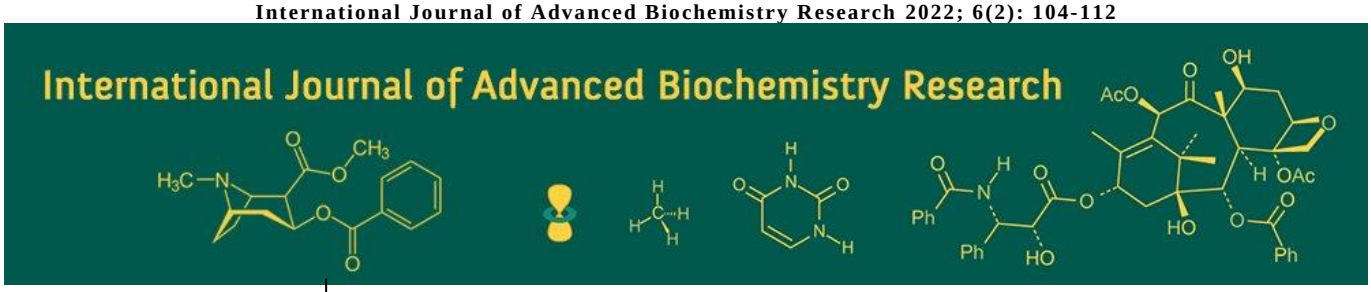

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Study the effect of antioxidants and ROS levels on antibacterial activity of some of modified pyrimidine derivatives

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Abstract
Recently, it was postulated that antibacterial substances kill bacteria by a common mechanism involving the formation of reactive oxygen species, in addition to particular drug-target interactions (ROS). However, there is a lot of controversy about this mechanism that produces hydroxyl radicals. Different experimental approaches are anticipated to be the root of the inconsistent results because the role of ROS to antibiotic-mediated death most likely varies on the circumstances. In the current work, the bacteria strains Escherichia coli, *Sarcina lutea*, Bacillus cereus, and Proteus mirabilis were treated with nucleoside-based compounds, and the production of ROS was measured using the markers araC, araCMP, TTU, and cCMP. It was shown over times that, the formation of intracellular reactive oxygen species (ROS) was increased by the examined modified pyrimidine nucleoside derivatives (validated via DCFA-DA probe assay). For instance, after treatment with araC and cCMP but not after treatment with araCMP and TTU, an increase in the ROS was detected in E. coli. Results also vary depending on the species studied and the experimental setup. Despite this, our data strongly imply that using antioxidants as therapeutic agents to treat some infections is a viable option that is starting to be used against bacterial strains.

Keywords: antioxidants, antibacterial activity, pyrimidine derivatives

Introduction
Reactive oxygen species (ROS) are a byproduct of aerobic respiration that are a normal cons equence [1]. These ROS are produced through repeated single-electron reductions and have the potential to harm lipids, proteins, and DNA, ultimately resulting in cell death. Aerobic ba cteria are equipped with enzymes that may detoxify ROS, such as catalases and superoxide d ismutases, as well as regulatory systems, including as SoxRS, OxyRS, and SOS regulons, to protect themselves from the harmful effects of ROS [2].

It's interesting to note that all bactericidal drugs can cause cell death via a common mechanism that Kohanski *et al*. discovered in 2007 [3]. This mechanism involves the creation of hydroxyl radicals. Actually, a mechanism is has been put out whereby bacterial membrane disruption causes envelope stress, which then causes the Arc regulating system to become perturbed and speed up respiration [4]. Superoxide and hydrogen peroxide are produced as a result of the hyperactivation of the electron transport chain, which harms iron-sulphur clusters and releases ferrous iron as a result. In the Fenton reaction, this iron can then combine with hydrogen peroxide to create hydroxyl radicals that can either harm DNA, lipids, and proteins directly or oxidize the deoxynucleotide pool and indirectly damage DNA. However, there is now a lot of discussion surrounding this notion [5-8]. Additionally, it was discovered that defense against ROS improves the viability of bacterial cells both before and after treatment with oxidizing agents and antibiotics [2, 13]. *In vitro* and in an experimental setting with bacterial peritonitis, antioxidants have a multidirectional impact on the effectiveness of antibacterial medicines. Preliminary *in vitro* experiments should be conducted in conjunction with the combination of antibiotics and antibacterial agents. Anti-infective chemotherapy is more successful and resistant strains from forming when antibacterial and antioxidant drugs are used in a sensible way.

Although antibiotics have received the majority of the attention in research examining the role of ROS in antibiotic-mediated death, there are other intriguing types of antimicrobial drugs, such as nucleoside-based compounds [7]. The kind and position of the substituents determine how effectively nucleoside analogs are as antibacterial agents. The enormous interest in developing antibacterial medications goes beyond just considering traditional methods; as new technologies emerge, new methods are also being developed [1-5]. A major issue for humanity is the development of drug resistance in harmful microbes as a result of the extensive, occasionally unnecessary use of antibiotics. The creation of novel antimicrobial agents has thus been and will continue to be important [6-9].

Natural compound-based medication development is a tried-and-true classic strategy. About 100 medicines have been developed thus far using nucleosides, half of which are antiviral and 25% of which are antitumor [10]. The structure of natural nucleosides is diverse; they are found in coenzymes, DNA, RNA, and nucleotides. tRNA yielded more than 140 minor nucleosides, while various natural sources yielded roughly 100 disaccharide nucleosides and 200 nucleoside antibiotics, the structures of which contain extra functional groups and hydrophobic residues. There are around 600 compounds in the library of natural nucleosides, which are used to build novel physiologically active molecules [11].

In connection with the recognition of the universal role of strengthening the processes of free radical oxidation in the development of inflammation of infectious etiology, the additional appointment of antioxidants is pathogenetically justified. Bacterial infection is accompanied by increased generation of reactive oxygen species that damage biomolecules and make a significant contribution to the development of cellular metabolism disorders, tissue and organ dysfunction [6]. At the same time, the bactericidal effect of many antibacterial agents has a common mechanism associated with the generation of OH-radicals and the development of oxidative stress in bacterial cells [8]. In this regard, antioxidants can reduce the effect of such drugs and, accordingly, reduce the effectiveness of treatment. It is also impossible to exclude the possibility of direct chemical interaction of antibacterial agents and antioxidants.

The aim of our work is to study the interaction between particular antioxidants (quercetin) and nucleoside–based compounds with promising antibacterial properties (2-F-araA, 2-F-araAMP, and NH_2-6-Cl-araPur) *in vitro.*

Materials and Methods

The used nucleosides and nucleotides were synthetized and characterized as described in our previous articles.

Bacteria strains and culture

Sarcina lutea, Bacillus cereus, Escherichia coli, and *Proteus mirabilis* were the bacterial strains used in the study. Different bacterial strains' colonies were transferred aseptically into a conical flask with 10 mL of MHB and a cap, where they were cultured at 37 °C overnight. Cells were centrifuged at 6000 rpm for 5 min after 18–24 h of incubation, the supernatant was discarded, and the cell pellet was then re-suspended in PBS before centrifugation. Following the removal of debris, a clean bacterial solution was obtained, and cells were then suspended in MHB. By using a UV-Visible spectrophotometer, the absorbance of the produced bacterial suspension was measured at 600 nm (OD600). The cells were modified to have an OD600 between 0.15 and 0.2, which was regarded as having 108 cells/mL of cells. To assess the action of nucleosides and nucleotides, this suspension was further diluted to a concentration of 107 cells per milliliter.

Resazurin reduction assay

The 96-well plates used for the resazurin metabolization tests were used as instructed [Travnickova *et al*. AMB Expr (2019) 9:183] [15]. In phosphate buffered saline, 200 L of resazurin at a concentration of 20 mol L1 were combined with a volume of 10 L of each suspension concentration (PBS). Using a multi-detection microplate reader, Synergy 4, the fluorescence (RFU) of microbially produced resorufin was recorded at ex = 520 nm/em = 590 nm after 60 min (BioTek Instruments Inc., USA). The mean and standard deviation (SD) of each concentration level were computed. In comparison to control wells without any compounds, the survival percentage for wells containing nucleosides/nucleotides was determined.

Detection of reactive oxygen species (ROS)

Utilizing the marker 2′-7′-dichlorodihydrofluorescein diacetate (DCFH-DA) (Sigma-Aldrich, UK), which can identify a variety of ROS including nitric oxide and hydrogen peroxide, after being exposed to altered nucleosides and nucleotides, it was examined whether or not certain bacterial strains produced ROS [12]. In the presence of DCFH-DA at a final concentration of 5 M in 0.85 percent saline, the modified bacterial culture (0.5 percent McFarland exponential phase bacteria culture) was exposed to various concentrations of the examined compounds and incubated at 37 °C aerobically for 24 hours. A negative control was provided in the form of untreated bacterial culture. The fluorescence emission of DCFH-DA was found at 525 nm using a Tecan microtiter plate reader with an excitation wavelength of 485 nm [13]. To determine the net fluorescence produced by the experiment, we assessed the autofluorescence of the bacterial cells grown without the probe and the background fluorescence of 0.85 percent saline. Three times the experiment was run.

Statistical analysis

Bacterial survival data and associated nucleosides/nucleotides concentrations from resazurin and plating were then fit to a log-logistic model with four parameters (b, c, d, e) LL.4 using R (Graph Pad Software, Inc.), affording the dose-response curves:

$$\varphi(\mathrm{x}) = c + \frac{d - c}{1 + e^{b\,(\log x - \log e)}}$$

The computed model parameters have a clear physical meaning. The parameters c and d specifically establish the lower and upper horizontal asymptotes of the sigmoid curve for the log-logistic model, e identifies the location of the inflection point, and d identifies the angle of inclination in the transition area. The generalized method of minimizing the sum of squares of deviations of model forecasts from the observed values, taking into account specifically chosen

weight coefficients, was used to fit the model parameters to the studied empirical data.

Using Student's t-test, which evaluated the idea that each coefficient is equal to zero and generated p-values to indicate the level of significance attained, the estimated parameters were statistically analyzed. The whole model's statistical significance was confirmed by contrasting it with an ANOVA-based simple regression with a slope coefficient of zero (the horizontal regression line indicates the absence of dose-effect correlation).

Results and discussion

Killing kinetics were used to assess the impact of various concentrations of modified nucleosides/nucleotides araC, araCMP, TTU and cCMP with or without antioxidant against different bacteria strains, e. g. *E. coli*, and *P. mirabilis* (gram-negative, facultative anaerobes), as well as *S. lutea* (gram-positive, obligate aerobe), and *B. cereus* (gram-positive, facultatively anaerobe) bacterial strains for 24 h.

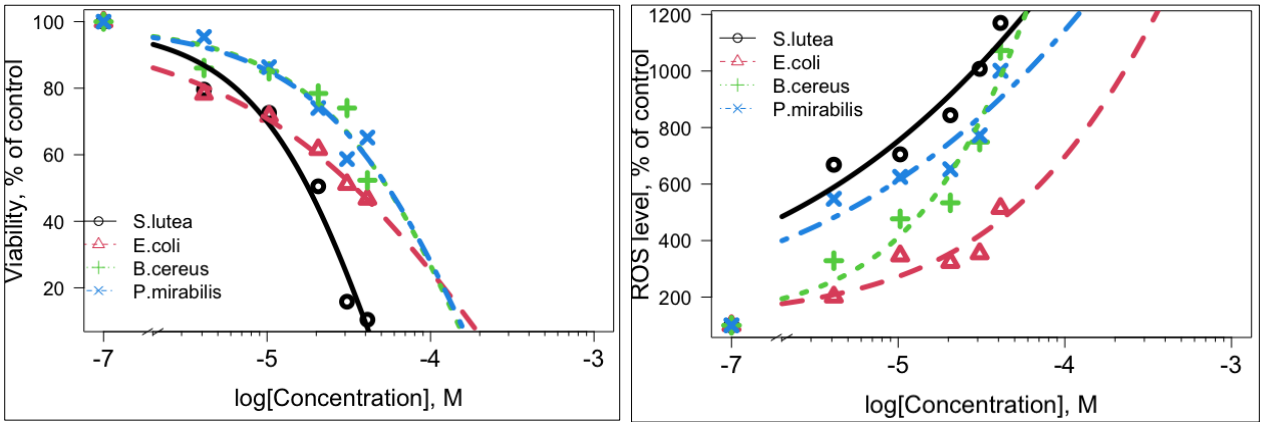

Fig 1: Effect of different concentrations of araC without antioxidant against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

Figure 1A showed that modified nucleoside araC achieved the same maximum effect against all strain *S. lutea*, *B. cereus*, *P. Mirabilis* and *E. Coli*, i.e. it had equal efficacy against all used bacterial strains. Anyway, araC achieved this effect at lower dose in case of action on the gram-positive strains *S. lutea* (ED_{50} = 5.5 * 10^{-4} M), compared to the other gram positive strain and *B. cereus* (ED_{50} = 2.1 * 10^{-3} M) and two gram negative strains *P. mirabilis* strain (ED_{50} = 2.2 * 10^{-3} M) and *E. coli* (ED_{50} = 3.3 * 10^{-3} M). The shape of dose-effect curve for all bacteria strain were not differed from each other.

As for figure 1B, we found a strong match between efficacy and potency of araC to bacteria cells growth inhibition and level of intracellular ROS burst after cells treatment in the same conditions. Actually, the lowest ROS level growth (500% compared to control without araC) was detected in case of the most resistant *E. coli* bacteria strain.

In the same time, both the most sensitive gram-positive strains *S. lutea*, and *B. cereus* showed the 12-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of araC (4.03 * 10^{-5} M and 4.01 * 10^{-5} M, respectively).

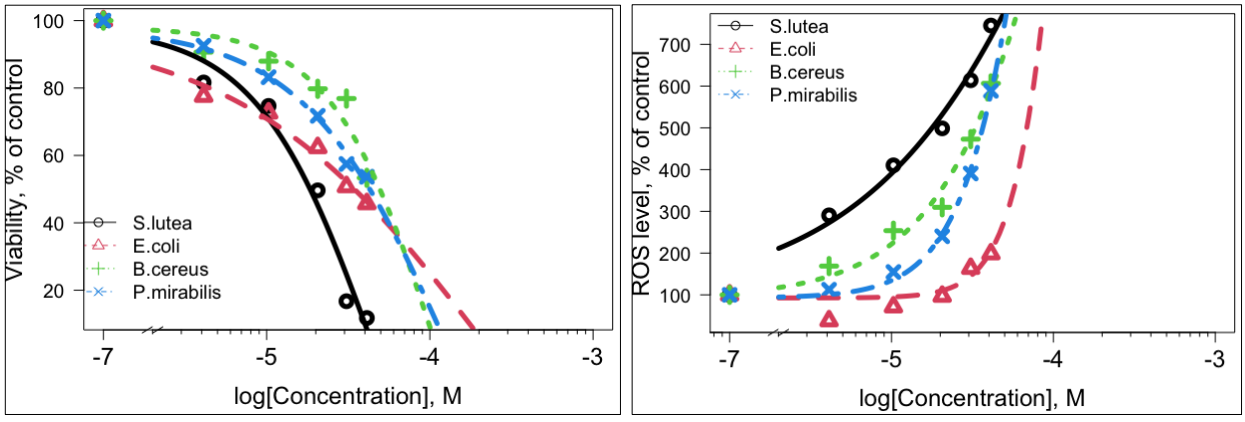

Fig 2: Effect of different concentrations of araC with equimolar antioxidant concentrations against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

In the study of the effect of antioxidant of the activity of araC, we note that the presence of antioxidant in the reagent mixture hasn't changed the dose-effect relationships in general, gram-positive and gram bacteria strains still have sensitives to araC, but we observed the decrease of ED_{50} values (i.e. increase of efficiency) in case of *S. lutea* 4.6 * 10^{-4} M, while the potency of araC against *B. cereus*, *E. coli* and *P. mirabilis* strains almost hasn't changed (ED_{50} = 1.1 * 10^{-3} M and 2.3 * 10^{-3} M, and 1.3* 10^{-4} M respectively).

The values of ROS levels in all bacteria strains after antioxidant adding have changed more o. The most resistant bacteria strain *E. coli* showed 2.5-fold decrease of intracellular level after treatment with araC combined with quercetin at the highest used concentration 4.1 * 10^{-4} M. The

same 1.5-1.8-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to araC bacteria strain was, the higher ROS level at the highest used compound concentration was showed.

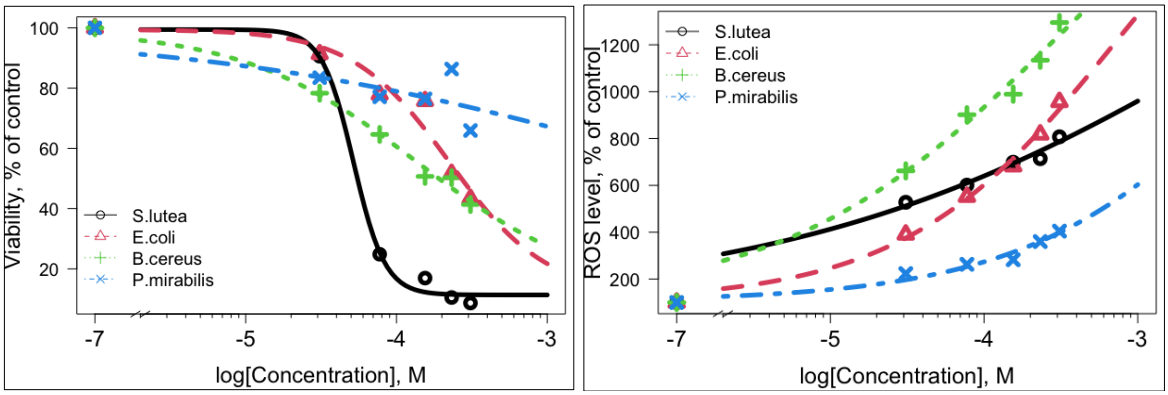

Fig 3: Effect of different concentrations of araCMP without antioxidant against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

According to (Figure 3A), the maximum effect modified nucleoside araCMP against all bactetial strains were same. araCMP had equal efficacy against both gram positive and gram negative strains. Anyway, araCMP achieved this effect at lower dose in case of action on gram-positive strains *S. lutea* (ED_{50} = 5 * 10^{-5} M), while *B. cereus* (ED_{50} = 1 * 10^{-4} M) compared to gram-negative strains *E. coli* bacteria strain ED_{50} = 2.2 * 10^{-4} M. The shape of dose-effect curve for *P. mirabilis* bacteria strain differed from all other used in experiments bacteria strains with calculated value of (ED_{50} = 7.3 * 10^{-3} M)what was closer to the value for another gram-negative bacteria strain.

For ROS levels, we found a strong match between efficacy and potency of araCMP to bacteria cells growth inhibition and level of intracellular ROS burst after cells treatment in the same conditions (figure 3B). The results showed that lowest ROS level growth (400% compared to control without araCMP) was detected in case of the most resistant *P. mirabilis* bacteria strain.

In the same time, the most sensitive gram-positive strains *B. cereus* showed the 12-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of araCMP (3 * 10^{-4} M).

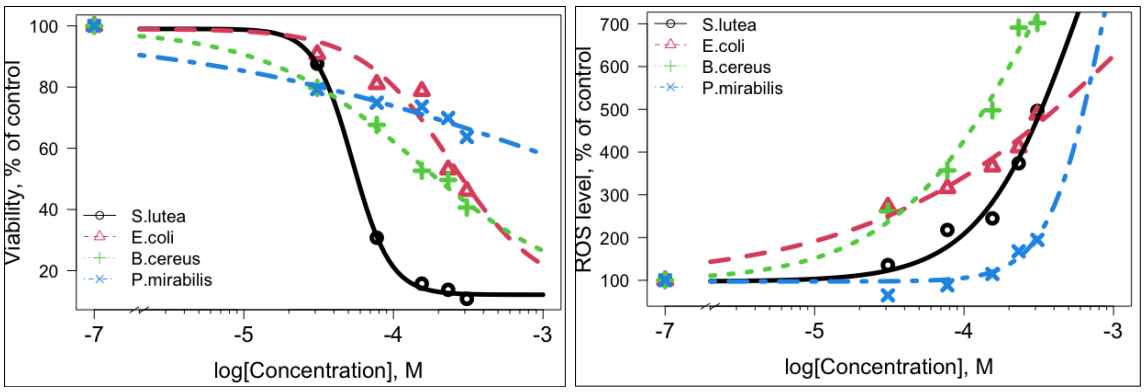

Fig 4: Effect of different concentrations of araCMP with equimolar antioxidant concentrations against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

Similar results to what was done previously for the presence of antioxidant in the reagent mixture, where antioxidant hasn't changed the dose-effect relationships in general, gram-negative and gram-positive bacteria strains remained have sensitive to araCMP, but we also observed the increase of ED_{50} values in case of all bacterial strains where S. *lutea* 5 * 10^{-5} M, *B. cereus* 1.4*10^{-4} M *E. coli* 2.4 * 10^{-4} M, and *P. mirabilis* 1.4 * 10^{-3} M, respectively).

The values of ROS levels in all bacteria strains after antioxidant adding have changed more dramatically. The most resistant bacteria strain *P. mirabilis* showed 2-fold decrease of intracellular level after treatment with araCMP combined with quercetin at the highest used concentration 2.7 * 10^{-4} M. The same 1.6-1.8-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to araCMP bacteria strain was, the higher ROS level at the highest used compound concentration was detected.

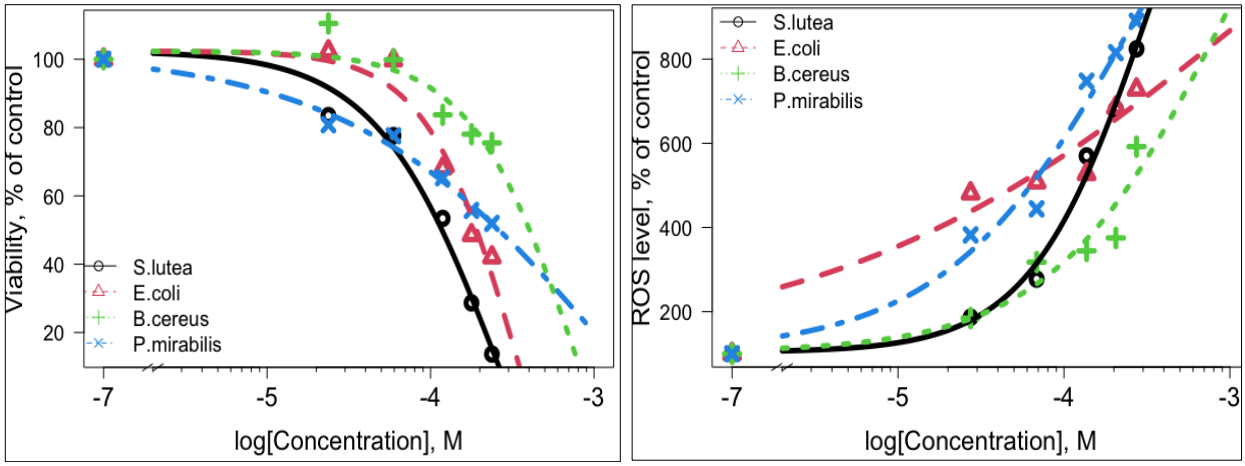

Fig 5: Effect of different concentrations of TTU without antioxidant against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

From (figure 5A), also we can observe that the maximum effect modified nucleoside TTU against all bactetial strains *S. lutea*, *B. cereus*, *P. Mirabilis* and *E. Coli* were same, i.e. it had equal efficacy against all the used strains.. However, TTU achieved this effect at lower dose in case of action on *S. lutea* (ED_{50} = 2.5 * 10^{-4} M) and *E. coli* strain (ED_{50} = 3.4 * 10^{-4} M) compare to *B. cereus* (ED_{50} = 7.9 * 10^{-4} M) and *P. Mirabilis* (ED_{50} = 1.4 * 10^{-3} M). The shape of dose-effect curve for *all* bacteria strain were same each other.

Next, we found a strong match between efficacy and potency of TTU to bacteria cells growth inhibition and level of intracellular ROS burst after cells treatment in the same conditions (figure 5B). Indeed, the lowest ROS level growth (400% compared to control without TTU) was detected in case of the most resistant *B. cereus* bacteria strain.

In the same time, both the most sensitive gram-positive strains *S. lutea*, and *P. Mirabilis* showed the 9-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of TTU (4 * 10^{-4} M).

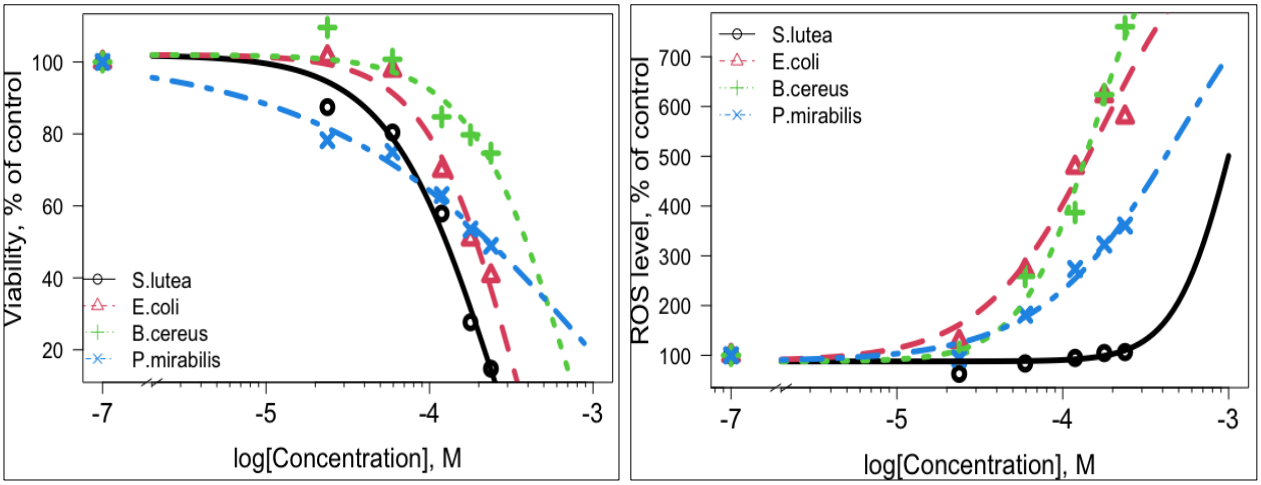

Fig 6: Effect of different concentrations of TTU with equimolar antioxidant concentrations against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

In general, the existence of antioxidant in the reagent mixture doesn't changed the dose-effect relationships, gram-positive and gram negative bacteria strains showed the same sensitive to TTU. ED_{50} values were exactly the same as what was described in(figure 6A).

The upsides of ROS levels in all bacteria strains after added antioxidant have changed more decisively. The most resistant bacteria strain *S. lutea* showed 8-fold decrease of intracellular level after treatment with TTU combined with quercetin at the highest used concentration 6 * 10^{-3} M. The 1.5-2.25-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to TTU bacteria strain was, the higher ROS level at the highest used compound concentration was detected.

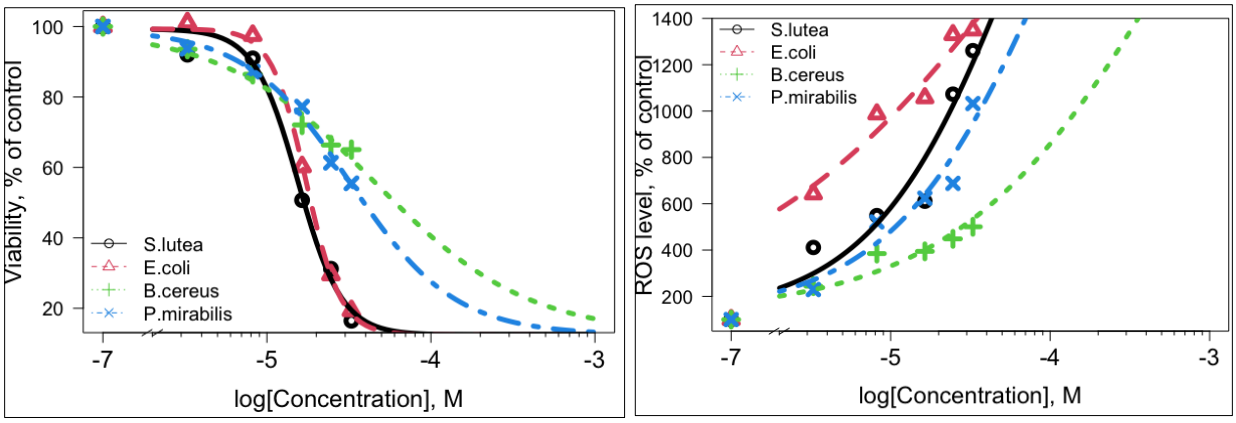

Fig 7: Effect of different concentrations of cCMP without antioxidant against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

Same to others modified pyrimidine derivatives, cCMP achieved the same maximum effect against all strain *S. lutea*, *B. cereus*, *P. Mirabilis* and *E. Coli*, i.e (figure 7). it had equal efficacy against that four strains but not. However, cCMP achieved this effect at lower dose in case of action on *S. lutea* (ED_{50} = 1.5 * 10^{-4} M) and *E. coli* strain (ED_{50} = 1.7 * 10^{-4} M), *P. Mirabilis* (ED_{50} = 3.1 * 10^{-3} M), and *B. cereus* (ED_{50} = 4.5 * 10^{-3} M). The shape of dose-effect curve for *all* bacteria strain were same each other.

Next, we found a strong match between efficacy and potency cCMP to bacteria cells growth inhibition and level of intracellular ROS burst after cells treatment in the same conditions (figure 7B). Indeed, the lowest ROS level growth (500% compared to control without TTU) was detected in case of the most resistant *B. cereus* bacteria strain.

In the same time, both the most sensitive gram-positive strains *E. Coli* showed the 14-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of cCMP (5 * 10^{-4} M).

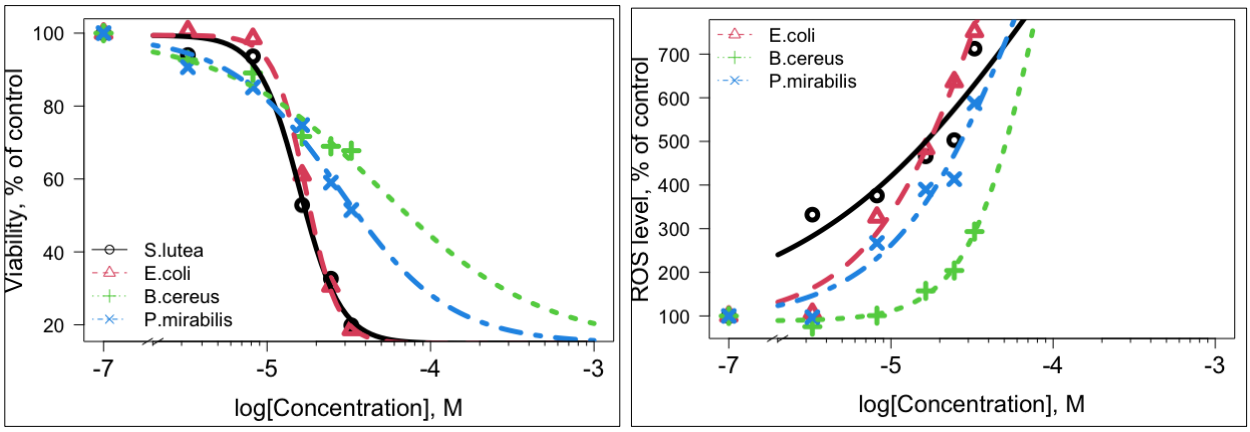

Fig 8: Effect of different concentrations of cCMP with equimolar antioxidant concentrations against exponential phase *E. coli*, *S. lutea*, *B. cereus*, and *P. mirabilis* (incubated aerobically) at 37 °C for 24 h

As it can be noted that the existence of antioxidant in the reagent mixture doesn't changed the dose-effect relationships, gram-positive and gram negative bacteria strains showed the same sensitive TTU. ED_{50} values were exactly the same as what was described in(figure 8A).

The upsides of ROS levels in all bacteria strains after added antioxidant have changed more decisively. The most resistant bacteria strain *B. cereus* showed 1.6-fold decrease of intracellular level after treatment with cCMP combined with quercetin at the highest used concentration 3.3 * 10^{-4} M. The 1.75-1.85-fold decrease of ROS levels was detected in case of all other bacteria strains, but what more important is that the dose-activity relationships remained the same, i.e. the more sensitive to cCMP bacteria strain was, the higher ROS level at the highest used compound concentration was detected.

Discussion

Due to chronic underfunding in anti-infective medication improvement, declining appeal of vaccines, and the increased prevalence and severity of treatment resistance, infectious illnesses may be said to be making a comeback [12, 13]. The majority of antibacterial, antifungal, and antiparasitic medications are decades old, and the continued decline in research and development jeopardizes the capacity to treat numerous infectious diseases. Even when new treatment methods are suggested, they are frequently created from existing antimicrobial agents, such as new penicillins, tetracyclines, diamidines, minor groove binders, etc. Although such tactics can (temporarily) prevent resistance, it was a feature of the strategy that resistance to the compound's class was already widespread in the microbial populations targeted.

One class of medicine that are critical from a scientific angle is nucleoside analogues, a pharmacologically numerous elegance of drugs that arose from chemically changed natural ribose or 2′-deoxyribose nucleosides [14]. Nucleoside analogues are a few of the most essential pills in the medical putting and are used widely as both anticancer and antiviral marketers [15]. By taking advantage of mobile metabolism,

nucleoside analogues resemble endogenous nucleosides and are integrated into both DNA and RNA. Their structural similarity to nucleosides and nucleotides worried in number one metabolism endows purine or pyrimidine nucleoside antibiotics with particular biochemical houses and talents; accordingly, those herbal merchandise can regularly be extraordinarily influential to the internal workings of dwelling organisms. Now not exceedingly, extensive effort has been directed to developing purine nucleosides natural merchandise and derivatives as pills. Indeed, a number of such compounds have visible clinical use for decades. As an example, carbocyclic nucleoside analogues, compounds wherein a methylene institution replaces the oxygen atom inside the farinose sugar moiety, have a prominent records as anti-infectious sellers, along with the food and Drug management (FDA)-authorised antiviral drugs abacavir, entecavir, and lobucavir, as well as the certainly taking place neplanocin and aristeromycin [16, 17, 18].

Its miles recognized that the primary mechanism of the harmful impact on eukaryotic cells below management of antimetabolites is the immoderate accumulation of reactive oxygen species as a result of activation of microsomal oxidation, respectively. The outcome of this is harm to the functioning of the antioxidant protection gadget (which include its enzymatic and non-enzymatic links). In this regard, we assessed the level of reactive oxygen species formed inside the bacteria cells under cultivation situations with changed purine nucleosides/nucleotides.

In this work we analyzed the pastime of a few modified purine nucleotides/nucleosides in opposition to one of a kind bacteria lines, e.G. *E. Coli* (gram-negative, facultative anaerobe), *S. Lutea* (gram-posotive, obligate aerobe), *B. Cereus* (gram-positive, facultatively anaerobe), and *P. Mirabilis* (gram-negative, facultative anaerobe). The section of exponential increase of bacterial culture was used on this work. Exponential section culture consists of actively developing cells which consume comfortably available oxygen and nutrients for growth.

The gram-negative bacterial celss wall lipopolysaccharide coat (LPS) gives a few safety from the toxic consequences of exogenous agents [19]. These microorganisms are able to thrive in places that would normally be considered unfriendly, such as the intestines of mammals, thanks to this capacity. LPS has previously been demonstrated to operate as a physical or chemical barrier that prevents ROS produced outside of cells from interacting with a critical target, together with membrane or cytoplasmic additions [20]. As a result, certain lines that are unable to deliver a significant portion of the LPS have shown more susceptibility to exogenous ROS than lines that are still able to do so. The majority of gram-positive bacteria don't have a protective structure like the gram-negative LPS or the outer membrane where it is fixed. This outer membrane, which is made up of unsaturated fatty acids and proteins that are substances known to chemically react with ROS, may operate as both a structural barrier to penetration and a chemical lure for ROS [21]. Since they can be eliminated without harming the cells, the outer membrane and LPS of gram-negative microorganisms nevertheless serve as significant targets for the fatal migration of ROS (Spheroplast formation). When the barrier is crossed with the help of ROS, the goals and mechanisms for cell killing for both gram-high quality and gram-bad bacteria can be expected to be similar or the same because the cellular wall shape of gram-tremendous and gram-bad bacteria represents the fundamental distinction between those cells.

Carotenoid pigments are acknowledged to physically quench ROS [22] and to defend micro-organism towards the deadly outcomes of photosensitization, whether by means of endogenous or exogenous photosensitizers [23]. Mathews-Roth and co-people [24] have correlated the protective results of carotenoids in opposition to photosensitization and singlet oxygen lethality in micro-organism. Carotenoids also had been observed to shield *Sarcina lutea* from killing by using leukocytes, probably by way of quenching singlet oxygen [25]. Administration of the carotenoid, -carotene has also been observed to shield mice from lethal exposure to hematoporphyrin by-product and mild and, in people, to mitigate the photosensitivity related to erythropoietic protoporphyria [26]. We've blanketed for look at a bacteria strain that produces high ranges of carotenoid pigments in order to verify what protective consequences the carotenoids may have against killing of those cells with the aid of publicity to pure exogenous ROS.

Antioxidants molecules work to reduce ROS. Antioxidants include substances that block the processes that produce ROS, directly scavenge ROS, and obstruct the routes that cause ROS to be degraded. ROS scavengers, NOX2 inhibitors, blockers of several ROS-generating pathways, and nuclear factor are examples of common antioxidants (erythroid-derived 2) the NRF2-like 2 (NRF2) activators, a class of chemicals that stimulate the expression of antioxidant enzymes and are therefore categorized as indirect antioxidants. Although NRF2-activators have recently piqued people's interest. The majority of study has focused on ROS-scavengers, which include N-acetyl-cysteine (NAC), a glutathione regenerating antioxidant. Many NRF2-activators are referred to as "nutraceuticals," naturally occurring food compounds with claimed health benefits. These include resveratrol, which is found in wine, pterostilbene, which is found in blueberries, sulforaphane, which is found in broccoli, curcumin, which is found in turmeric, cafestol, which is found in coffee, quercetin, which is found in red onions, and epigallocatechin-3-gallate (found in green tea), and carn Tert-butylhydroquinone, a food preservation ingredient, is a strong NRF2 activator. The medication cobalt-protoporphyrin (CoPP), which is frequently employed in experimental studies, has the ability to trigger the production of heme-oxygenase 1 (HO-1) via activating NRF2. However, NOX2 inhibitors have received less attention, perhaps as a result of their less focused effects on NOX family proteins. The most researched NOX2 inhibitor, apocynin, is derived from vanillin and is benign, however it is not yet used in medical settings [28].

Constitutive antioxidant defenses often negate the housekeeping creation of ROS. When antioxidant defenses are overpowered by ROS generation, oxidative stress results. The oxidative damage then encourages the separation of NRF2 from kelch-like ECH-associated protein (Keap), enabling NRF2 to go to the nucleus and activate cytoprotective and antioxidant defenses by triggering the transcription of genes with ARE motifs in their promoters. The capacity of NRF2 to interact with numerous additional transcription factors has recently been explored in the literature [29]. The oxidants that make up the majority of the indirect antioxidants that work by triggering NRF2-dependent pathways frequently encourage brief spikes in the generation of ROS, and some of them may even act as pro-

oxidants in high concentrations. The majority of NRF2 activators fall under the general category of "hormetic" agents since they cause little stress that activates the antioxidant defenses and has a generally positive effect on the organism [30].

The phase II enzymes: HO-1, NAD (P) H quinione oxidoreductase 1, glutathione peroxidase, glutamate cysteine ligase, and glutathione S-transferases are among the NRF2-target genes. Not all the genes regulated by NRF2 produce enzymes with direct antioxidant activity, though. For instance, the proteins H-ferritin and ferroportin (FPN)-1, which control the amount of labile iron in the body, have ARE motifs in their promoters but are very loosely related to redox control. NRF2 affects genes involved in lipid metabolism, DNA repair, and tissue regeneration. Some ARE-motif-containing promoters, like the CD36 promoter, also contain PPARE-controlled PPARE motifs that can be activated by both factors simultaneously [31]. ATF-1 and NRF2 simultaneously controlled the genetic program that gave rise to the macrophage phenotype MHem, which has recently been identified as having the ability to prevent foam cell production. This suggests that antioxidant defenses are not always activated by an NRF2-dependent event [32].

The current results showed that both gram-negative (*E. coli* and *P. mirabilis*) and gram-positive (*S. lutea* and *B. cereus*) bacteria stains were sensitive to the exposure of such modified purines nucleosides and/or nucleotides derivatives as araC, araCMP, TTU, and cCMP. Besides that our results consider to set up some structure-function relationships in the range of modified pyrimidine nucleosides and/or nucleotides derivatives by the bacteria cell growth inhibition. Gram-negative (*E. coli* and *P. mirabilis*) bacteria stains were more sensitive to the exposure of TTU and cyclo-CMP and less sensitive to the exposure of ara-C and ara-CMP compared to gram-positive ones. The most effective cells growth inhibitor for gram-positive strains (*S. lutea*, *B. cereus*) was ara-CMP. *Sarcina lutea* appeared to be the most sensitive bacteria strain to the exposure of all studied compounds.

Next it was shown that the ROS production in bacteria strains was improved following treatment with all investigated drugs in a dose-dependent manner. Both the most sensitive gram-positive strains *S. lutea*, and *B. cereus* showed the *S. lutea*, and *B. cereus* showed the 12-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of araC (4.03 * 10^{-5} M and 4.01 * 10^{-5} M, respectively). the most sensitive gram-positive strains *B. cereus* showed the 12-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of araCMP (3 * 10^{-4} M). While *S. lutea*, and *P. Mirabilis* showed the 9-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of TTU (4 * 10^{-4} M), and *E. Coli* showed the 14-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of cCMP (5 * 10^{-4} M).

The results showed that the existence of antioxidant in the reagent mixture doesn't changed the dose-effect relationships, gram-positive and gram negative bacteria strains showed the same sensitive to all modified pyrimidine derivatives.

Conclusion

1. In conclusions, araC had higher potency against all strains *S. lutea* all strain *S. lutea* (ED_{50} = 5.5 * 10^{-4} M), *B. cereus* (ED_{50} = 2.1 * 10^{-3} M), *P. Mirabilis* (ED_{50} = 2.2 * 10^{-3} M), and *E. Coli* (ED_{50} = 3.3 * 10^{-3} M)., both the most sensitive gram-positive strains *S. lutea*, and *B. cereus* showed the 12-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of araC (4.03 * 10^{-5} M and 4.01 * 10^{-5} M, respectively). The presence of antioxidant in the reagent mixture hasn't changed the dose-effect relationships in general, gram-positive and gram bacteria strains still have sensitives to araC, but we observed the decrease of ED_{50} values. The most resistant bacteria strain *E. coli* showed 2.5-fold decrease of intracellular level after treatment with araC combined with quercetin at the highest used concentration 4.1 * 10^{-4} M. The same 1.5-1.8-fold decrease of ROS levels was detected in case of all other bacteria strains.

2- For araCMP, it achieved this effect at lower dose in case of action on gram-positive strains *S. lutea* (ED_{50} = 5 * 10^{-5} M), while *B. cereus* (ED_{50} = 1 * 10^{-4} M) compared to gram-negative strains *E. coli* bacteria strain ED_{50} = 2.2 * 10^{-4} M. The shape of dose-effect curve for *P. mirabilis* bacteria strain differed from all other used in experiments bacteria strains with calculated value of (ED_{50} = 7.3 * 10^{-3} M)what was closer to the value for another gram-negative bacteria strain, and *B. cereus* showed the 12-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of araCMP (3 * 10^{-4} M). The presence of antioxidant in the reagent mixture, where antioxidant hasn't changed the dose-effect relationships in general. The most resistant bacteria strain *P. mirabilis* showed 2-fold decrease of intracellular level after treatment with araCMP combined with quercetin at the highest used concentration 2.7 * 10^{-4} M. The same 1.6-1.8-fold decrease of ROS levels.

3- TTU also showed that that the maximum affection against all bactetial strains S. *lutea*, *B. cereus*, *P. Mirabilis* and *E. Coli* where same, i.e. it had equal efficacy against all the used strains. However, TTU achieved this effect at lower dose in case of action on *S. lutea* (ED_{50} = 2.5 * 10^{-4} M) and *E. coli* strain (ED_{50} = 3.4 * 10^{-4} M) compare to *B. cereus* (ED_{50} = 7.9 * 10^{-4} M) and *P. Mirabilis* (ED_{50} = 1.4 * 10^{-3} M), thus *S. lutea*, and *P. Mirabilis* showed the 9-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of TTU (4 * 10^{-4} M). The existence of antioxidant in the reagent mixture doesn't changed the dose-effect relationships against the strains. The most resistant bacteria strain *S. lutea* showed 8-fold decrease of intracellular level after treatment with TTU combined with quercetin at the highest used concentration 6 * 10^{-3} M. The 1.5-2.25-fold decrease of ROS levels.

4-Finally, also cCMP achieved the same maximum effect against all strain *S. lutea*, *B. cereus*, *P. Mirabilis* and *E. Coli*, *where* cCMP achieved this effect at lower dose in case of action on *S. lutea* (ED_{50} = 1.5 * 10^{-4} M) and *E. coli* strain (ED_{50} = 1.7 * 10^{-4} M), *P. Mirabilis* (ED_{50} = 3.1 * 10^{-3} M), and *B. cereus* (ED_{50} = 4.5 * 10^{-3} M). *E. Coli* showed the 14-fold burst of intracellular ROS after treatment with near-ED_{50} concentrations of cCMP (5 * 10^{-4} M). the existence of antioxidant in the reagent mixture doesn't changed the dose-effect relationships. The most resistant bacteria strain *B. cereus* showed 1.6-fold decrease of intracellular level after treatment with cCMP combined with quercetin at the highest used concentration 3.3 * 10^{-4} M. The 1.75-1.85-fold decrease of ROS levels.

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