RESEARCH ARTICLE

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Cytotoxic Effect of *Stenotrophomonas maltophilia* Isolated from Prostate and Bladder Cancer Patients in Iraq

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Abstract

Objective: This study investigated the cytotoxic effects of *Stenotrophomonas maltophilia* on normal human cells. **Methods:** Seven isolates of *S. maltophilia* were obtained from 120 urine samples collected from prostate and bladder cancer patients, with diagnoses confirmed via Vitek. The cytotoxicity of these bacterial isolates was assessed on normal human fibrocyte cells (NHF) using various concentrations of bacterial filtrate (3.125, 6.25, 12.5, 25, 50, and 100 µg/ml). **Results:** The bacterial filtrate exhibited significant toxicity to NHF cells, with the highest cell death rate of 68% and the lowest optical density (OD) of 0.25 at a concentration of 100 µg/ml. The IC_{50} value, indicating the concentration at which 50% inhibition of cell viability occurred, was determined to be 49.35 µg/ml. Further research is necessary to explore the potential role of these bacterial isolates in promoting cancer through inflammation. **Conclusion:** The clinical isolates of *S. maltophilia* demonstrated substantial cytotoxic activity against normal human fibrocyte cells, leading to a notable reduction in both cell number and optical density, with the highest percentage of cell death observed. These findings suggest the need for further investigation into the specific toxins or enzymes involved, which could pave the way for future studies.

Keywords: Bladder cancer- Cytotoxic activity- Prostate cancer- *Stenotrophomonas maltophilia*

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Introduction

Stenotrophomonas maltophilia, a Gram-negative bacterium, has emerged as a significant nosocomial pathogen, particularly in critically ill patients, causing bloodstream infections (bacteremia), pneumonia, and urinary tract infections [1]. It ranks as the third most common opportunistic pathogen in hospitals, following Pseudomonas aeruginosa and Acinetobacter baumannii. *S. maltophilia* plays a crucial role in polymicrobial infections, contributing to increased mortality rates in pneumonia patients [2]. This bacterium is commonly found in hospital environments, including dialysis machines, blood pressure monitors, faucets, sphygmomanometers, disinfectants, and ventilators, and it can spread between patients or even from patients to healthy individuals [3].

Although *S. maltophilia* is not highly pathogenic, it is capable of causing a range of infections, from mild to life-threatening, including wound infections, endocarditis, cellulitis, and, in rare cases, meningitis. Key risk factors for these infections include immunodeficiency, chronic pulmonary disease, a history of inappropriate or excessive antibiotic use, prolonged hospital or ICU stays, renal failure, catheterization, diarrhea, and transplant rejection [4, 5]. Immunocompromised individuals should be particularly vigilant about community-acquired *S. maltophilia* infections due to the high fatality rates associated with this bacterium, which is a significant nosocomial pathogen linked to mortality rates ranging from 14% to 69% in bacteremia patients [6, 7]. In cancer patients, *S. maltophilia* is a particularly important opportunistic pathogen because it can undermine clinical treatment through complex bacterial-host interactions. This extracellular parasitic bacterium typically modulates the host's immune response via outer membrane proteins such as fibrin or flagellin [8-10], posing a significant threat to immunocompromised patients. Consequently, this study aims to investigate the cytotoxic effects of *Stenotrophomonas maltophilia* on normal human cells, given its recent widespread isolation from patients, particularly those with compromised immune systems, such as cancer patients.

Materials and Methods

Bacterial sampling and identification

A total of 120 mid-stream urine samples were collected as follows: 27 urine sample collected from prostate cancer patients, 42 bladder cancer etc urine samples collected from 27 prostate cancer patient, 42

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bladder cancer patient, 46 renal failure patient and 5 from urinary tract infection patient. Initial diagnosis of isolates based on morphological characteristic of the colonies that includes colony shape, colony texture, color and edges then, the diagnosis confirmed by Vitec 2 compact system (Biomerieux, France) according to the instruction provided by the company, unless otherwise specified results were reported with a high degree of certainty (excellent or very good identification).

Inclusion criteria

Patients with bladder and prostate tumor and patients with kidney dialysis as well as patients with other complications of urinary tract.

Exclusion criteria

Patients treated with chemotherapy, immunotherapy or antibiotics for UTIs for at less than 1 month.

Current study was done based to the Declaration of Helsinki (1964) and was approved by Ethical Committee of the College of Medicine-AL-Iraqia University.

Maintenance of cell cultures

The MCF-7 cell line was grown in MEM (Sigma, USA) containing 10% Fetal bovine, 100 units/mL penicillin, and 100 g/mL streptomycin. Cells were passaged twice a week with Trypsin-EDTA (Sigma, USA), reseeded at 50% confluence, and incubated at 37 °C [11].

Cytotoxicity Assays

The MTT cell viability assay (MTT stain, Bio-rad, USA) was performed on 96-well plates to determine the cytotoxic effect. Cell lines were seeded at a density of $1*10^4$ cells per well. Cells were treated with the bacterial filtrate after 24 hours or when a confluent monolayer was achieved. After 72 hours of treatment, cell viability was determined by removing the medium, adding 28 µL of a 2 mg/mL MTT solution, and incubating the cells for 1.5 hours at 37 °C. After removing the MTT solution, the crystals in the wells were solubilized by adding 130 µL of DMSO (Dimethyl Sulphoxide, Santacruz biotechnology, USA), then a 15-minute incubation at 37 °C with shaking. The absorbency was measured using a microplate reader using ELISA reader (Human, German) at 492 nm (test wavelength), in triplicate. The rate of inhibition for cell growth (cytotoxicity percentage) was calculated as [12]:

% Cell viability= (Absorbance of treated cell/ Absorbance of non-treated cell) * 100

% Cytotoxicity= 100-cell viability

Statistical analysis

GraphPad Prism 6 was used to perform a statically analysis of the acquired data using an unpaired t-test. The values were shown as the triple measurements' mean ± standard deviation.

Results

Patients Demography

In this study, general demographic criteria including gender as follows: Age range of patients from (24-86) years, mean age 55.5 ±5. Male to female ratio of patients were (76/120) 63.3% and (44/120) 26.7% respectively.

Culturing of urine sample obtained from patients revealed that 87/120 (72.5%) samples were positive for bacterial growth including different species of bacteria. Bacterial growth was obtained from ordinary culture media including blood agar media, nutrient agar media, MacConkey agar media and chromogenic agar UTI media. Seven isolates were suspected to be *S. maltophilia* depending on the primary diagnosis bacteria were diagnosed by Vitec 2 compact system which confirm the identification of bacteria depending on 49 biochemical tests.

Cytotoxicity assay

The assay of MTT was used to determine the cytotoxic effect of *S. maltophilia* culture filtrates on Normal human fibroblast from skin (NHF) at different concentrations (3.125, 6.25, 12.5, 25, 50, 100 µg/ml) the results indicated that the cytotoxicity *S. maltophilia* culture filtrates increased with concentration were observed 68.70%, 54.90%, 39.30%, 33.00%,29.20% and 21.70% of cell death occurred when 100, 50, 25, 12.5, 6.25 and 3.125 µg/ml respectively. The higher decrease in number of cells and optical density with highest percentage killing and lower optical density 68% and 0.25 O.D respectively at

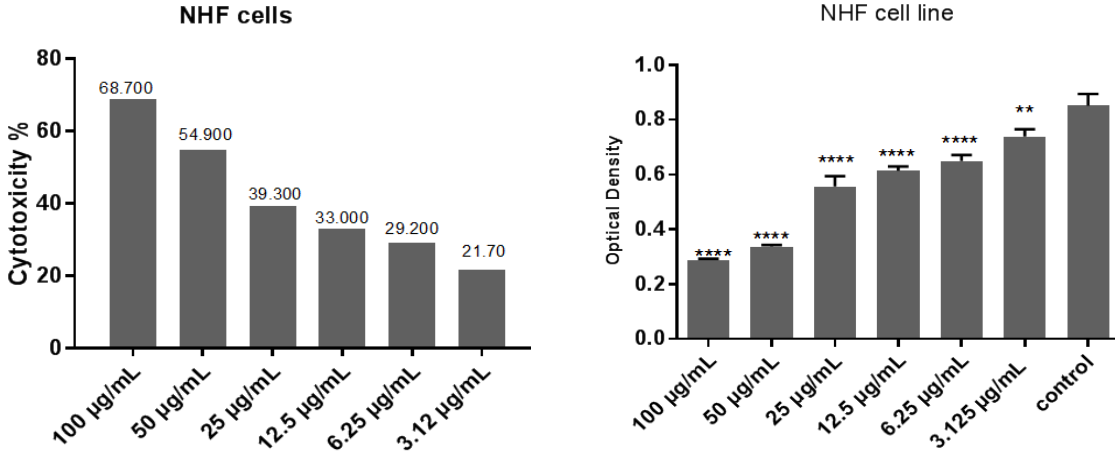

Figure 1. Cytotoxicity and Optical Density of *Stenotrophomonas maltophilia* on Normal Human Fibroblast Cell Line

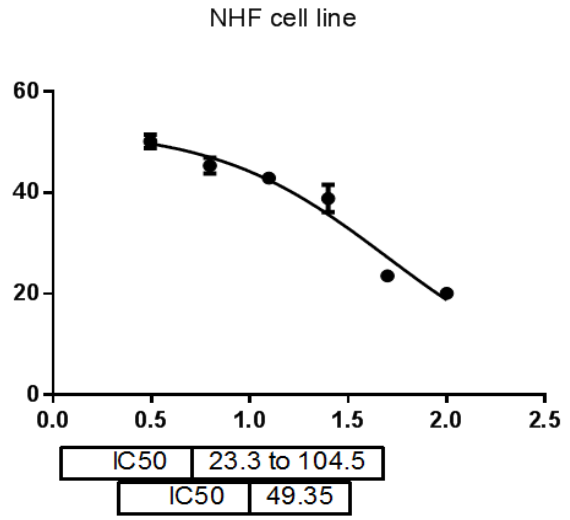

Figure 2. Half maximal inhibitory concentration IC_{50} effect of *Stenotrophomonas maltophilia* on normal human fibroblast cell line

concentration 100µg/ml of bacterial filtrate, while optical density recorded, Half maximal inhibitory concentration (IC_{50}) was 49.35, Figure 1,2.

Cytopathic effects were observed in *S. maltophilia* culture filtrates. After incubation with culture filtrates, the cells changed from flat elongated shaped cells typical of human fibrocyte cells to round and shriveled cells, with gradual destruction of the monolayer as in Figure 3.

Discussion

Following the culture of patient urine samples, 87 out of 120 samples (72.5%) tested positive for bacterial growth, encompassing a variety of bacterial species. The isolated microorganisms were divided into 53/87 (61%) Gram negative bacteria and 34/87 (61%) Gram positive bacteria (39%). Form total bacterial growth only seven isolated was *S. maltophilia*.

The result of the current study shows that the extract of *S. maltophilia* was significant to killed the cell. In natural people, infecting with *S. maltophilia* bacteria cause the killing of epithelial cells, and every time they kill these cells, they were regenerate and grow new cells, so it was produced new cells quickly, and this speed of division and cell regeneration can lead to an error in DNA multiplication, and this error by doubling can accumulate and cause progress in the occurrence of bladder cancer or any other type of cancer.

A study in USA in 2015 investigated the cytotoxic activity of *S. maltophilia* secreted proteins on host cell viability their results suggest that extracellular matrix protein degradation, IL-8 degradation, and A549 cell rounding are primarily caused by StmPr1 and StmPr2, but other Xps substrates are also involved in these processes. Together, these findings shed new light on the potential virulence of the *S. maltophilia* Xps type II secretion system and the substrates StmPr1 and StmPr2 [13].

A study in Korea at 2016 was examined the cytotoxic potential of *S. maltophilia* outer membrane vesicles (OMVs) as well as their capacity to elicit inflammatory reactions both in vitro and in vivo. During in vitro culture, spherical OMVs were discovered to be secreted by *S. maltophilia* ATCC 13637 and two clinical isolates. Human lung epithelial A549 cells were cytotoxic by *S. maltophilia* ATCC 13637 OMVs. This study provides evidence for the first time that *S. maltophilia* OMVs cause cytotoxicity in epithelial cells grown in culture and

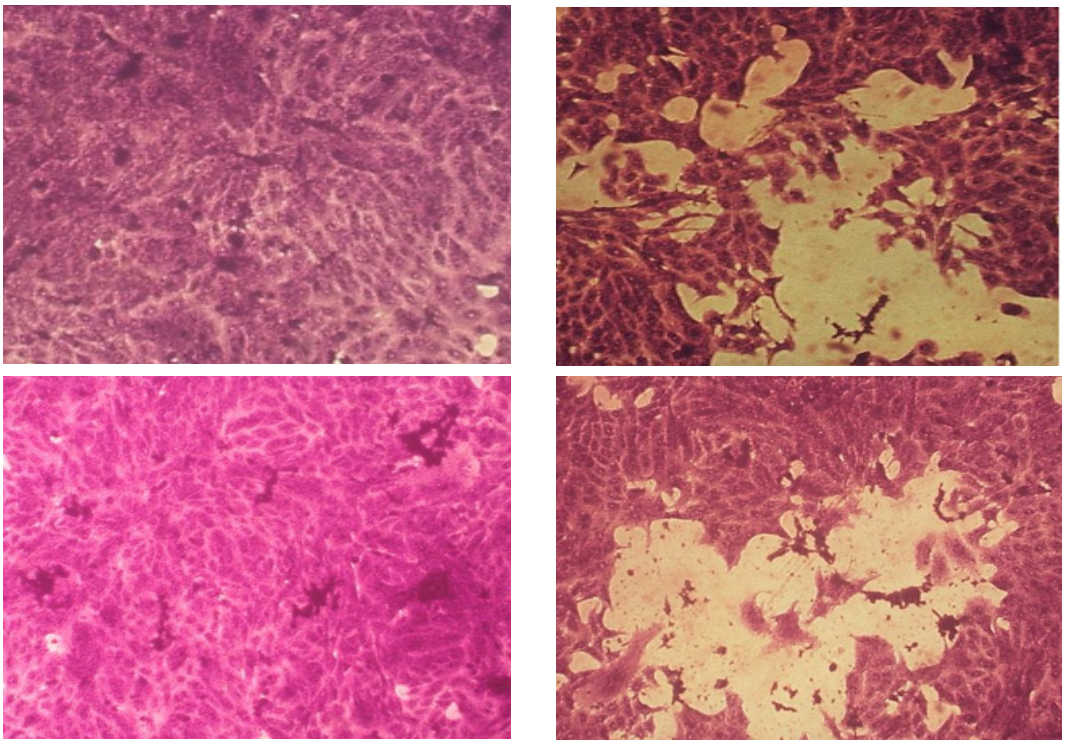

Figure 3. Cytotoxic Effects of *Stenotrophomonas maltophilia* to Normal Human Fibrocyte Cells at Half Maximal Inhibitory Concentration after 24hr. under inverted microscope (10 X), left: untreated, right: treated

provoke inflammatory reactions both in vitro and in vivo. Further research is needed to evaluate whether OMVs released from *S. maltophilia* constitute a therapeutic target because they are significant secretory nanocomplexes linked to bacterial pathogenesis [14].

Other study in Brazil at 2006 was demonstrated that the Vero (African green monkey), HEp-2 (human larynx epidermoid carcinoma) and HeLa (human cervix), cell lines were used to test cytotoxic activities. Microscopic examinations showed that HEp-2 cells had extensive rounding, loss of intercellular connections, and membrane changes (blebbing) before dying. Strong endocytosis and cell aggregation were the hallmarks of the cytotoxic effects in Vero and HeLa cells. Neutral red was used to test the vitality of grown mammalian cells and showed that each cell's sensitivity varied. HeLa and Vero cells had intense endocytosis and cell aggregation in response to *S. maltophilia* culture supernatants, which also resulted in hemolytic and enzymatic activities [15].

As previous study indicated that the cellular changes in cell line were comparable to those observed in viral and intracellular membrane fusion proteins, that contain a minimal set of domains that must be deployed at the right time during the fusion process [16]. A fusogenic protein that binds to the target membrane receptors and alters the conformation of the lipid bilayer is a key factor in membrane fusion. The result of these modifications is a hydrophobic defect that symbolizes the committed step of fusion by exposing the acyl chains and some transmembrane domains of the fusogenic protein to the aqueous media [17].

Conclusion and recommendation

The clinical isolate of *S. maltophilia* exhibits a highly significant cytotoxic effect on normal human fibrocyte cells, leading to a reduction in cell number and optical density, with the highest observed percentage of cell death. This finding could motivate future research focused on characterizing the bacterium's toxins or enzymes. Although studies have begun exploring *S. maltophilia* infections in both mammalian and nonmammalian animal models, our current understanding of its pathogenesis remains limited. Further research is needed to elucidate the role of its toxins or enzymes in disease development.

Author Contribution Statement

All authors contributed equally in this study.

Acknowledgements

Approval

The study protocol was approved by protocol committee of collage of medicine in Al-Iraqia university.

Ethics Committee Approval

This study was approved by ethical committee of the College of Medicine- AL- Iraqia University in 2022.

Availability of data (if apply to your research)

The datasets generated and/or analyzed during the current study are not publicly available.

Declaration of Conflict of interest

The authors declare that they have no conflict of interests.

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