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The assessment of the antimicrobial effect of gemfibrozil alone or in combination with ceftriaxone or gentamycin on several types of bacteria

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ABSTRACT

The present work aimed to investigate the potential antimicrobial action of gemfibrozil alone and in combination with ceftriaxone or gentamycin against specific bacterial strains isolates (*S. aureus, S. epidermidis, Streptococcus spp.,* and *E. coli*) with an evaluation of minimum inhibitory concentration (MIC) values, which revealed that gemfibrozil demonstrated the lowest MIC values against all studied bacterial isolates and the combination of gemfibrozil with either ceftriaxone or gentamycin results in an improvement in the MIC values to levels lower than those obtained with ceftriaxone or gentamycin alone, which revealed that there is a synergistic effect of the gemfibrozil combination with ceftriaxone or gentamycin on antibacterial activity against the studied pathogens, which appear more pronouncedly in the effect of combined antibacterial effect upon *Staphylococcus aureus, Staphylococcus epidermis,* and *Streptococcus spp*. In conclusion, the current study demonstrated a synergistic effect between gemfibrozil and both ceftriaxone and gentamycin, indicating that the combination of these compounds is a potential therapeutic option for treating resistant bacterial strains.

Keywords: ceftriaxone, gemfibrozil, gentamycin, synergism

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INTRODUCTION

The continual demand for novel antibacterial agents for the treatment of bacterial infections that should be effective against multidrug-resistant bacteria has prompted researchers to evaluate a variety of antimicrobial development strategies[1]. The extensive and frequent use of antibacterial agents favors the generation of resistant bacteria, which may cause serious infections[2]. In spite of the availability of antibacterial agents, they may also be associated with a limited number of factors such as side effects, adverse effects, and the emergence of new strains of resistant bacteria[3].

Moreover, resistance to antibacterial agents has led to the generation of worldwide infections, causing an increase in morbidity and mortality, with about thirteen million deaths per year within the past century[4].

Antibiotic-resistant pathogens such as *Staphylococcus* species are the most prevalent multidrug-resistant pathogens in both community and hospital infections, and they are the most prevalent in hospitals[5]. Therefore, it is mandatory to investigate new strategies in the treatment of bacterial infections.

Gemfibrozil is peroxisome proliferator-activated receptor-alpha (PPAR-alpha)[6]. It acts by inducing changes in the biometabolism of fats, subsequently lowering triglyceride (TG) levels and also increasing the level of high-density lipoprotein (HDL)[7]. PPAR-alpha causes activation of the lipoprotein lipase (LPL) in both adipose tissues and muscles, leading to a downregulation of the TG concentration. In addition, gemfibrozil also decreases lipolysis and the removal of free fatty acids via the liver, leading to decreased TG production. Gemfibrozil also inhibits the synthesis and stimulates the catabolism of very-low-density lipoprotein (VLDL)[8]. The reduction of the levels of VLDL can cause a drop in serum TG levels to be decreased by about 30–60 percent. On the other hand, gemfibrozil can also upregulate HDL by different mechanisms[9].

Studies showed that gemfibrozil reduces the export of different organic anions, such as penicillin and quinolone antibiotics, in murine macrophages, so it can increase the intracellular levels of these agents and improve their ability to inhibit the intracellular growth of certain bacterial pathogens, such as Listeria monocytogenes[10]. In addition, gemfibrozil can play an essential role in the potentiation of host immune defense activity against different pathogenic bacteria[11,12]. Especially, it has a significant inhibitory effect on the replication of *Pseudomonas aeruginosa*[13] Furthermore, studies showed that gemfibrozil has a significant effect on the treatment of sepsis associated with acute kidney injury [14]. So, by exploring this system, we can investigate the promoting ef-

fect of gemfibrozil on the antimicrobial action of ceftriaxone and gentamycin antibiotics on several types of bacteria. Gentamicin is an antibacterial agent, considered the prototype of the aminoglycoside group, used in the eradication of various bacterial infections; it acts through binding to the 30s subunit of susceptible bacterial ribosomes, leading to inhibition of protein synthesis[15].

Ceftriaxone sodium, a third-generation cephalosporin[16], has been used to treat different bacterial events; however, it is associated with increased resistance that may contribute to treatment failure[17].

The present study was to investigate the possible direct or adjuvant antibacterial effect of gemfibrozil alone or in combination with Ceftriaxone and Gentamycin in an *in vitro* study to identify its spectrum of action in the treatment of infections with major pathogens such as *Staphylococcus aureus, Staph epidermidis, Streptococcus spp., Pseudomonas aeruginosa,* and *E. coli*.

METHODOLOGY

Microorganisms

Bacterial species, including *Staphylococcus aureus* and *epidermidis, Streptococcus sp., Pseudomonas aeruginosa,* and *Escherichia coli*, were carefully isolated from patients. The samples were collected, then carefully enriched in selective media and examined by microscopic and biochemical identification.

Procedure

The antibacterial activity of gemfibrozil alone, gemfibrozil with ceftriaxone, and gemfibrozil with gentamycin was tested against bacterial isolates (*S. aureus, S. epidermidis, Streptococcus spp.,* and *E. coli*). The agar-well diffusion method was used. and compare the result with the antimicrobial activity of ceftriaxone and gentamycin alone.

1. Prepare a bacterial plate by using Mueller-Hinton agar to measure the inhibition zone.

2. Sterile swabs were used to spread the bacterial inoculum ($1.5x10^8$ CFU/ml McFarland standard) onto Mueller-Hinton agar in three directions.

a. A sterile stainless-steel borer was used to punch out wells (6 mm in diameter) in the plates. There were three wells each containing 0.10 µl of gemfibrozil solution with concentrations of 100, 50, and 25 µg/ml.

The plates were kept at 37^oC for a period of 24 hours, and a ruler was used to measure the inhibitory zone width in mm.

b. Repeat the step (a.) with another prepared bacterial plate for *S. aureus, S. epidermidis, Streptococcus spp.,* and *E. coli*, and put 0.10 µl of gemfibrozil solution with concentrations of 100, 50, and 25 µg/ml separately with a ceftriaxone disc (30 µg) in each well. The plates were also kept at 37°C for a period of 24 hours. A ruler was used to measure the inhibitory zone width in mm.

c. Repeat the step (a.) with another prepared bacterial plate for *S. aureus, S. epidermidis, Streptococcus spp.,* and *E. coli* and put 0.10 l of gemfibrozil solution with concentrations of 100, 50, and 25 µg/ml separately with gentamycin disc (10 µg) in each well; the plates were then kept at 37°C for a period of 24 hours. A ruler was used to measure the inhibitory zone width in mm.

d. With another prepared bacterial plate for *S. aureus, S. epidermidis, Streptococcus spp.,* and *E. coli*, a sterile stainless-steel borer was used to punch out one well (6 mm in diameter) in the plates and put a ceftriaxone disc (30 µg) alone in this well. The plates were kept at 37°C for a period of 24 hours. A ruler was used to measure the inhibitory zone width in mm.

e. with another prepared bacterial plate (*S. aureus, S. epidermidis, Streptococcus spp.,* and *E. coli*). A sterile stainless-steel borer was used to punch out one well (6 mm in diameter) in the plates. Put the gentamycin disc (10 µg) alone in this well. The plates were kept at 37°C for a period of 24 hours. A ruler was used to measure the inhibitory zone width in mm.

Determination of minimum inhibitory concentration (MIC)

The MIC was determined accurately by serial dilutions according to the National Institute of Clinical Laboratory Standards[18]. Briefly, the investigated drugs were diluted serially and then added to well plates containing molten Muller-Hinton Gold II agar. Then, the plates were set aside to cool, then dried thoroughly. Finally, bacterial species were distributed among the wells of a plate using a steer replicator, with each drop containing $5x10^4$ colony units. At the end of an 18-hour incubation at 37°C, well plates are then accurately read. MIC of an antibacterial agent is defined as the lowest concentration of the agent, in milligrams per liter (µg/mL), that completely inhibits the growth of the designated bacterial strain under *in vitro* conditions. Well plates were read in duplicate, and then the highest MIC value was determined. The reference number is indicated in the tables of the National Committee of Laboratories, which were used to decide whether it was susceptibility or resistance.

Chemicals

All drugs used in this study were purchased from Sigma-Aldrich, and prior to MIC testing, they were diluted to a concentration of 1 mg/ml in dimethyl sulfoxide (DMSO). All substances were used in their purest forms. As a surfactant, dimethyl sulfoxide helped the evaluated drugs dissolve better. However, DMSO has zero antibacterial activity and serves as a negative control because of this fact.

Statistics

GraphPad Prism was used to conduct the statistical analysis (version 4.0, GraphPad Software, CA). To test for statistical significance, we used both one-way ANOVA and Tukey's post-hock test. Significant results were defined as p-values ≤ 0.05[19].

RESULTS and DISCUSSION

Results obtained in the current study regarding the antibacterial effect of either Gemfibrozil alone or in combination with ceftriaxone or gentamycin on standard bacterial strains: *Staphylococcus aureus, Staphylococcus epidermis, Streptococcus spp., Pseudomonas aeruginosa,* and *E. coli* revealed that gemfibrozil alone or in combination with either ceftriaxone or gentamycin induced variable degrees of antibacterial action, with ceftriaxone plus gemfibrozil and gentamycin plus gemfibrozil being more potent than either ceftriaxone or gentamycin alone.

Results illustrated in Table 1 and Figure 1 showed that gemfibrozil demonstrated the lowest MIC values against all studied bacterial isolates. The results also showed that the combination of gemfibrozil with either ceftriaxone or gentamycin results in an improvement in the MIC values to levels lower than those obtained with ceftriaxone or gentamycin alone, which revealed that there is a synergistic effect of the gemfibrozil combination with ceftriaxone or gentamycin on antibacterial activity against the studied pathogens, which appear more pronouncedly in the effect of combined antibacterial effect upon *Staphylococcus aureus, Staphylococcus epidermis,* and *Streptococcus spp.*

The antibacterial activity of gemfibrozil was evaluated against five important bacterial strains by measuring MIC values. In Table 1, results showed that various responses of antibacterial activity were induced by bacterial statins, where ceftriaxone plus gemfibrozil and gentamycin plus gemfibrozil were the most potent combinations compared to either gentamycin or ceftriaxone alone ($p \leq 0.05$). Nevertheless, *Staphylococcus aureus, Staphylococcus epidermis,*

and Streptococcus spp. were more sensitive to ceftriaxone plus gemfibrozil compared to gentamycin alone or in combination with gemfibrozil (P≤0.05). In contrary, *Pseudomonas aeruginosa* and *E. coli* showed to be more sensitive to gentamycin plus gemfibrozil compared to ceftriaxone alone or in combination with gemfibrozil (p≤0.05).

Table 1. Minimum inhibitory concentrations (MIC; µg/mL) of different bacterial strains

Bacterial spp	Gemfibrozil	Ceftriaxone	Gentamycin	Ceftriaxone + Gemfibrozil	Gentamycin + Gemfibrozil
Staphylococcus aureus	63.3 ± 16	350 ± 24	143 ± 12	233.6 ± 14	113.5 ± 12
Streptococcus spp	69.9 ± 12	321 ±12	143 ± 16	250 ± 19	80.5 ± 15
Pseudomonas aeruginosa	83.8 ± 22	159 ± 14	243 ± 15	140 ± 12	223.5 ± 5.8
Staphylococcus epidermis	70 ± 10	388 ± 12	113 ± 17	310 ± 19	70.9 ± 14
Escherichia coli	130 ± 13	190 ±15	290 ± 16	180 ± 11	270.4 ± 24

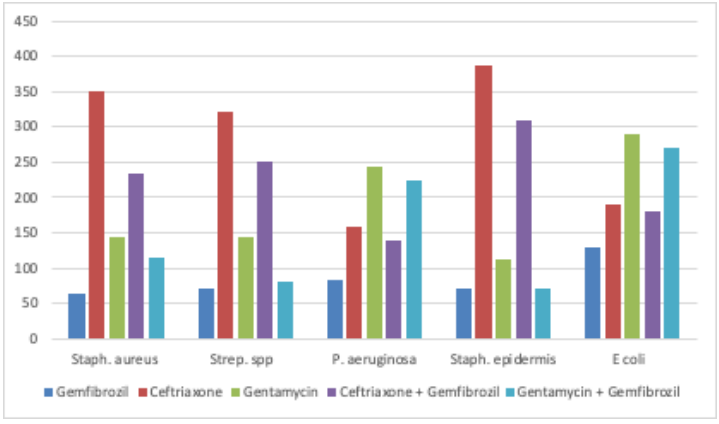

Figure 1. Minimum inhibitory concentrations (MIC; µg/mL) of different bacterial strains

The emergence of drug resistance, antibacterial adverse effects, and poor patient compliance indicates a potential need for a therapy regimen with similar or higher antibacterial beneficial activity yet with fewer side effects. Studies involving combinations for synergism have been prescribed as mandatory for multidrug-resistant bacteria species[20]. Therefore, there is an urgent need to investigate new antibacterial modalities. Results of this study showed potential antibacterial effects for gemfibrozil and indicated the superior antibac-

terial effect of adding gemfibrozil to both ceftriaxone and gentamycin compared with both of them alone. Gemfibrozil is a member of the fibrate group of medications, which were used to lower cholesterol and plasma triglycerides 40 years ago[20]. It is an agonist of the peroxisome proliferator-activated receptors (PPARs)[22]. This agent has recently been demonstrated to have an additional effect through reducing inflammation, decreasing serum interferon and tumor necrosis factor (TNF)[23], as well as interleukin-6 (IL-6)[24].

Gemfibrozil also produces a variety of anti-inflammatory cytokines, such as IL-4, and protects against autoimmune encephalitis[25]. Furthermore, gemfibrozil inhibits TNF, IL-1, IL-6, and nitric oxide[26]. In addition to its antihyperlipidemic effects, gemfibrozil was shown to have other, related effects, including an anticoagulant property, antioxidative activity, and immunomodulatory effect[27,28]. The current study indicated the antimicrobial effect of gemfibrozil against *Staphylococcus aureus, Staphylococcus epidermis, Streptococcus spp., Pseudomonas aeruginosa,* and *E. coli.* Results of the present study also showed that the use of gemfibrozil could potentially improve the antibacterial activity of both ceftriaxone and gentamycin. The antibacterial action of the investigated drugs was validated through the measurement of MIC values against the bacterial species involved in this study. As mentioned earlier, MIC of an antibacterial agent is defined as the lowest concentration of the agent, in milligrams per liter (μg/mL), that completely inhibits the growth of the designated bacterial strain under *in vitro* conditions[29].

Other studies showed that gemfibrozil noncompetitively abolished the growth of Legionella pneumophila and Mycoplasma tuberculosis by inhibiting their enoyl reductases[10]. It is well known that bacterial fatty acid synthesis occurs within membrane phospholipid synthesis, so substances that inhibit fatty acid synthesis will inhibit bacterial growth[30]. Moreover, gemfibrozil could aid the action of ceftriaxone and gentamycin through their reported pleiotropic actions[31]. Additionally, our study showed that gemfibrozil plus ceftriaxone or gentamycin was superior to both drugs alone. These distinct actions could be related to the antimicrobial effect of gemfibrozil, which is unrelated to its lipid-lowering action[32]. The MIC value for gemfibrozil plus ceftriaxone was lower for *E. coli* than pseudomonas strains as compared with gemfibrozil plus gentamycin, while the first combination was better against *Staphylococcus aureus, Staphylococcus epidermis,* and *Streptococcus spp*.

In conclusion, current study demonstrated synergistic activity between gemfibrozil and both ceftriaxone and gentamycin. The results depicted that the combination of these compounds is a potential therapeutic option for treatment resistant bacterial strains. This combination has essentially to be studied in pharmaceutical industry and clinical studies.

STATEMENT OF ETHICS

Not applicable.

CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

All authors have contributed equally to the conception, drafting, and critical revision of the manuscript, and approve of the final version to be published.

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