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INTRACELLULAR, BIOFILM-INHIBITORY AND MEMBRANE DAMAGING ACTIVITIES OF NIMBOLIDE ISOLATED FROM *AZADIRACHTA INDICA A. JUSS (MELIACEAE)* AGAINST METICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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Staphylococcus aureus is a leading aetiologic agent of nosocomial- and community-acquired infectious diseases worldwide. *S. aureus* causes several human diseases, ranging from minor skin and soft tissue infections to more severe conditions such as toxic shock syndrome, glomerulonephritis, pneumonia, meningitis, endocarditis, osteomyelitis and septicaemia. The public health concern regarding staphylococcal infections is inflated by the increasing occurrence of multidrug-resistant strains, e.g. multidrug- and meticillin- resistant *S. aureus* (MDR MRSA). This study was designed to evaluate the intracellular bactericidal activity and *in vitro* membrane-damaging and biofilm-inhibitory activities of nimbolide isolated from Azadirachta indica against MDR MRSA. *In vitro* antibacterial activity of nimbolide was determined by performing MIC, MBC and time-kill kinetic studies. It showed much lower MIC (8 µg ml^{-1}) and MBC (32 µg ml^{-1}) values than other antibiotics. Biofilm-inhibitory activities were determined by SEM. Cellular drug accumulation and assessments of intracellular activities were performed using Vero cell culture. SEM findings revealed that exposure to nimbolide at 1X MBC on *S. aureus* resulted in the disintegration of the bacterial cell envelope, severe bacterial membrane perturbation, significant membrane damage, bursting of cells and cell lysis. The biofilm structure was disrupted, and the biofilm formation was greatly reduced in the presence of nimbolide as examined by SEM. The level of accumulation of nimbolide in Vero cells incubated for 24 h is relatively higher than that of ciprofloxacin and nalidixic acid. The viable number of intracellular *S. aureus* was decreased [reduction of ~2 log10 c.f.u. (mg Vero cell protein)$^{-1}$] in a time-dependent manner in the presence of nimbolide (4 X MBC) that was comparable to that of tetracycline and nalidixic acid. The significant intracellular, biofilm-inhibitory and bacterial membrane-damaging activities of nimbolide demonstrated here suggested that it has potential as an effective antibacterial agent for the treatment of severe infections caused by MDR MRSA.

BIOGRAPHY

Anirban Banerjee has completed his PhD at the age of 29 years from Visva Bharati Santiniketan, India. He is now Post-Doctoral fellow in Department of Biochemistry, University of Calcutta, India. He is engaged in research in microbiology and parasitology and has many publications in reputed Journals.

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