شناسایی و حذف عفونت‌های *پروتئوس وولگاریس* و *کلپسیلا پنومونیا* در موش‌های نود

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چکیده

مقدمه: موش‌های نود نقص سیستم ایمنی دارند و بیشتر به‌عنوان مدل‌های جانوری برای بیماری‌های انسانی استفاده می‌شوند. باوجود ارزش زیاد این حیوانات و هزینه‌های گزاف نگهداری آنها در تحقیقات مختلف، مطالعات انجام‌شده درخصوص حذف موانع عفونی اجتناب‌ناپذیری که ممکن است حین پژوهش رخ دهد، بسیار محدود است. مطالعهٔ حاضر، یک راهنمای عملی برای شناسایی و حذف عفونت‌های احتمالی ایجادشده در مدل‌های موشی نود ارائه می‌دهد.

مواد و روش‌ها: در این تحقیق پس از پیوند رده‌سلولی سرطان خون انسانی به موش‌های نود *C57BL/6*، وزن بدن حیوانات به‌صورت روزانه اندازه‌گیری شد. پس از بروز مرگ ناگهانی و پی‌درپی ۷ موش، بستر آنها به سرعت تعویض شد و قفس‌ها، غذا و ظروف آب مجددا ضدعفونی شدند. همچنین، نمونه خون با استفاده از سیستم رادیومتریک بک‌تک بررسی شد و آنتی‌بیوتیک‌های مؤثر بر مبنای آزمون‌های مهار رشد میکروبی انتخاب شدند.

نتایج: *پروتئوس وولگاریس* و *کلپسیلا پنومونیا* به‌ترتیب با آزمون‌های رادیومتریک شناسایی شدند. براساس آزمایش‌های حساسیت ضدمیکروبی، موش‌های بیمار، با سیپروفلوکساسین خوراکی (۱۳۲ mg/kg) به مدت دو هفته و سپس با سیفکسیم (۵۰۰ mg/kg) به مدت یک هفته تیمار شدند. در عفونت دوم، دوز پایینی از سیفکسیم (۱۳۲ mg/kg) نیز به‌صورت پروفیلاکتیک تجویز شد. پس از پایان دورهٔ درمان، حذف عفونت میکروبی در موش‌های بیمار گزارش شد. همچنین تا پایان دورهٔ آزمایش هیچ نوع عفونتی در موش‌های سالم مشاهده نشد.

بحث و نتیجه‌گیری: نتایج این تحقیق نشان می‌دهند نظارت دقیق بر سلامتی مدل‌های موشی امکان شناسایی عفونت‌های احتمالی در آنها را فراهم می‌کند. سیپروفلوکساسین و سیفکسیم، آنتی‌بیوتیک‌های انتخابی برای حذف عفونت‌های *پروتئوس وولگاریس* و *کلپسیلا پنومونیا* هستند. همچنین، استفاده از دوز پایین آنتی‌بیوتیک به‌صورت پروفیلاکتیک، از بروز عفونت‌های محتمل جلوگیری می‌کند و ضمن حفظ جان این مدل‌های حیوانی پرهزینه، محیطی مناسب برای انجام آزمایش‌های برون‌تنی فراهم می‌آورد.

واژه‌های کلیدی: آنتی‌بیوتیک، موش‌های نود فاقد غده تیموس، عفونت، عاری از پاتوژن‌های خاص

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Detection and elimination of *Proteus vulgaris* and *Klebsiella pneumoniae* in nude mice

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Abstract

Introduction: Nude mice are immunodeficient, commonly used model organisms for studying human diseases. Despite the high purchase price and maintenance cost of these animals, few studies have aimed to eradicate inevitable infectious complications which may arise during *in-vivo* studies. The current project is a practical guideline for detecting and elimination of possible infections developed in nude mice.

Materials and Methods: Followed by transplantation of human leukemic cell lines into *C57BL/6* nude mice, body weights were monitored daily. After a sudden death of 7 mice during 4 consecutive days, all beddings were discarded and cages, food and water bottles were disinfected immediately. Automated blood culture analyses were carried out using radiometric BACTEC systems, and specific antibiotics were administered based on applied microbial growth inhibition assays.

Results: *Proteus vulgaris* and *Klebsiella pneumoniae* were identified, respectively, according to the radiometric assays. According to the antimicrobial susceptibility tests, 132mg/kg ciprofloxacin was administered orally for 2 weeks, followed by 500mg/kg cefixime for one week. Meanwhile, low-dose cefixime (132 mg/kg) was applied as a prophylactic treatment. Automated blood cultures showed elimination of both bacterial infections followed by demand treatments. Low-dose antibiotic prophylaxis saved uninfected mice from developing infections.

Discussion and Conclusion: Results showed that precise monitoring of nude mice health conditions help early detection of the possibly grown infections. Ciprofloxacin and cefixime are the antibiotics of choice for eradication of *Proteus vulgaris* and *Klebsiella pneumoniae*. On the other hand, low-dose antibiotic prophylactic treatment may rescue these expensive animals from possible infections and provide suitable environment for performing *in-vivo* experiments.

Key words: Antibiotic, athymic nude mouse, infection, specific-pathogen-free

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Introduction

Immunodeficient mice are introduced as principal sources of animal models in a wide range of biomedical experiments, especially in oncology researches (1). Nude (nu) mouse harbors a single-gene mutation in *Foxn1* gene, winged-helix/forkead transcription factor, which causes the lack of body hair. In 1966, it was unveiled that homozygote nu mutations contribute to a rudimentary thymus, called athymic state. An athymic state generally leads to inappropriate T-cell-mediated functions, including rejection of transplantation and deficiency, or lack of defense against infections. On the other hand, upon their different strains and microbiological status, nude mice possess natural killer (NK) cells and macrophages with different variety of numbers and activities. Xenograft transplants can be successfully generated from nude mice (2). However, because of their inhibited immune system, nudes are extremely vulnerable to infection and, in spite of the advanced monitoring systems and high level of expertise applied for nude mice maintenance, development of infectious agents is unavoidable. Along with diverse mice strains (3-5), several papers have introduced nude mice as mouse models of bacterial or viral infections (6, 7). However, there is a lack of information for guidelines preventing unwanted infections, and developing *in-vivo* experiments with least mice fatality. One of the few researches performed to solve this problem is the study of Burr and colleagues. They introduced a method of treatment for *Corynebacterium*-associated murine hyperkeratosis (8). Giving a particular emphasis to the impact of close monitoring of mice health status, the current study presents a guideline for the detection and eradication of bacterial infections, *Proteus vulgaris* and *Klebsiella pneumoniae*, in nude mice.

Materials and Methods

Chemicals and reagents: BACTEC automated blood culture and pediatric vials were purchased from Becton Dickinson, Inc (Towson, USA). Ciprofloxacin (500 mg) and cefixime (400 mg) were obtained from Ariya (Tehran, Iran) and Loghman (Tehran, Iran), respectively.

Mice, maintenance and xenographt transplantation: 48 athymic nude mice (*C57BL/6* Nude, 4–6 week-old female, 12.7 ± 0.17 gr (mean ± SEM) were purchased from Pasteur Institute (Amole, Iran). Mice were placed in a standard specific-pathogen-free (SPF) room and xenograft transplantation was performed at week 2 according to the approved national patent number IR.UI.REC.1396.056 (for more information, related protocols and procedures for confirmatory tests are introduced in the supplementary information). The study was in accordance with the ethical standards of the responsible Ethics Committee of the University of Isfahan (ethics number: IR.UI.REC.1396.056 for animal handling). Food, beddings, water bottles and cages were sterilized and replaced twice weekly.

Detection of infectious agents and antimicrobial susceptibility tests: Four weeks followed by transplantation, mice started losing weight and 7 mice died during 4 days. Autopsies were performed and automated blood cultures were performed using radiometric BACTEC system. 200 µl of mice heart-blood samples were subjected into separate BACTEC tubes and sent to a pathology reference laboratory (Isfahan, Iran) for investigating the origin of infection. Antimicrobial susceptibility tests were performed using disc diffusion method on Mueller-Hinton agar culture media. The diameter of the inhibition zone around each disc was measured and the degree of sensitivity was interpreted as susceptible, intermediate or resistant. Same procedure

was performed while we encountered a second infection at week 6 post-transplantation. Selected antibiotic tabs were dissolved in 2% dextrose in water then filtered. Dextrose was added to the solvent because of the antibiotics bitter taste. Water bottles containing antibiotics were replaced twice a week.

Mice isolation and precautionary managements: Followed by each time identification of infectious pathogens, cages, food and water bottles were replaced with newly disinfected resources and daily change of beddings were carried out in order to prevent any possible spread of infection through feces. Mice showing torpid or aggressive behavior, hunched posture or sudden weight loss were considered as suspected carriers of infection and transferred to separate cages.

Results

Post-mortem examination of infected mice: Four weeks followed by transplantation (Figure 1S), body weight was decreased in 7 mice (-2.2 ± 0.75 gr, mean ± SEM) resulting in their sudden death during 4 days. Autopsies showed a couple of abscesses in corpse abdominal cavities (Figure 1).

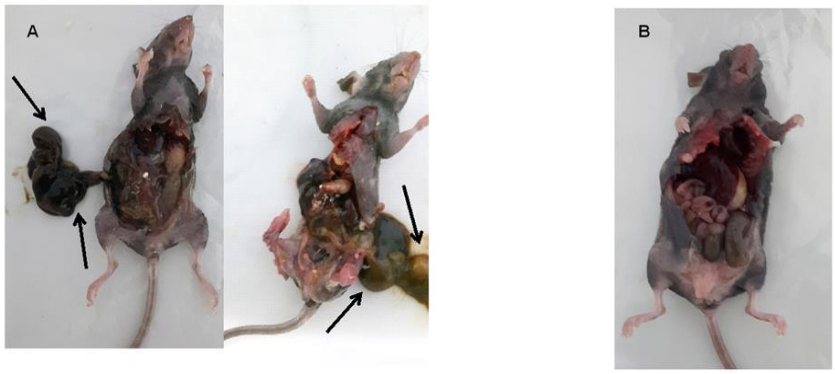


Fig. 1- Comparison between the infected and healthy mice. Followed by ventral dissections, the abdominal cavities of infected mice were compared with that of a healthy mouse. Infected nudes showed several abscesses (indicated by arrows). Corps are representatives of infected (A) and healthy (B) mice.

Demand-treatment and low-dose prophylaxis: At week 4 post-transplantation, BACTEC analyses determined the presence of *Proteus vulgaris* in the heart-blood samples of infected mice. Antibiogram tests demonstrated bacteria susceptibility to several antibiotics, among which, ciprofloxacin was chosen (Figure 2A, B). Mice with altered behavior were kept in separate cages from healthy mice and given 132 mg/kg oral ciprofloxacin (9) for 2 weeks. Blood culture analyses showed negative contamination post demand-treatment. At week eight, *Klebsiella pneumoniae* was identified in the blood samples of two dead mice. Antibiotic susceptibility tests showed *Klebsiella* susceptibility to cephalosporins (Figure 3A, B). Mice with signs of infection were treated with 500 mg/kg oral cefixime, for 1 week. At the same time, 132 mg/kg cefixime was given to the remained mice as prophylaxis. At week nine, automated blood culture analysis results showed no bacterial contamination, indicating complete elimination of bacteria. Subsequently, the prophylactic treatment was terminated and no more infection was observed until the end of the experiment (Figure 4). According to the protocol introduced in this study, the infection rate was decreased from 29.16 to 0 percent in the SPF room.

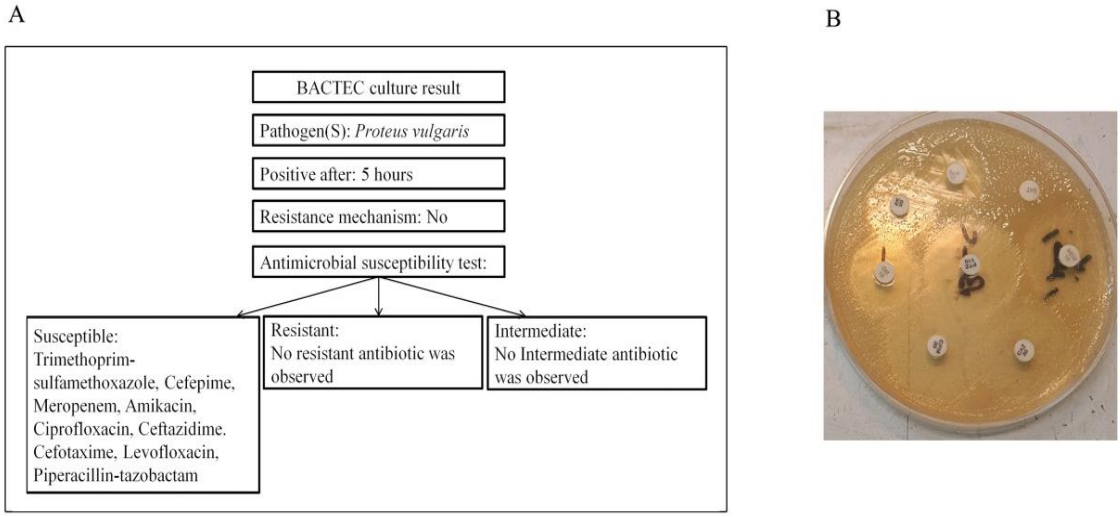


Fig. 2- Representatives of an automated blood culture (A) and an antimicrobial susceptibility test (B) followed by first time murine infection. BACTEC analyses detected *Proteus vulgaris* in the heart-blood samples of dead mice. Antimicrobial susceptibility tests determined a wide range of antibiotics, among which the injectable antibiotics were ignored to prevent any mouse skin damage and stressful conditions. Oral ciprofloxacin was therefore selected and administered to the infected mice.

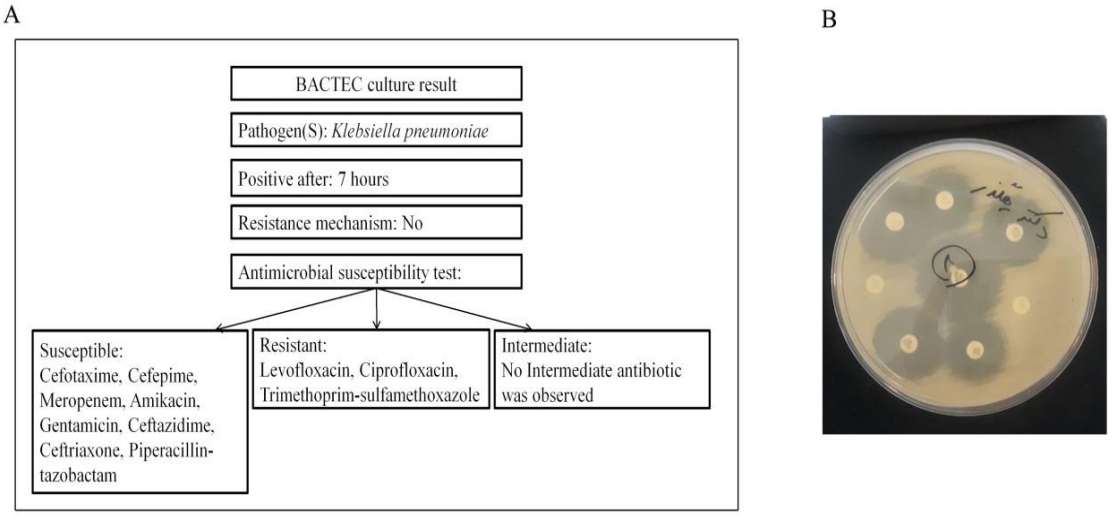
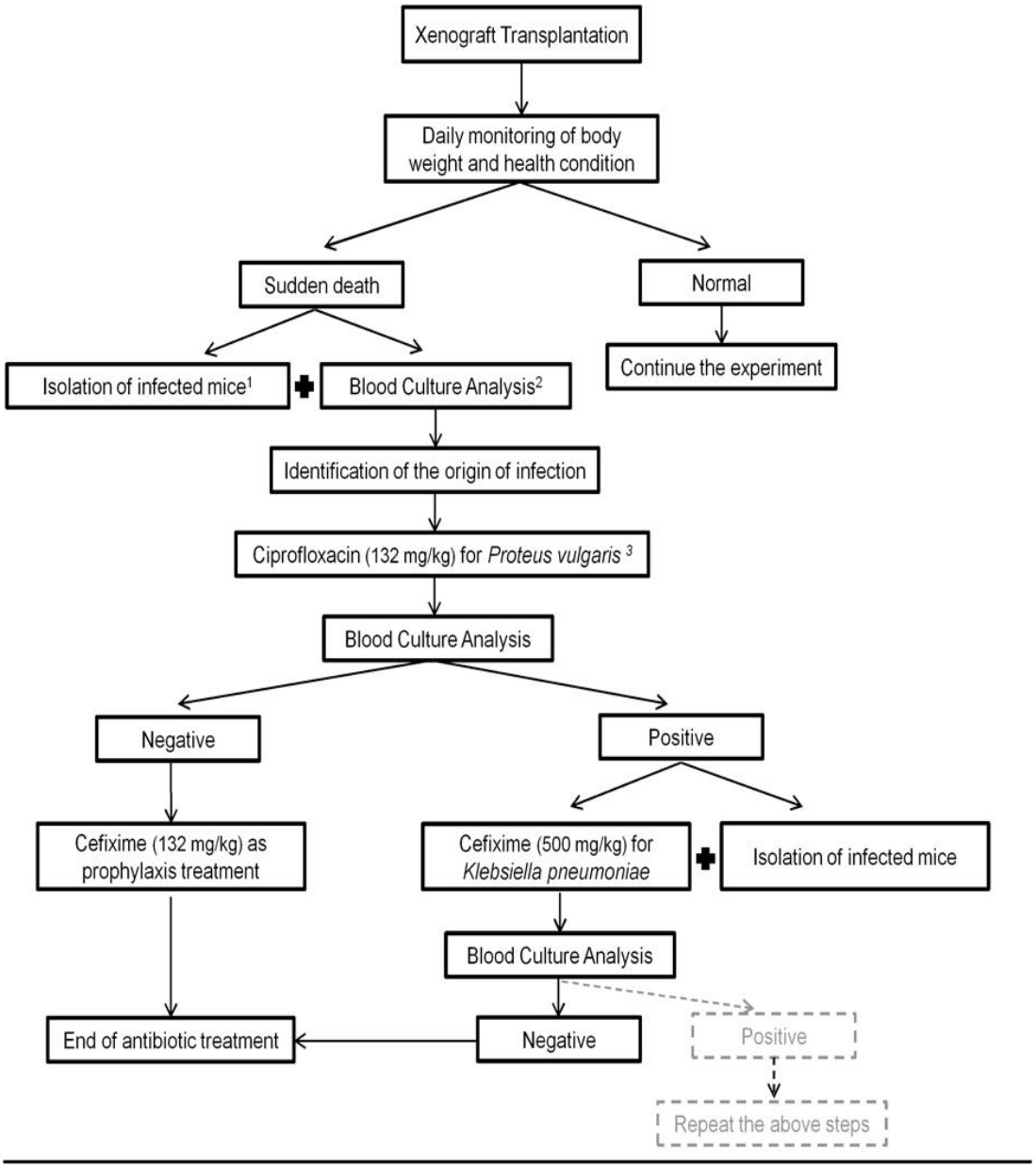


Fig. 3- Representatives of an automated blood culture (A), and an antimicrobial susceptibility test (B) followed by second time murine infection. BACTEC analyses detected eradication of *Proteus vulgaris*, but detection of *Klebsiella pneumoniae* in the heart-blood samples of dead mice. Antimicrobial susceptibility tests determined resistance to the previously administered ciprofloxacin, but susceptibility to the cephalosporin family of antibiotics.



[1] Discard dead bodies, separate the infected mice, change the beddings, replace cages, food and water bottles with newly disinfected resources
[2] Automated blood culture analysis using a radiometric BACTEC system
[3] Select oral antibiotics with no interference with the reagents/medicines used in the project
Dashed line: Possible condition

Fig. 4- Guideline for detection and elimination of bacterial infections in nude mice

Discussion and Conclusion

Nude mice can be used as experimental animal models for human diseases, especially in cancer research (1). Due to the lack of T cells and severely impaired immune response, nudes are susceptible to infection (10, 11) and they mostly have short life span. Weight loss or failure to gain weight is one of the symptoms of infection. Considering that the immune system is not completely inactive in nude mice, providing a specific, but not absolute, pathogen-free living condition for these animals is adequate for maintaining them in good health, in order to perform *in-vivo* experiments (12). However, keeping nude

mice in an SPF room must be accompanied with the closely monitoring of mice health conditions, in order to prevent any possible infections during the examinations. In the current study, after confirmation of the bacterial infection in blood cultures using radiometric BACTEC system, specific antibiotic was selected amongst those suggested by antimicrobial susceptibility tests. The rationale behind this selection was the absence of any interference between the chosen antibiotic and the chemotherapy regimen applied during the study. Furthermore, the feasibility of administering the anti-bacterial treatment was concerned. Therefore, oral antibiotics were preferred in this regard. Eventually, complete bacterial elimination was reported and the experiment was terminated at the predicted time.

The bacterial infections identified in this study were *P. vulgaris* and *K. pneumoniae*. *Proteus vulgaris* is a rod-shaped, gram-negative bacterium, which belongs to the family of Enterobacteriaceae, and can cause gastrointestinal infections (13, 14). *K. pneumoniae* is a gram-negative bacterium inducing pneumonia, urinary tract infections and liver abscesses (15). *K. pneumoniae* is considered as a fatal, virulent bacterium for nude mice. Our results demonstrated that *Proteus vulgaris* was sensitive to ciprofloxacin. Chauhan and Pathiranage found similar sensitivity pattern for *Proteus vulgaris* (16, 17). However, Bilal and Akerele claimed that *Proteus vulgaris* was resistant to ciprofloxacin (18, 19). On the other hand, according to the present study *Klebsiella pneumonia* is susceptible to cefixime. This information is not in compliance with previous studies performed by Bokaeian and Gurung (20, 21). Variation in bacteria resistance patterns may be attributed to the antibiotics manufacturers' protocols of application and their possible different formulations.

The prophylactic antimicrobial treatment introduced in this study helped decrease the infection rate from 29.16 to 0 percent. Prophylaxis is defined as a preventive care which is recommended in different vulnerable conditions including surgical procedures (22), cancer chemotherapy-induced neutropenia (23) and several hematological disorders such as hemophilia (24). The immune system of nude mice is impaired; hence these animals are easily prone to various infections, even while maintained in SPF laboratories. Prophylaxis was previously administered to prevent *streptococcus pneumoniae* and chikungunya virus infections in mice models (25, 26).

The possible impact of different infections on the biomedical parameters of *in-vivo* experiments, recommend exclusion of the infected mouse from the study. However, the developed infection may easily spread amongst the whole number of mice, and eventually terminate the entire project. Unless, practical guidelines are used for treating the infected mice and protecting the rest of animals from possible contaminations. The current study introduces a simple, rapid and reliable approach to easily detect the infected mice, eradicate the origin and prevent the spread of infection in nude mice in order to keep the safety of results and ensure cost-effectiveness of the study.

Supplementary information

Protocol for establishing human leukemia xenograft mouse models

Materials: Roswell Park Memorial Institute-1640 (RPMI1640), fetal bovine serum (FBS) and penicillin-streptomycin (Pen/Strep) were from Bioidea (Tehran, Iran). CCRF-CEM (T-ALL) human cell line was obtained from Pasteur Institute (Tehran, Iran). Cells were cultured in RPMI1640 containing 10% heat-inactivated FBS and 1% Pen/Strep. 48 athymic nude

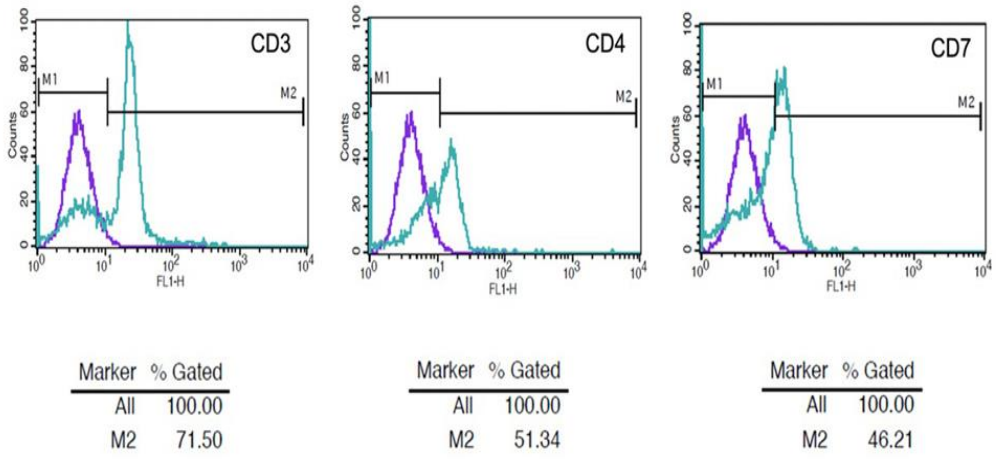
mice (*C57BL/6* Nude, 4 to 6-week-old female, 12.7 ± 0.17 gr (mean ± SEM) were purchased from Pasteur Institute (Amole, Iran). Mouse anti-human monoclonal antibodies against human CD3, CD4 and CD7 markers were purchased from Dako (Les Ulis, France).

Methods: After a 2-week delay for giving mice enough time for adaptation, 300 mg/kg cyclophosphamide was injected intraperitoneally. 72h later, mice undergo transplantation receiving 15×10^6 CCRF-CEM cells in 100 µl FBS, subcutaneously.

Immunophenotyping assay: To confirm the xenograft transplantation, heart blood samples were collected from nude mice transplants. Immunophenotyping assays were performed using mouse anti-human monoclonal antibodies against human CD3, CD4 and CD7 markers. 1×10^5 cells were subsequently applied to Partec CyFlow ML Flow Cytometer (Munster, Germany) and data were analyzed using FloMax® software.

Results

Twenty days post-transplantation, followed by the observation of few small nodules at the site of injected grafts, flow cytometry was performed on blood samples. Results demonstrated the presence of human lymphoblasts CCRF-CEM markers (71.50% CD3, 51.34% CD4 and 46.21% CD7) in mice blood samples (Figure 1S).



Marker	% Gated
All	100.00
M2	71.50

Marker	% Gated
All	100.00
M2	51.34

Marker	% Gated
All	100.00
M2	46.21

Fig. 1S- Confirmation of transplantation in nude mice models using flow cytometry. Transplantation confirmatory test was performed on mice heart-blood samples using flow cytometry. Identification of 71.50% CD3, 51.34% CD4 and 46.21% CD7 human markers confirmed transplantation.

Conflict of Interests

Authors declare no conflict of interests.

Ethical considerations

All investigations were conducted in accordance with the principles outlined in the Declaration of Helsinki and approved by the responsible Ethics Committee of the University of Isfahan (agreement number IR.UI.REC.1396.056 for animal studies).

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References

(1) Szadvári I, Krizanova O, Babula P. Athymic nude mice as an experimental model for cancer treatment. *Physiological*

research. 2016;65.

(2) Belizário JE. Immunodeficient mouse models: an overview. *The open immunology journal*. 2009;2(1).

(3) Rowell J, Lo C-Y, Price GE, Misplon JA, Epstein SL, Garcia M. Conventional influenza vaccines influence the performance of a universal influenza vaccine in mice. *Vaccine*. 2018;36(7):1008-15.

(4) Ng GZ, Solomatina A, van Driel IR, Hartland EL. The Mouse as a Model for Pulmonary *Legionella* Infection. *Legionella*: Springer; 2019. p. 399-417.

(5) Velazquez EM, Nguyen H, Heasley KT, Saechao CH, Gil LM, Rogers AW, et al. Endogenous Enterobacteriaceae underlie variation in susceptibility to *Salmonella* infection. *Nature microbiology*. 2019;4(6):1057-64.

(6) Speranza M-C, Kasai K, Lawler SE. Preclinical mouse models for analysis of the therapeutic potential of engineered oncolytic herpes viruses. *ILAR journal*. 2016;57(1):63-72.

(7) Hu J, Cladel NM, Budgeon LR, Balogh KK, Christensen ND. The mouse papillomavirus infection model. *Viruses*. 2017;9(9):246.

(8) Burr HN, Lipman NS, White JR, Zheng J, Wolf FR. Strategies to prevent, treat, and provoke *Corynebacterium*-associated hyperkeratosis in athymic nude mice. *Journal of the American Association for Laboratory Animal Science*. 2011;50(3):378-88.

(9) Kang SS, Bloom SM, Norian LA, Geske MJ, Flavell RA, Stappenbeck TS, et al. An antibiotic-responsive mouse model of fulminant ulcerative colitis. *PLOS Medicine*. 2008;5(3):e41.

(10) Huang F, Zhang W, Gong G, Yuan C, Yan Y, Yang S, et al. Experimental infection of Balb/c nude mice with Hepatitis E virus. *BMC infectious diseases*. 2009;9(1):1-8.

(11) Romano R, Palamaro L, Fusco A, Iannace L, Maio S, Vigliano I, et al. From murine to human nude/SCID: the thymus, T-cell development and the missing link. *Clinical and Developmental Immunology*. 2012;467101.

(12) Gullino P, Ediger R, Giovanella B, Merchant B, Outzen Jr H, Reed N, et al. Guide for the care and use of the nude (thymus-deficient) mouse in biomedical research. *ILAR News*. 1976;19(2):M1-M20.

(13) Drzewiecka D. Significance and roles of *Proteus* spp. bacteria in natural environments. *Microbial ecology*. 2016;72(4):741-58.

(14) Hamilton AL, Kamm MA, Ng SC, Morrison M. *Proteus* spp. as putative gastrointestinal pathogens. *Clinical microbiology reviews*. 2018;31(3).

(15) Siu LK, Yeh K-M, Lin J-C, Fung C-P, Chang F-Y. *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. *The Lancet infectious diseases*. 2012;12(11):881-7.

(16) Chauhan A, Sadaf D, Koul S, Mir K, Sharma N, Singh R. Drug's resistant activity of *Proteus vulgaris* isolated from gut of Mystus seenghala of northern Punjab region. *Journal of Chemical and Pharmaceutical Research*. 2015;7(12):284-8.

(17) Pathiranage S, Debatapitiya D, Gunathilake M, Nadishani H, Sithara U, Hasintha K, et al. Isolation of *Salmonella* spp, *E. coli* and *Proteus* spp from Broiler Chicken Meat and Their Antibiotic Sensitivity to Commonly used Antibiotics. 2020.

(18) Akerele J, Ayinde B, Ngiagah J. Phytochemical and antibacterial evaluations of the stem bark of *Newbouldia laevis* against isolates from infected wounds and eyes. *Tropical Journal of Pharmaceutical Research*. 2011;10(2).

(19) Bilal S, Anam S, Mahmood T, Abdullah RM, Nisar S, Kalsoom F, et al. Antimicrobial profiling and molecular characterization of antibiotic resistant genes of *Proteus vulgaris* isolated from tertiary care hospital, Islamabad, Pakistan. *Pakistan journal of pharmaceutical sciences*. 2019;32.

(20) Bokaeian M, Saeidi S, Shahi Z, Kadaei V. TetA and tetB Genes in *Klebsiella pneumoniae* isolated from clinical samples. *Gene, Cell and Tissue*. 2014;1(2).

(21) Gurung S, Kafle S, Dhungel B, Adhikari N, Shrestha UT, Adhikari B, et al. Detection of OXA-48 gene in carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* from urine samples. *Infection and Drug Resistance*. 2020;13:2311.

(22) McHugh S, Collins C, Corrigan M, Hill A, Humphreys H. The role of topical antibiotics used as prophylaxis in surgical site infection prevention. *Journal of antimicrobial chemotherapy*. 2011;66(4):693-701.

(23) Bisson J, Argyle D, Argyle S. Antibiotic prophylaxis in veterinary cancer chemotherapy: A review and recommendations. *Veterinary and comparative oncology*. 2018;16(3):301-10.

(24) Petrini P, Valentino LA, Gringeri A, Re WM, Ewenstein B. Individualizing prophylaxis in hemophilia: a review. *Expert review of hematology*. 2015;8(2):237-46.

(25) Bernardshaw S, Johnson E, Hetland G. An extract of the mushroom *Agaricus blazei* Murill administered orally protects against systemic *Streptococcus pneumoniae* infection in mice. *Scandinavian Journal of Immunology*. 2005;62(4):393-8.

(26) Couderc T, Khandoudi N, Grandadam M, Visse C, Gangneux N, Bagot S, et al. Prophylaxis and therapy for Chikungunya virus infection. *The Journal of infectious diseases*. 2009;200(4):516-23.