosporins against pneumococci in the mouse protection test

Antibiotic (T>MIC)	Strain 1064		Strain 2916	
	MIC	ED50	MIC	ED50
Penicillin	0.25	11.2	4	47.8
Cephalothin	1	40.7	64	>150
Cefuroxime	0.25	8.7	8	17.7
Cefotaxime	0.06	1.1	1	20.4
Ceftriaxone (200-260 min)	0.125	0.61	1	4.2
Cefepime (10-40 min)	0.5	0.7	2	1.1

Mouse protection test: Synergy between penicillin and gentamicin against pneumococci

		Penicillin, mg/mouse				Total
		None	0.75	1.5	3.0	
Genta- micin mg/ mouse	None		0	0	0	0/15
	0.1	0	3	2	5	10/15
	0.2	0	5	2	4	11/15
	0.4	0	5	5	5	15/15
	Total	0/15	13/15	9/15	14/15	36/45

Screening of antibacterial substances in experimental animals: Conclusion

- screening models are used extensively for first time testing of antibacterial substances
- care should be taken to get as much information as possible from the animals
- the use of ED50/CD50 should be reduced
- quantitative bacteriology from relevant sites should be performed