Repurposing of Tamoxifen Against the Oral Bacteria

Tamoxifenin Oral Bakterilere Karşı Yeniden Konumlandırılması

Ali Abdul Hussein S. AL-Janabi
Karbala University College of Medicine, Department of Microbiology, Karbala, Iraq

ABSTRACT

Objectives: Tamoxifen (TAM), which is used for treating breast cancer, has exhibited another important function as an antimicrobial agent. The objective of this study is to investigate the antibacterial action of TAM against the bacteria present in the human oral cavity.

Materials and Methods: The bacteria present in the human oral cavity were isolated from healthy individuals. Different concentrations of TAM were tested against the isolated bacteria. Additionally, bactericidal and bacteriostatic effects of TAM were also determined.

Results: Out of 23 isolated bacteria, a greater number of Gram-positive bacteria were highly susceptible to the low concentrations of TAM than Gram-negative bacteria. *Kytococcus sedentarius*, which is Gram-positive bacterium, and *Pseudomonas stutzeri*, which is Gram-negative bacterium, needed a high minimum inhibitory concentration value of TAM (2.5 mg/mL) to be inhibited by TAM’s bacteriostatic action. Resistance to TAM was also observed in three strains of Gram-positive and four strains of Gram-negative bacteria.

Conclusion: TAM has shown a potential antibacterial effect against the bacteria present in the oral cavity, especially against Gram-positive bacteria. This effect is mostly bacteriostatic. This study also found bacterial resistance toward TAM.

Key words: Bacteria, oral cavity, tamoxifen, Gram-positive, Gram-negative

ÖZ

Amaç: Meme kanserinin tedavisinde kullanılan tamoksifenin (TAM), antimikrobiyal ajan olarak önemli bir etkisi daha vardır. Bu çalışmanın amacı, TAM’ın insan ağrız boşluğunda bulunan bakterilere karşı antibakteriyel etkinliğini araştırmaktır. 

Gereç ve Yöntemler: İnsan ağrız boşluğunda bulunan bakteriler sağlıklı bireylerden izole edildi. İzole edilen bakterilere karşı farklı TAM konsantrasyonları test edildi. Ek olarak, TAM’ın bakterisidal ve bakteriyostatik etkileri de belirlendi.

Bulgular: İzole edilen 23 bakteriden, Gram-negatif bakterilere kıyasla daha fazla sayıda Gram-pozitif bakterinin düşük TAM konsantrasyonlarına yüksek oranda duyarlı olduğu saptandı. *Kytococcus sedentarius*, Gram-pozitif bakteri olarak adlandırılan, *Pseudomonas stutzeri*, Gram-negatif bakteri olarak adlandırılan, TAM’ın bakteriyostatik etkisiyle inhibe edilebilecekleri için yüksek bir minimum inhibitory konsantrasyon değeri (2,5 mg/mL) ihtiyaç duydular. TAM’ya direnç, üç Gram-pozitif ve dört Gram-negatif bakteri suşunda da gözlemlemiştir.

Sonuç: TAM, özellikle Gram-pozitif bakteriler olmak üzere ağrız boşluğunda bulunan bakterilere karşı potansiyel antibakteriyel etki göstermiştir. Bu etki çoğunlukla bakteriyostatiktir. Bu çalışmada aynı zamanda TAM’ya karşı bakteriyle direnç gösterilmemiştir.

Anahtar kelimeler: Bakteri, ağrız boşluğu, tamoksifen, Gram-pozitif, Gram-negatif
INTRODUCTION

As a member of selective estrogen receptor modulators, tamoxifen (TAM) is mainly used in the treatment and prevention of estrogen-positive breast cancer.\(^1\)\(^2\) It was first introduced by AstraZeneca, UK as a more effective therapy for estrogen-positive breast cancer in women of Pakistan and Australia.\(^3\) After more than four decades, TAM is considered as a golden drug for the treatment of breast cancer and extending the lives of approximately 500,000 women every year worldwide.\(^3\)\(^5\) The chemopreventive usage of TAM is another approach approved by the US Food and Drug Administration to protect women from breast cancer for at least five years.\(^4\)\(^6\) After two years of postoperative application, a study also found that TAM had the ability to reduce mortality resulting from coronary heart disease.\(^7\)

The potential antimicrobial activities of TAM against various pathogenic organisms have prompted researchers to conduct drug repurposing.\(^8\) Various studies have confirmed the direct and indirect antibacterial activities of TAM against different types of bacteria. TAM has shown a direct inhibitory action on the growth of *Staphylococcus aureus* and methicillin-resistant *S. aureus* (MRSA) strains,\(^9\) and also against *Mycobacterium tuberculosis*.\(^10\)\(^11\) The growth of *Enterococcus faecium* and its pathogenesis was found to be reduced by TAM after its administration in a *Galleria mellonella* infection model.\(^12\) Meanwhile, the indirect antibacterial action of TAM was observed when it enhanced the activity of immune cells represented by neutrophils against various pathogenic bacteria such as MRSA, *Pseudomonas aeruginosa*, and *Escherichia coli* through the agonist activity of the G protein-coupled estrogen receptor.\(^13\) Moreover, the antibacterial action of standard antibiotics such as polymyxin B also increased against various bacteria after combination with TAM.\(^14\)

The treatment of breast cancer by TAM usually takes a longer period that could extend up to five years;\(^2\) therefore, the normal bacterial flora of the human body, such as in the oral cavity, could be affected by the potential antibacterial activity of TAM. Hence, the objective of this study is to investigate the *in vitro* antibacterial activity of TAM against the oral flora of the human body.

MATERIALS AND METHODS

**Chemicals**

TAM citrate was purchased from Ebewe Pharma, Austria. Müller Mueller-Hinton agar (MHA) and Müller Mueller-Hinton broth (MHB) were purchased from HiMedia, India.

**Isolation of bacteria**

The swab samples were collected from the oral cavity of the healthy volunteers by sterilized cotton swabs. Then, the collected samples were cultured on blood agar and MacConkey agar (HiMedia, India). Inoculated media were incubated at 37°C for 24 hours for growing suspected bacteria. Diagnosis of the isolated bacteria was performed by the Vitek® 2 system using Vitek® 2 YST ID diagnostic cards for Gram-positive and Gram-negative bacteria (BioMérieux, France).

**Determination of minimum inhibitory concentration (MIC) of TAM**

The MIC of TAM was determined by the methods of dilution antimicrobial susceptibility tests for bacteria that grow aerobically as mentioned by CLSI-M07-A10 (2015).\(^15\) A broth culture of isolated bacteria was prepared by the selected inoculum bacteria in MHB and was incubated at 37°C for 24 hours. The turbidity of growing bacterial cells was adjusted with 0.5 McFarland standard to contain approximately 1x10⁸ CFU/mL. Serial concentrations of TAM (5, 2.5, 1.25, and 0.625 mg/mL) were prepared from a stock solution (10 mg/mL). Plastic microdilution plates (96-well plates) were used to determine the MIC values of TAM. Each well of the plate received 50 µL from the standard count of each bacterial suspension and 100 µL from MHB, followed by adding 50 µL of specific concentration of TAM. Several controls were used within a microdilution plate, including MHB with only bacteria, MHB without bacteria, and MHB with only TAM. The inoculated plate was incubated at 37°C for 24 hours. The results were visually read observed as the presence or absence of bacteria growth.

Bactericides and the bacteriostatic effect of TAM were determined by re-culturing the growth-inhibited bacteria from the microdilution plate on MHA and then incubating the same at 37°C for 24 hours. The growth of inhibited bacteria indicated bacteriostatic action, whereas the absence of growth showed bactericidal effects.

**Ethical approval**

The study was ethically approved by the local Ethics Committee of the College of Medicine of the University of Karbala in July 20, 2019.

**Statistical analysis**

Data of all the tests were expressed as mean ± standard deviation. The values were statistically analyzed with One-Way ANOVA using Microsoft Excel for Windows version 10. The minimum level of p value >0.05 was considered as statistically significant.

RESULTS

In total, 23 isolated strains of bacteria were diagnosed after culturing the swab samples from the oral cavity. There were 13 strains of Gram-positive and 10 strains of Gram-negative bacteria. Most of the Gram-positive bacteria were highly susceptible to the low concentration of TAM as compared to the Gram-negative bacteria. *Kytococcus sedentarius* as one Gram-positive and *Pseudomonas stutzeri* as one Gram-negative bacterium needed higher concentrations of TAM (MIC: 2.5 mg/mL) to be inhibited by the same. Additionally, the effect of TAM on these two types of bacteria was determined as bacteriostatic action (Table 1 and 2).

The effective low concentrations of TAM on susceptible bacteria, which showed MIC at 0.625 mg/mL, were mostly determined as bacteriostatic action. This bacteriostatic action was clearly shown in the five isolated strains of Gram-positive and in the two strains of Gram-negative bacteria, whereas TAM
demonstrated bactericidal action against three strains of Gram-positive and one strain of Gram-negative bacteria at the same concentration (Table 1 and 2).

The resistance to TAM was observed in three strains of Gram-positive bacteria, namely *Granulicatella elegans*, *Kocuria kristinae*, and *K. varians*, and in four strains of Gram-negative bacteria, namely *Acinetobacter baemolyticus*, *E. coli*, *Enterobacter cloacae*, and *Klebsiella pneumonia* (Table 1 and 2).

**DISCUSSION**

TAM is an effective drug for the treatment of breast cancer in both women and men. A clinical trial study conducted worldwide demonstrated that TAM can reduce the incidence of breast cancer by 50% in high-risk pre- and post-menopausal women. It has also been used as an adjuvant therapy with an efficacy of more than five years for treating postmenopausal, node-positive, and estrogen or progesterone receptor-positive women since the mid-1980s. Hence, TAM is commonly used by more than 7 million patients in a year and had saved the lives of approximately 500,000 women. In addition to the antagonist binding property of TAM with estrogen receptor to prevent the development of breast cancer, it also has another mechanism to prevent this type of cancer via the stimulated production of transforming growth factor (TGF)-calmodulin and protein kinase C, and also by blocking the angiogenesis process by lowering the production of IGF-1 and TGF.

In recent times, the resistance of bacteria to the most common antibacterial agents has increased progressively because of the massive overuse of these types of agents. The misuse and overprescription of antibacterial agents is considered as the most important factor that has contributed to the rise of resistant bacterial strains for such type of agents. The bacteria present in the oral cavity have also developed drug resistance toward many common types of antibiotics because of the genetic changes in their genomic structure. The resistance of the oral bacteria to erythromycin due to the activity of *mef* and *erm* (B) genes is one example of such antibiotic resistance. However, the list of antibiotics has hardly changed in four decades, and most of the pharmaceutical companies have left the antibiotic field due to the absence of a new class of antibacterial agents. Hence, antibiotic resistance is emerging as one of the modern crises, and now is the right time for a global commitment to develop new antibacterial drugs. The repurposing of existing drugs can be introduced as a solution to resolve the limited number of antimicrobial agents and for the enhancement of the treatment of most severe bacterial and fungal infections. The repurposing process is usually employed to discover a new therapeutic action of a specific drug to add to its previously known usage. TAM is one of those drugs, whose potential antimicrobial effect against various bacterial strains has been determined by many studies. These studies have presented promising data to repurpose the use of TAM from an anticancer drug into an antimicrobial agent. Low side-effect profile and cheaper price are other important characteristics that could encourage the continued usage of TAM for the treatment of cancer and microbial infections.

Out of 23 strains of the oral bacteria isolated in this study, 19 of them revealed susceptibility to TAM with variable MIC values. Low concentration of TAM (7.1 μg/mL) exhibited moderate antibacterial effect against *M. tuberculosis*, whereas a high concentration of TAM (MIC$_{50}$: 5-10 mg/mL) is required to suppress its growth. However, drug-sensitive strains of *M. tuberculosis* could be inhibited by low concentrations of TAM (3.125-6.25 μg/mL) as compared with drug-resistant strains (6.25-12.5 μg/mL). Antibacterial effects of TAM were also recorded against various other types of bacteria such as *E. faecium* and *S. aureus* and also against more drug-resistant bacterial strains such as MRSA and *M. tuberculosis*. The chemical derivatives of TAM have also shown potent antibacterial action.
ametabolic derivative of TAM, exerted an inhibitory action against *M. tuberculosis* with an MIC value of 5-10 mg/mL. The newly synthesized triaryl butane, which is an analog of TAM, exhibited antibacterial activity against Gram-positive and Gram-negative food-borne pathogens, such as *Listeria monocytogenes*, *Listeria ivanovii*, *Enterococcus faecalis*, *S. aureus*, and *E. coli*. Triphenylethylenne, which is considered as a backbone of TAM, has also shown antibacterial activity against various types of pathogens including bacteria. Otherwise, TAM had shown synergistic action with many known antibiotics to make them more effective against pathogenic bacteria as with polymyxin B against the polymyxin-resistant *P. aeruginosa*, *K. pneumoniae*, and *Acinetobacter baumannii* and the three first-line antibiotics (rifampin, isoniazid, and ethambutol) against *M. tuberculosis*. The activity of chitosan against *E. coli* and *Staphylococcus* spp. was increased in the presence of TAM when they were prepared in the nano-fiber polycaprolactone structure. Moreover, TAM can increase the defensive ability of the immune cells. This process is well proven when the bactericidal activity of neutrophils is increased by TAM against various bacteria such as MRSA, *P. aeruginosa*, and *E. coli*. TAM can also enhance the bacterial clearance by this immune cell with 2.4-4.2 log reductions in bacterial counts in several types of tissue samples. Intracellular tuberculosis in macrophages was also decreased after its treatment with TAM in a dose-dependent manner.

The results of this study have shown that Gram-positive bacteria are more susceptible to TAM than Gram-negative bacteria. The resistance rate in Gram-negative bacteria was also found to be higher (36-73%) toward many antibiotics as compared to that in Gram-positive bacteria. Generally, the resistance of Gram-negative bacteria is clearly identified toward various types of antibiotics because of its cell wall components, which make them a formidable barrier against any dangerous materials. The inhibition function of the bacterial cell membrane is the proposed mechanism of TAM action against bacteria. This type of antibacterial action is mostly related to the hydrophobicity of TAM because of the presence of alkyl groups that are attached to the amino group in its structure. Ultrastructural alterations in the components of the cell membrane of *Bacillus stearothermophilus*, which cause bacterial cell killing after treatment with TAM shows evidence that TAM is a membrane-active drug. This type of alteration, which leads to high K+ and Na+ efflux from bacterial cells and causes severe damage in the inner and outer membranes, is also recognized after the treatment of *E. coli* and *L. ivanovii* with an analog of TAM (triaryl butane). The mitochondrial membrane of *M. tuberculosis* was also observed to be collapsing by the ionic protonophore uncouplers of TAM and its lipophilic nature.

Seven of the isolated bacteria, including three types of Gram-positive and four types of Gram-negative bacteria, in this study showed resistance to TAM. This resistance may related to the modification of the lipid or protein composition of the outer membrane of bacterial cells as observed in most of the antibiotic-resistant bacteria. Other membrane modifications may include changes in the action of efflux pumps, the expression of various drug-deactivating agents, and proteolytic degradation.

As one of four members of Gram-negative bacteria that was resistant to TAM, *A. haemolyticus* also showed resistance against other antibiotics. This bacterium, which usually causes nosocomial infections and is frequently isolated from the intensive care unit (ICU) of the hospitals, is emerging to be one of the bacteria that are to most of antimicrobial agents. Isolates of *A. haemolyticus* from patients with immunocompromised status revealed a high level of resistance toward a wide range of antibiotics, including ampicillin-sulbactam, ampicillin, aztreonam, cefuroxime, and ceftazidime. This resistance was also observed among isolated strains from patients receiving treatment at ICU against ciprofloxacin, cefepime, ceftazidime, piperacillin, and amikacin. Like clinical isolates, *A. haemolyticus* isolated from the natural environment also showed resistance toward antibiotics such as cefotaxime and ceftazidime. However, the resistance of *A. haemolyticus* mainly depends on its acquisition of beta-lactamase and cephalosporinase enzymes, whereas resistance to quinolone is related to the mutations in *gyrA* and/or *parC* genes.

*E. coli* was a second Gram-negative bacterium that was resistant to TAM. This type of bacterium is usually sensitive to almost all the relevant antibiotics, but it also has the ability to acquire resistance genes from other species of bacteria via horizontal gene transfer. Another source of multiple antibiotic resistance may result from the change in amino acids of *mar* locus regulator or mutation in the operator-promoter region *marO* of the bacterium. In total, 137 *E. coli* isolates extracted from the cases of urinary tract infection (UTI) exhibited a high resistance (51.1-94.3%) toward ten types of antibiotics, whereas 17 non-pathogenic *E. coli* strains extracted from different sources revealed multiple antibiotic resistance because of the genes carried by class 1 and class 2 integrons. However, the transfer of resistance genes acquired by *E. coli* could result from plasmids and from other mobile genetic elements, such as transposons and gene cassettes in class 1 and class 2 integrons.

Our study results showed that *Enterobacter cloacae* complex (ECC), which contains common nosocomial bacteria that can cause various types of infection, exhibited resistance to TAM. The greatest antibiotic resistance by *E. cloacae* was against penicillin, cephalosporins (cefotaxime, ceftazidime, ceftriaxone), aminoglycosides, colistin, and fluoroquinolones. Moreover, a study recorded an emerging resistance of ECC to new generation of carbapenems. The pathogenic strains of *E. cloacae* that caused bacteremia were found to be resistant against cefalothin and ampicillin and a smaller number to these strains against aminoglycosides. However, this bacterium becomes resistant by acquiring resistance genes just like the other members of Gram-negative bacteria. *K. pneumoniae*, which causes various nosocomial infections, sepsis in neonates and bacteremia, is another bacterium of the Gram-negative group that showed resistance against
TAM. A study conducted from 1998 to 2010 in the USA also showed such resistance of *K. pneumoniae* against a wide range of antibiotics. The emergence of such types of multidrug-resistant bacteria have been increasing nowadays because of their production of extended-spectrum beta-lactamases and a mutation in the *mgrB* regulatory gene that lead to resistance against colistin.

Our results also exhibited resistance of three isolates of Gram-positive bacteria, namely *Granulicatella elegans*, *K. kristinae* and *K. varians*, and *G. elegans*, to TAM. These bacteria are related to the nutritionally variant streptococci that is usually found as one of the oral flora with an ability to cause infections and endocarditis under some conditions. It is considered as the most sensitive species to antibiotics other than species of its genus, especially *G. adiacens*. The resistance of *G. elegans* to macrolide and beta-lactam antibiotics was recently noticed because of the presence of *erm* and *mef* genes. This resistance could be increased in the case of biofilm formation by this bacterium.

Our results also showed that the two isolates of *Kocuria* spp., namely *K. kristinae* and *K. varians*, were resistant to TAM. This Gram-positive cocci bacterium is mostly non-pathogenic, and it mostly causes infection in the patients with immunocompromised status such as those with cancer diseases. Antibiotic resistance had been hardly recognized in this bacterium because of very limited available data. Thus, the absence of useful guidelines for determining the antibiotic resistance of *Kocuria* spp. makes the susceptibility test necessary according to the *Staphylococcus* guidelines. However, *K. kristinae* was found to be more resistant to antibiotics than *K. varians*. All the isolates of *K. varians* extracted from patients with endophthalmitis were found to be sensitive to all the tested antibiotics, whereas *K. kristinae* showed resistance against amikacin and cefazolin. Other isolates of *K. kristinae* from patients with UTI, immunosuppressive conditions, and cancer diseases also exhibited resistance against a wide range of antibiotics. Meanwhile, all the isolates of *K. varians* from periodontitis and brain abscess exhibited sensitivity to all the tested antibiotics.

**CONCLUSION**

The antibacterial action of TAM was clearly observed against oral bacteria, especially Gram-positive bacteria. The action was mostly determined as a bacteriostatic effect. The repurposing of TAM from cancer therapy to antimicrobial treatment could be encouraged by many factors; for example, TAM is a cheap drug with a few adverse effects. Although some bacteria show resistance, most of the known virulent species of isolated bacteria were found to be sensitive to TAM. This result will provide a promising view to use TAM in the treatment of infections caused by such types of bacteria.

**REFERENCES**

1. Osborne CK. Tamoxifen in the treatment of breast cancer. N Engl J Med. 1998;339:1609-1618.
2. Bekele RT, Venkatraman G, Liu R, Tang X, Benesch MG, Mackey JR, Godbout R, Curtis JM, McMullen TP, Brindley DN. Oxidative stress contributes to the tamoxifen-induced killing of breast cancer cells: implications for tamoxifen therapy and resistance. Sci Rep. 2016;6:21164.
3. Shahbaz K. Tamoxifen: Pharmacokinetics and pharmacodynamics. J Pharm Res. 2017;11:1-8.
4. Jordan VC. Tamoxifen: a most unlikely pioneering medicine. Nat Rev Drug Discov. 2003;2:205-213.
5. Bogush TA, Polezhaev BB, Mamuchey IA, Bogush EA, Polotsky BE, Tjulandin SA, Ryabov AB. Tamoxifen never ceases to amaze: New findings on non-estrogen receptor molecular targets and mediated effects. Cancer Invest. 2018;36:211-220.
6. Jordan VC. Tamoxifen. In: Schwab M, ed. Encyclopedia of Cancer. Philadelphia: Springer; 2017.
7. Rosell J. Long-term effects of adjuvant tamoxifen treatment on cardiovascular disease and cancer. Linköping University Medical Dissertations No. 1430. Linköping University, Faculty of Health Sciences, Department of Clinical and Experimental Medicine, Sweden; Division of Clinical Sciences, Oncology; 2014.
8. Montoya MC, Krysan DJ. Repurposing estrogen receptor antagonists for the treatment of infectious disease. mBio. 2018;9:1-10.
9. Levinson NS. Towards the elucidation of the mechanism of the antibiotic activity of tamoxifen. MS dissertation in Chemistry, College of Science, Georgia Institute of Technology; 2017. https://smartgchatech.edu/bitstream/handle/1853/58251/LEVINSON-THESIS-2017.pdf.
10. Jang WS, Kim S, Podder B, Jyoti MA, Nam KW, Lee BE, Song HY. Anti-Mycobacterial Activity of Tamoxifen Against Drug-Resistant and Intra-Macrophage Mycobacterium tuberculosis. J Microbiol Biotechnol. 2015;25:946-950.
11. Chen FC, Liao YC, Huang JM, Lin CH, Chen YY, Dou HY, Hsiung CA. Pros and cons of the tuberculosis drugome approach--an empirical analysis. PLoS One. 2014;9:e100829.
12. Jacobs AC, Didone L, Jobson J, Sofia MK, Krysan D, Dunman PM. Adenylate kinase release as a high-throughput-screening-compatible reporter of bacterial lysis for identification of antibacterial agents. Antimicrob Agents Chemother. 2013;57:26-36.
13. Flores R, Insel PA, Nizet V, Corrinder R. Enhancement of neutrophil antimicrobial activity by the breast cancer drug tamoxifen. FASEB Journal. 2016;30:969.
14. Hussein M, Han M, Zhu Y, Schneider-Futschik EK, Hu X, Zhou QT, Lin Y, Anderson D, Creek DJ, Hoyer D, Li J, Velkov T. Mechanistic insights from global metabolomics studies into synergistic bactericidal effect of a polymyxin B combination with tamoxifen against cystic fibrosis MDR Pseudomonas aeruginosa. Compt Struct Biotechnol J. 2018;16:587-599.
15. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved standard 10th ed. Document M07-A10. Wayne, Pennsylvania; 2015:29.
16. Karn A, Jha AK, Shrestha S, Acharya B, Poudel S, Bhandari RB. Tamoxifen for breast cancer. JNMA J Nepal Med Assoc. 2010;49:62-67.
17. Bartlett JG, Gilbert DN, Spellbergs B. Seven ways to preserve the miracle of antibiotics. Clin Infect Dis. 2013;56:1445-1450.
18. Nature Editorial. The antibiotic alarm. Nature. 2013;495:141.
19. Roberts MC. Antibiotic resistance in oral/respiratory bacteria. Crit Rev Oral Biol Med. 1998;9:522-540.
20. Villedieu A, Diaz-Torres ML, Roberts AP, Hunt N, McNab R, Spratt DA, Wilson M, Mullany P. Genetic basis of erythromycin resistance in oral bacteria. Antimicrob Agents Chemother. 2004;48:2298-2301.

21. Infectious Diseases Society of America. The 10 x ’20 Initiative: pursuing a global commitment to develop 10 new antibacterial drugs by 2020. Clin Infect Dis. 2010;50:1081-1083.

22. Miró-Canturri A, Ayerbe-Algaba R, Smani Y. Drug repurposing for the treatment of bacterial and fungal infections. Front Microbiol. 2019;10:41.

23. Feng X, Zhu W, Schurig-Briccio LA, Lindert S, Shoen C, Hitchings R, Li J, Wang Y, Baig N, Zhou T, Kim BK, Crick DC, Cynamon M, McCammon JA, Gennis RB, Oldfield E. Anti-infectives targeting enzymes and the proton motive force. Proc Natl Acad Sci U S A. 2015;112:E7073-7082.

24. El Arbi M, Théolier J, Pigeon P, Jellali K, Trigui F, Top S, Aifa S, Fliss I, J, Wang Y, Baig N, Zhou T, Kim BK, Crick DC, Cynamon M, McCammon JA, Gennis RB, Oldfield E. Anti-infectives targeting enzymes and the proton motive force. Proc Natl Acad Sci U S A. 2015;112:E7073-7082.

25. Hussein MH, Schneider EK, Elliott AG, Han M, Reyes-Ortega F, Morris F, Blastovich MAT, Jasim R, Currie B, Mayo M, Baker M, Cooper MA, Li J, Velkov T. From breast cancer to antimicrobial: Combating extremely resistant Gram-negative “superbugs” using novel combinations of polymyxin B with selective estrogen receptor modulators. Microb Drug Resist. 2017;23:640-650.

26. Saeidi Z, Ashjaran A, Dabirsiyaghi SAR. Study of anti-cancer drug release (tamoxifen) of the nanofibers made of poly-caprolactone-chitosan. International J Advanced Biotechnology and Research. 2016;7:997-906. https://bipublication.com/files/ijabr20167304Morteza.pdf

27. Zorgani AA, Belgasim Z, Ziglam H, Ghenghesh KS. Antibacterial susceptibility profiles of Gram-negative Bacilli and Gram-positive cocci isolated from cancer patients in Libya. Archives of Clinical Microbiology. 2012;31-8.

28. Delcour AH. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta. 2009;1794:808-816.

29. Luxo C, Jurado AS, Madeira VM, Silva MT. Tamoxifen induces ultrastructural alterations in membranes of Bacillus stearothermophilus. Toxicol In Vitro. 2003;17:623-628.

30. Steinbuch KB, Fridman M. Mechanisms of resistance to membrane-disrupting antibiotics in Gram-positive and Gram-negative bacteria. Med Chem Comm. 2016;7:86-102.

31. Tripathi PC, Gajbhiye SR, Agrawal GN. Clinical and antimicrobial profile of Acinetobacter spp.: An emerging nosocomial superbug. Adv Biomed Res. 2014;3:13.

32. Dimple R, Nupur S, Mahawal BS, Ankit K, Ajay P. Speciation and antibiotic resistance pattern of Acinetobacter species in a tertiary care hospital in Uttarakhand. Int J Med Res Health Sci. 2016;5:89-96.

33. Kumari M, Batra P, Malhotra R, Mathur P. A 5-year surveillance on antimicrobial resistance of Acinetobacter isolates at a level-1 trauma center of India. J Lab Physicians. 2019;11:34-38.

34. Kittinger C, Kirschner A, Lipp M, Baumert R, Mascher F, Farnleitner AH, Zarfel GE. Antibiotic resistance of Acinetobacter spp. isolates from the river Danube: Susceptibility stays high. Int J Environ Res Public Health. 2017;15:52.

35. Espinal P, Roca I, Vila J. Clinical impact and molecular basis of antimicrobial resistance in non-baumannii Acinetobacter. Future Microbiol. 2011;6:495-511.

36. Poirel L, Madec JY, Lupo A, Schink AK, Kieffer N, Nordmann P, Schwarz S. Antimicrobial resistance in Escherichia coli. Microbiol Spectr. 2018;6.

37. Sáenz Y, Briñas L, Domínguez E, Ruiz J, Zarazaga M, Vila J, Torres C. Mechanisms of resistance in multiple-antibiotic-resistant Escherichia coli strains of human, animal, and food origin. Antimicrob Agents Chemother. 2004;48:3996-4001.

38. Olorunmola FO, Kolawole DO, Lamikanra A. Antibiotic resistance and virulence properties in Escherichia coli strains from cases of urinary tract infections. Afr J Infect Dis. 2013;7:1-7.

39. Annavajhala MK, Gomez-Simmonds A, Uhlemann A. Multidrug-resistant Enterobacter cloacae complex emerging as a global, diversifying threat. Frontiers in Microbiology. 2019;10:1-8.

40. Uzunović S, Ibrahimagić A, Bedenić B. Antibiotic resistance in Enterobacter cloacae strains with derepressed/partially derepressed/inducible AmpC and extended-spectrum beta-lactamases in Zenica-Doboj Canton, Bosnia and Herzegovina. Med Glas (Zenica). 2018;15:35-45.

41. Lima TB, Silva ON, de Almeida KC, Ribeiro SM, Motta D, Maria-Neto S, Lara MB, Filho CR, Ombredane AS, de Faria Junior C, Parachin NS, Magalhães BS, Franco OL. Antibiotic combinations for controlling colistin-resistant Enterobacter cloacae. J Antibiot (Tokyo). 2017;70:122-129.

42. John JF, Jr Sharbaugh RJ, Bannister ER. Enterobacter cloacae: Bacteremia, epidemiology, and antibiotic resistance. Rev Infect Dis. 1982;4:13-28.

43. Khaertynov KS, Anokhin VA, Rizvanov AA, Davydov YD, Semenova DR, Luba SA, Skvortsova NN. Virulence factors and antibiotic resistance of Klebsiella pneumoniae strains isolated from neonates with sepsis. Front Med (Lausanne). 2018;5:225.

44. Garbati MA, Godhair AI. The growth resistance of Klebsiella pneumoniae: the need to expand our antibiogram. Case report and review of the literature. Afr J Infect Dis. 2013;7:8-10.

45. Sanchez GV, Master RN, Clark RB, Fyyaz M, Duvvuri P, Ekta G, Bordon J. Klebsiella pneumoniae antimicrobial drug resistance, United States, 1998-2010. Emerg Infect Dis. 2013;19:133-136.

46. Kidd TJ, Mills G, Sá-Pessoa J, Dumigan A, Frank CG, Ensua JL, Ingram R, Hobløy L, Bengoechea JA. A Klebsiella pneumoniae antibiotic resistance mechanism that subdues host defenses and promotes virulence. EMBO Mol Med. 2017;9:430-447.

47. Madison G, Golamari R, Bhattacharya P. Endocarditis caused by Abiotrophia and Granulicatella species. In: Michael S, ed. Advanced Concepts in Endocarditis. United Kingdom; IntechOpen; 2018:41-58.

48. Cargill JS, Scott KS, Gascoyne-Binzi D, Sandoe JA. Granulicatella infection: diagnosis and management. J Med Microbiol. 2012;61:755-761.

49. Alberti MO, Hindler JA, Humphries RM. Antibiotic susceptibility of Abiotrophia defectiva, Granulicatella adiacens, and Granulicatella elegans. Antimicrob Agents Chemother. 2015;60:1411-1420.

50. Kanamoto T, Terakubo S, Nakashima H. Antibacterial susceptibility of oral isolates of Abiotrophia and Granulicatella according to the consensus guidelines for fastidious bacteria. Medicines (Basel). 2018;5:129.

51. Zheng X, Freeman AF, Villafranca J, Shotridge D, Beyer J, Kabat W, Dembkowski K, Shulman ST. Antibacterial susceptibility of invasive pediatric Abiotrophia and Granulicatella isolates. J Clin Microbiol. 2004;42:4323-4326.
52. Moreno MG, Wang L, De Masi M, Winkler T, Trampuz A, Di Luca M. *In vitro* antimicrobial activity against *Abiotrophia defectiva* and *Granulicatella elegans* biofilms. J Antimicrob Chemother. 2019;74:2261-2268.

53. Savini V, Catavitello C, Masciarelli G, Astolfi D, Balbinot A, Blanco A, Febbo F, D-Amario C, D-Antonio D. Drug sensitivity and clinical impact of members of the genus *Kocuria*. J Med Microbiol. 2010;59:1395-1402.

54. Purty S, Saranathan R, Prashanth K, Narayanan K, Asir J, Sheela Devi C, Kumar Amarnath S. The expanding spectrum of human infections caused by *Kocuria* species: a case report and literature review. Emerg Microbes Infect. 2013;2:71.

55. Dave VP, Joseph J, Pathengay A, Pappuru R. Clinical presentations, management outcomes, and diagnostic dilemma in *Kocuria* endophthalmitis. J Ophthalmic Inflamm Infect. 2018;8:21.

56. Lakshmikantha M, Devki V, Yogesh C. Is *Kocuria kristinae* an upcoming pathogen?. Int J Curr Microbiol App Sci. 2015;4:885-889.

57. Tewar R, Dudeja M, Das AK, Nandy S. *Kocuria kristinae* in catheter associated urinary tract infection: A case report. J Clin Diagn Res. 2013;7:1692-1693.

58. Tsai CY, Su SH, Cheng YH, Chou YL, Tsai TH, Lieu AS. *Kocuria varians* infection associated with brain abscess: a case report. BMC Infect Dis. 2010;10:102.

59. Meletis G, Gogou V, Palamouti M, Spiropoulos P, Xanthopoulou K, Tantou P, Rizou A, Thomoglou V. Catheter-related relapsing peritonitis due to *Kocuria varians* in a patient undergoing continuous ambulatory peritoneal dialysis. Nefrologia. 2012;32:541-542.