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Investigation of antimicrobial activities of newly synthesized sulfonamide based menthol

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Abstract

The need of new antimicrobial agents is justified because more microorganisms are being resistance to the currently available antibacterial drugs and this is bringing alarming threat to public health and causing growing concern among people across the globe. At the same time as the old antibiotics are losing their effectiveness, the supply of new drugs is drying up. Sulfonamides are an important class of synthetic bacteriostatic antibiotics still used today for the treatment of bacterial infections and those caused by other microorganisms[1]. In this article, newly synthesized sulfonamide based-menthol antibacterial activity was studied against Gram-positive bacteria *Staphylococcus aureus* and *Pseudomonas aeruginosa* and Gram-negative bacteria *Escherichia coli* and *Bacillus cereus*.

Antibacterial properties of sulfonamide 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 6000, 4000, 2000, and 3000 milligram per milliliter of sulfonamide, respectively.

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

Keywords: Antibacterial activity, Gram-positive, Gram-negative, Sulfonamide.

Introduction

An infection is the successful colonization of a host by a microorganism. Infections can lead to disease, which causes signs and symptoms resulting in a deviation from the normal structure or functioning of the host. Microorganisms that can cause disease are known as pathogens.

The term antibiotic was coined from the word „antibiosis“ which literally means „against life“. In the past, antibiotics were considered to be organic compounds produced by one

microorganism which are toxic to other microorganisms (Russell, 2004). As a result of this notion, an antibiotic was originally, broadly defined as a substance, produced by one microorganism (Denyer et al., 2004), or of biological origin (Schlegel, 2003) which at low concentrations can inhibit the growth of, or are lethal to other microorganisms (Russell, 2004). However, this definition has been modified in modern times, to include antimicrobials that are also produced partly or wholly through synthetic means. Whilst some antibiotics are able to completely kill other bacteria, some are only able to inhibit their growth. Those that kill bacteria are termed bactericidal while those that inhibit bacterial growth are termed bacteriostatic (Walsh, 2003). Although antibiotic generally refers to antibacterial, antibiotic compounds are differentiated as antibacterials, antifungals and antivirals to reflect the group of microorganisms they antagonize (Brooks et al., 2004; Russell, 2004). Penicillin was the first antibiotic discovered in September 1928 by an English Bacteriologist, late Sir Alexander Fleming who accidentally obtained the antibiotic from a soil inhabiting fungus Penicillium notatum but its discovery was first reported in 1929 (Aminov, 2010), and clinical trials first conducted on humans in 1940 (Russell, 2004; Schlegel, 2003).

The discovery and development of the first significant antibiotic “penicillin” in 1920‟s, and subsequent introduction into man‟s health care system in the 1940‟s has continued to transform the management and fight against bacterial infections (White and Cox, 2013). However, antibiotics are not totally selective in their antibacterial activity. Whilst antagonizing disease causing bacteria, they also antagonize the normal and useful microbiota that we all have and need in our systems as those in the gastrointestinal tract (Walsh, 2003). Prescription and administration of any given antibiotics is therefore predicated on the overall intended benefit, taking into consideration the attendant side effects. For this reason, it is pertinent to understand the mechanism of action of every identified antibiotic before introduction into our health care delivery system, and recent molecular biological approaches have played very significant roles to elucidate our understanding in this regard. Hence this paper aimed to review the classification of antibiotics and their mode of action with emphasis on molecular perspectives.

Some common classes of antibiotics based on chemical or molecular structures include Beta-lactams, Macrolides, Tetracyclines, Quinolones, Aminoglycosides, Sulphonamides, Glycopeptides and Oxazolidinones (van Hoek et al., 2011; Frank and Tacconelli, 2012; Adzitey, 2015)

sulfa drugs are important class of synthetic bacteriostatic antibiotics still used today for the treatment of bacterial infections and those caused by other microorganisms. They were the main source of therapy against bacterial infections before the introduction of penicillin in 1941.

After a few years, bacteria started to develop resistance to the drugs, and eventually penicillin replaced them as a first-line treatment. While antibiotic resistance remains a problem for this class of antibiotics, sulfa drugs are still commonly used to treat a variety of bacterial infections.

Sulfa drugs work by binding and inhibiting a specific enzyme called dihydropteroate synthase (DHPS). This enzyme is critical for the synthesis of folate, an essential nutrient. Mammals get folate from their diet, but bacteria must synthesize this vitamin. Folate synthesis requires a chemical reaction between 2 molecules, DHPP and PABA, that is catalyzed by DHPS.

Although sulfa drugs have for the most part been replaced by other agents, they still maintain considerable action in certain types of infection, for example in the urinary tract, eye and ear, and bronchitis.

2. Materials and methods

2.1 Disc-diffusion assay

Antimicrobial activity testing was performed according to the protocol described by Vuddhakul et al. 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.. 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. 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. 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

This new synthesized drug is a dual drug which is a sulfonamide that linked to menthol. The majority of sulfonamides prevent bacterial reproduction by acting as an antimetabolite to para-aminobenzoic acid (PABA), where PABA is a vital component in the biosynthesis of tetrahydrofolic acid. Competitive inhibition of PABA processing enzymes by sulfonamides ultimately blocks the action of dihydrofolic acid synthetase, and therefore prevents dihydrofolic acid formation .As bacteria are unable to take up tetrahydrofolic acid from their surroundings, inhibition of dihydrofolic acid synthetase will starve the bacteria of thymidine and uridine. These two nucleosides are required for DNA replication and transcription, therefore cell growth and division is disrupted, and thus provides enough time for the body's own immune system to eliminate the bacterial threat.

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