



**UNIWERSYTET MEDYCZNY**  
IM. PIASTÓW ŚLĄSKICH WE WROCŁAWIU

Andrzej Szuba MD PhD  
Professor of Medicine  
Division of Angiology, Wrocław Medical University & Department of Internal  
Medicine, 4th Military Hospital in Wrocław  
Weigla 5 street; 50- 981 Wrocław, Poland phone/fax: [+48 261 660599](tel:+48261660599)  
mobile: [+48 504 085 101](tel:+48504085101)  
email: [szubaa@yahoo.com](mailto:szubaa@yahoo.com);  
email2: [andrzej.szuba@umed.wroc.pl](mailto:andrzej.szuba@umed.wroc.pl)

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**Opinion**

**on doctoral dissertation of Adnan Saeed, MSc. entitled  
“Structural and immunochemical studies of polysaccharides from some  
pathogenic actinomycetal strains”**

The PhD candidate has prepared his thesis on structural and immunochemical analysis of polysaccharides from selected pathogenic actinomycetal strains.

The thesis consist of ten main sections. The thesis is written on 114 pages with 10 Tables and 36 Figures. The „Introduction“ covers characteristics of bacteria of the genus *Actinomyces* and *Tsukamurella*, the architecture of the cell wall of bacteria, etiology, epidemiology , pathophysiology and diagnosis od actinomycosis.

Mycobacterioses, nocardioses and actinomycetoses remain important and serious medical problem. *Actinomyces* belong to oportunistic bacteria which cause several diseases broadly called actinomycoses. Actinomycetes are present in the normal

flora of the oral cavity, less prominent in the lower gastrointestinal tract and female genital tract. These microorganisms are considered not virulent and require a break in the integrity of the mucous membranes to invade deeper body structures and to cause human illness. Recently significant growth in endo- and exogenous infections caused by Actinomycetacea is noted. This is caused by many factors including increased number of immunocompromised patients as well as environmental pollution and stress. Actinomycoses are difficult to diagnose for frequently inspecific presentation, unreliable diagnostic tests including histopathology and PCR. Therefore actinomycoses and nocardioses are frequently misdiagnosed and mistreated. Also the incidence especially in Poland is not known. The chapter demonstrates that the candidate acquired extensive knowledge of the Actinomycetales, its biology and clinical significance and provided important scientific background justifying his research.

The „Aims of Research“ section describes the goals of the study and its importance for both basic and clinical science. Being a clinician I highly appreciate the choice of the study. Opportunistic infections have become a quickly growing problem around the world. Infections caused by Actinomycetales due to difficulties in clinical and laboratory diagnostics are underdiagnosed and undertreated. Therefore studies on these these poorly known bacteria are necessary and greatly appreciated.

In the „Materials and Methods“ chapter the PhD candidate provides detailed description of advanced and novel methods including scanning ion-electron microscopy and production of monoclonal antibodies from the growing hybridoma, used in his studies. The candidate used MALDI-TOF mass spectrometry for identification of bacterial species and several techniques to isolate and analyze polysaccharides. He used also GLC-MS, methylation analysis and NMR spectroscopy for further analysis. Specific monoclonal antibodies against selected exopolysaccharides of four studied bacterial species were produced with hybridoma cells. The candidate performed also in vivo experiments on mice to assess formation of abscess after intraperitoneal injection of bacteria.

The „Results“ are clearly presented on 36 pages. The candidate describes in results of all experiments including detailed characterization of bacterial exopolysaccharides and successful creation of hybridomas producing monoclonal antibodies. Monoclonal

antibodies against bacterial exopolysaccharides are characterised and their reactivity is presented.

In the „Discussion“ the PhD candidate critically discuss his results and related publications. An interesting and important was finding that Tsukamurella pulmonis could be a very invasive bacterial species capable of causing fulminant infection with multiorgan involvement.

The PhD candidate has correctly drawn six conclusions from his work.

The most important findings of this study in my opinion are identification of immunogenic polysaccharides of high diagnostic value. It may help in development of new diagnostic tests useful in identification of Actinomyces and Tsukamurella sp. The work of the candidate provide a substantial and novel contribution to knowledge on Actinomycetales and might be soon transferred to clinical practice.

The manuscript of Mr Adnan Saeed meets requirements for doctoral thesis and I recommend it for further stages of doctoral proceeding.

I declare hereby that this doctoral thesis of MSc Adnan Saeed entitled “Structural and immunochemical studies of polysaccharides from some pathogenic actinomycetal strains” is written according to rules for doctoral theses (Ustawa z dnia 14 marca 2003 r. O stopniach naukowych i tytule naukowym oraz o stopniach i tytule w zakresie sztuki, i późn. zmianami), and I apply to Scientific Council of Hirszfeld Institute of Immunology and Experimental Therapy of Polish Academy of Sciences in Wrocław for admission to further steps of processing of doctoral degree. I also recommend to distinguish Adnan Saeed’s doctorate thesis

A handwritten signature in blue ink, appearing to read 'Adnan Saeed', is positioned in the lower right quadrant of the page.