Investigation of antimicrobial activities of newly synthesized sulfite based menthol

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Abstract

Antibacterial properties of sulfite drug against bacteria were assessed using dilution method. Bacteriological tests were performed using the initial concentration of (1 -1.5× 10^{-5}) CFU / ml of each type of bacteria. The minimum inhibitory concentration (MIC) of growth and minimum bactericidal concentration (MBC) was determined for each bacteria in the agar medium[2].

The results showed that MIC for bacteria of *E. coli*, *Bacillus cereus*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were at a concentration of 7000, 5000, 3000, and 4000 milligram per milliliter of sulfite, respectively.

Based on the results of this study, bacteria *E.coli* showed the lowest sensitivity and *Staphylococcus aureus* the greatest sensitivity to newly synthesized sulfite based-menthol.

Introduction

Some infectious diseases can be passed from person to person. Some are transmitted by insects or other animals. And you may get others by consuming contaminated food or water or being exposed to organisms in the environment.
Signs and symptoms vary depending on the organism causing the infection, but often include fever and fatigue. Mild infections may respond to rest and home remedies, while some life-threatening infections may need hospitalization.
The invasion and multiplication of microorganisms such as bacteria, viruses, and parasites that are not normally present within the body. An infection may cause no symptoms and be subclinical, or it may cause symptoms and be clinically apparent. An infection may remain localized, or it may spread through the blood or lymphatic vessels to become systemic (bodywide). Microorganisms that live naturally in the body are not considered infections. For example, bacteria that normally live within the mouth and intestine are not infections.
The Centers for Disease Control and Prevention estimates that 2 million patients suffer from hospital-acquired infections every year and nearly 100,000 of them die. Most of these medical errors are preventable. Hospital-acquired infections result in up to $4.5 billion in additional healthcare expenses annually. The U.S. government has responded to this financial loss by focusing on healthcare quality report cards and by taking strong action to curb healthcare spending.
Antibiotics, either are cytotoxic or cytostatic to the micro-organisms, allowing the body's natural defenses, such as the immune system, to eliminate them. They often act by inhibiting the synthesis of a bacterial cell, synthesis of proteins, deoxyribonucleic acid

(DNA), ribonucleic acid (RNA), by a membrane disorganizing agent, or other specific actions.(Levy and Marshall 2011) Antibiotics may also enter the cell wall of the bacteria by binding to them, using the energy-dependent transport mechanisms in ribosomal sites, which subsequently leads to the inhibition of the protein synthesis [2].
To combat against infections or microbes, undoubtedly antibiotics are a blessing to human civilization that has saved millions of people [3]. Multiple varieties of the antibiotics have been used for therapeutic purposes over time. Antibiotics were seen as the 'wonder drug' in the mid20th century. At the time, there was an optimistic belief that communicable disease was nearly coming to a complete halt. The beginning of modern "antibiotic era" was synonymously associated with two names Alexander Fleming and Paul Ehrlich [4]. Antibiotics were considered a magic bullet that selectively targeted microbes that were responsible for disease causation, but at the same time would not affect the host. Fleming was the first who cautioned about the potential resistance to penicillin if used too little or for a too short period of treatment [4]. The period from the 1950s to 1970s was thus considered as the golden era for the discovery of novel antibiotics classes [5].

Millions of metric tons of newer classes of antibiotics have been produced in last 60 years since its inception. Increased demand for antibiotics across many sectors has allowed for less expensive and off-label uses of drugs. Conversely, due to the enormous and irresponsible use of the antibiotics, has contributed significantly to the advent of the resistant strains [6]. In the previous days, the production of new antibiotics was directly proportional to the development of resistant strains. However, the mainstream approach in fighting against the diseases is now focused on the modification of existing antibiotics to combat emerging and re-emerging resistance of pathogens globally [5].
Class of antibiotics
Antimicrobial resistance in bacterial pathogens is a worldwide challenge associated with high morbidity and mortality [2]. Multidrug resistant patterns in Gram-positive and -negative bacteria have resulted in difficult-to-treat or even untreatable infections with conventional antimicrobials. Because the early identification of causative microorganisms and their antimicrobial susceptibility patterns in patients with bacteremia and other serious infections is lacking in many healthcare settings, broad spectrum antibiotics are liberally and mostly unnecessarily used [2]. Dramatic increases in emerging resistance occurs and, when coupled with poor infection control practices, resistant bacteria can easily be disseminated to the other patients and the environment [2]. Availability of updated epidemiological data on antimicrobial resistance in frequently encountered bacterial pathogens will be useful not only for deciding on treatment strategies but also for devising an effective antimicrobial stewardship program in hospitals[2].
Resistance of important bacterial pathogens to common antimicrobial therapies and emergence of multidrug-resistant bacteria are increasing at an alarming rate. There are challenges in the combat of bacterial infections and accompanied diseases and the current shortage of effective drugs, lack of successful prevention measures and only a few new antibiotics in the clinical pipeline will require the development of novel treatment options and alternative antimicrobial therapies [3]. The authors stated that increasing understanding of bacterial virulence strategies and induced molecular pathways of the infectious disease provides novel opportunities to target and interfere with crucial pathogenicity factors or virulence-associated traits of the bacteria while bypassing the evolutionary pressure on the

bacterium to develop resistance [3]. The authors took a closer look at the bacterial virulence-related factors and processes that present promising targets for anti-virulence therapies, recently discovered inhibitory substances, their promises and discussed the challenges and problems that need to be faced [3].
Finding strategies against the development of antibiotic resistance is a major global challenge for the life sciences community and for public health. The past decades have seen a dramatic worldwide increase in human-pathogenic bacteria that are resistant to one or multiple antibiotics [4]. More infections caused by resistant microorganisms fail to respond to conventional treatment, and even last-resort antibiotics have lost their power. Multidrug-resistant bacteria have increased at an alarming rate over recent decades and cause serious problems [6]. The emergence of resistant infections caused by these bacteria has led to mortality and morbidity and there is an urgent need to find solutions to combat bacterial resistance [6].

2. Materials and methods

2.1 Disc-diffusion assay

Antimicrobial activity testing was performed according to the protocol described by Vuddhakul et al. [3] For the experiments, a loopful of the microorganisms working stocks were enriched on a tube containing 3 mL of Mueller-Hinton broth(for bacteria) and Sabouraud Chloramphenical broth (for Yeast strains), then incubated at 37 C for 18-24 h. The overnight cultures were used for the antimicrobial activity of the new sulfa drug used in this study, and optical density was adjusted at 0.5 McFarland turbidity standards with a DENSIMAT (Biom erieux). The inoculums of the respective bacteria and yeast were streaked onto MH or SB agar plates using a sterile swab.
Sterile filter discs (diameter 6.4 mm, Whatman paper N3) were impregnated with 30 µl of new sulfa drug placed on the appropriate agar media (SB, MH and MHþ 1% NaCl). Sulfamethoxazole trimethoprim (1.25/23.75 µg/disc), Imipenem (10 µg/disc), Amikacin (30 µg/disc), Ampicillin (10 µg/disc) and Penicillin(10µg/disc) were used as positive reference standards to determine the sensitivity of one strain/isolate to each of the tested microbial species. Antibiotic susceptibility was determined using the Kirby-Bauer method. [4]. After incubation at 37 C for 18-24 h, the diameter of inhibition zone was measured with 0.1 mm coliseum, and diameters were interpreted according to the Clinical and Laboratory Standards Institute of the antibiogram [5]. The diameter of inhibition zones around each of the discs was taken as measure of antimicrobial activity. Each experiment was carried out in triplicate, and the mean diameter of the inhibition zone was recorded.

2.2 Micro-well determination of MIC, MBC and MFC

Minimal inhibition concentration (MIC), minimal bactericidal concentration (MBC), and minimal fungicidal concentration (MFC) values were determined for all bacterial and fungal strains used in this study as described by Gulluce et al. [6] the inoculums of the bacterial and fungal strains were prepared from 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. 0.001 mol of sulfite was dissolved in one ml double distilled water (DDW) and diluted to the 1 molar concentrations. Serial two-fold dilutions were then prepared in 5 mL sterile test tubes containing nutrient broth. The 96-well plates were prepared by dispensing into each well 95 µl of nutrient broth and 5 µl of the inoculum. A 100 µl aliquot from the stock solutions of sulfite was added into the first

wells. Then, 100 µl from the serial dilutions were transferred into eleven consecutive wells. The last well containing 195 µl of nutrient broth without sulfa drug and 5 µl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 µl. The plates were incubated at 37 C for 18-24 h. The sulfa drug tested in this study was screened two times against each organism. The MIC value was defined as the lowest concentration of the compounds to inhibit the growth of the microorganisms. The MBC and MFC values were interpreted as the highest dilution (lowest concentration) of the sample, which showed clear fluid with no development of turbidity and without visible growth. MBC/MIC and MFC/MIC ratios were also calculated. All tests were performed three times.

3. Conclusion

These compounds based menthol increase bacterial and fungal cell permeability and membrane fluidity and inhibit medium acidification. Moreover, terpenes are thought to be inducing alterations in cell permeability by entering between the fatty acyl chains that make up the membrane lipid bilayers, thus disrupting lipid packing and causing changes to membrane properties and functions [12,13]. The results obtained in the present study showed that gram-positive bacteria were more sensitive than gram-negative bacteria, which is in agreement with some previous reports in the literature [14,15]. Among the tested microorganisms, bacteria, including Gram-positive and Gram-negative strains, were less sensitive to the new synthesized sulfonamide drug[16]. As shown, the obtained data showed that Gram-positive bacteria were more sensitive than Gram-negative ones. These differences could be due to the differences in the cell membrane of those bacterial groups with the presence of the lipopolysaccharides in the outer membrane for Gram-negative bacteria, which makes them inherently resistant to external agents, such as hydrophilic dyes, antibiotics, detergents, and lipophilic compounds [17,18]. Without this barrier, the membrane in Gram-positive bacteria can be permeated more easily and disrupt the proton motive force, electron flow, active transport, and coagulation of the cell contents [19].

References:

1. Organisation for Economic Co-operation and Development, OECD Health Data 2006. from the OECD Internet subscription database updated October 10, 2006. Copyright OECD 2006, http://www. oecd.org/health/healthdata.

2. National Center for Health Statistics. (2002) Health, United States, 2002 with Chartbook on Trends in the Health of Americans, Table 32. In: JW Henderson, ed. Health Economics and Policy. 3rd ed. Boston: Cenage Learning;2005.

3. Healthcare-Associated Infection Working Group of the Joint Public Policy Committee, Essentials of Public Reporting of Healthcare Associated Infections: A Tool Kit. Centers for Disease Control and Prevention Web site. http://www.cdc.gov/ncidod/dhqp/pdf/ar/ 06_107498_Essentials_Tool_Kit.pdf. Accessed Jan 16, 2009.

4. Chaudhuri AK. Infection control in hospitals: has its quality enhancing and cost-effective role been appreciated? J Hosp Infect. 1993;25(1):1–6.

5. Hughes JH. Study on the efficacy of nosocomial infection control (SENIC Project): results and implications for the future. Chemotherapy. 1988;34(6):553–561.

6. Edwards JR, Peterson KD, Andrus ML, et al. National Healthcare Safety Network Report, data summary for 2006, issued June 2007. Am J Infect Control. 2007;35(9):290–301.

7. Levy SB: The challenge of antibiotic resistance. Scientific American. 1998, 278:32–39.
8. Grigoryan L, Burgerhof JG, Haaijer-Ruskamp FM, et al.: Is self-medication with antibiotics in Europe driven by prescribed use?. J Antimicrob Chemother. 2007, 59:152–156. 10.1093/jac/dkl457.
9. Alabi K.A., Owolabi B.J. (2012). Synthesis and antimicrobial property of 2-(2-nitrovinyl)furan. J Pure Appl Microbio. 6, 131-134.
10. Barra F., Roscetto E., Soriano A.A., Vollaro A., Postiglione I., et al. (2015). Photodynamic and antibiotic therapy in combination to fight biofilms and resistant surface bacterial infections. Int J Mol Sci. 16, 20417-20430.
11. Buommino E., Donnarumma G., Scognamiglio M., D'Abrosca B., Fioren□tino A. (2014). Recent advances in natural product-based anti-biofilm approaches to control infections. Mini Rev Med Chem. 14, 1169-1182.
12. Chua S.L., Liu Y., Yam J.K., Chen Y., Vejborg R.M., et al. (2014). Dispersed cells represent a distinct stage in the transition from bacterial biofilm to planktonic lifestyles. Nat Commun. 5, 4462.
13. Fan M., Yan Z., Liu W., Liang Y. (2005). DABCO-Catalyzed reaction of a-ha lo carbonyl compounds with dimethyl acetylenedicarboxylate: a novel method for the preparation of polysubstituted furans and highly func tionalized 2H-Pyrans. J Org Chem. 70, 8204-8207.
14. Gutierrez K., Halpern M.S., Sarnquist C., et al. (2013). Staphylococcal in fections in children, California, USA, 1985-2009. Emerg Infect Dis. 19, 10-20.
15. Hall C.W., Mah T.F. (2017). Molecular mechanisms of biofilm-based antibi otic resistance and tolerance in pathogenic bacteria. FEMS Microbiol Rev. 4, 1-26.
16. Iesce M.R., Sferruzza R., Cermola F., Della Greca M. (2016). Furanyl alco hols as alkylating reagents in Friedel-Crafts Reaction of arenes. Helv Chim Acta. 99, 296-301.
17. Kayumov A.R., Khakimullina E.N., Sharafutdinov I.S. Inhibition of biofilm formation in Bacillus subtilis by new halogenated furanones. J Antibi ot. 68, 297-301.
18. Keay B.A., Hopkins J.M., Dibble P.W. (2008). Furans and their benzo de rivatives: Applications. In Comprehensive Heterocyclic Chemistry III,
19. ed. Katritzky A.R., Ramsden C.A., Scriven F.V., Taylor R.J.K. 571-624. Amsterdam: Elsevier.
20. Li J.J.. (ed. 2013). Heterocyclic Chemistry in Drug Discovery Hoboken-New Jersey: Wiley