The Enchanced Sensitivity of *Acinetobacter baumannii* Strains Resistant to Bactericidal Activity of Normal Human Serum After Exposure to the Subminimal Inhibitory Concentrations of Antibiotics

Maja Bogdan[1], Domagoj Drenjancevic [2], Jasminka Talapko [2], Aurora Antolovic Amidzic [3],Dubravka Vukovic[1]

[1]Department of Microbiology, Institute of Public Health for Osijek-Baranja County, Osijek, Croatia, [2] Osijek University Hospital and Faculty of Medicine, University of Osijek, Department of Microbiology and Parasitology, Osijek, Croatia[3] The Hospital Pharmacy, Osijek Unversity Hopital, Osijek, Croatia.

Background:

Serum bactericidal activity has been regarded as one of the most important host defense mahanisms against bacterial infections (1). The complement system is a crucial constituent of the innate immunity required to control pathogenic microorganisms. Pathogenic bacteria via diverse mechanisms are capable of effectively evading immune responses (2). Gram-negative organisms that give rise to bacteremia are more resistant to normal human serume (NHS) and phagocytosis than bacteria causing other types of infection (3). *A.baumannii* is an opportunistic pathogen a leading cause of nosocomial infections, including pneumonia, bacteremia, meningitis, urinary tract infections and wound infections that tipically infect critically ill patients (2, 4). The pathogenicity of *A.baumannii* is complex, but yet only few virulence factors have been described that, besides exceptional drug resistance, makes it so successful pathogen (5,6). At the site of infections, antibiotics are often present at subinhibitory concentrations (5). Exposure to subMICs of diverse antibiotics, although not able to kill bacteria, can modify their biological characteristics, may cause alteration in bacterial morphology, proliferation, also can modulate bacterial gene expression, and may interfere with some essential bacterial functions thus influencing bacterial virulence(7). Further one *in vitro*, studies showed that serum-resistant, Gram-negative organisms grown in subminimal inhibitory concentrations of certain antibiotics became susceptible to complement-mediated serum bactericidal activity (8). It has been shown that most clinical isolates of *A.baumannii* exhibit intrinsic resistance to normal human serum (3). The aim of this study is to evaluate the *in vitro* effect of subminimal inhibitory concentrations (subMICs) of antibiotics on the sensitivity of *Acinetobacter baumannii* clinical isolates to the bactericidal activity of the normal human serum .

Material/methods:

Sixty five non duplicate clinical isolates of *A.baumannii,* resistant to the bactericidal activity of NHS were included in the study. They were exposed to the 1/2, 1/4, 1/8 and 1/16 of previousely determned minimal inhibitory concentrations (MICs) of imipenem, ampicillin/sulbactam, azithromycin, rifampicin and colistin. After the overnight exposure of the tested bacterial strains to the subMICs of antibiotics, suspensions were pelleted, twice washed in the phosphate buffered saline (PBS) and afterwards exposed to the bactericidal effect of the NHS at 37˚ C. After two hour exposure to the serum, 100µ of the prepared suspensions were transferred to Mueller-Hinton agar plate and overnight incubated at 37˚ C in aerobic conditions. The colony forming units (cfu) was determined and the percentage of the bacterial reduction was calculated in comparison to the bacterial suspension exposed to the heat inactivated serum (HIS) within the same conditions (2 h/37˚ C/air). The reduction of the cfu ≥ 90% was considered as significant.

Results:

The observed frequencies of the enchanced bactericidal activity of the NHS to tested *A.baumannii* strains after their exposure to the subMICs of antibiotics are presented in Table 1.

Table 1. Frequencies of observed changes of serum resistant *A.baumannii* strains

	1/2		1/4		1/8		1/16	
ANTIBIOTIC	**N**	**χ^2**	**N**	**χ^2**	**N**	**χ^2**	**N**	**χ^2**
IMIPENEM	4	2.25	1	0	0	-	2	0,5
AMPICILLIN/ SULBACTAM	14	12.07*	12	10.08*	10	8.01*	10	8.01*
AZITHROMYCIN	14	12.07*	11	9.09*	11	9.09*	10	8.01*
RIFAMPICIN	5	3.2	3	1.33	5	3.2	5	3.2
COLISTIN	5	3.2	3	1.33	0	-	1	0

*Chi square test with Yates' correction in frequency is less than 20, p>0.05; N= number of serum sensitive strains after exposure to subMIC of selected antibiotic.

Conclusion:

The results of this study indicate that the subminimal inhibitory concentrations of ampicillin/sulbactam and azithromycin enhances the bactericidal activity of the normal human serum, whereas imipenem, rifampicin and colistin have not exhibited the same ability.

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