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Examination of various antibiotics on mycoplasma bacterial contamination in the human K562 chronic myeloid leukemia cell line culture

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Abstract

Mycoplasma is one of the most common contaminants in the mammalian cell culture. The contamination of the chronic myeloid leukemia cell line was tested with a different type of antibiotics (Cocktail of 10000 U/mL Penicillin and 10000 µg/mL *Streptomycin, Ciprofloxacin, Gentamycin, Tetracycline*). The bacteria responsible for this contamination are morphologically identified as *mycoplasma*. Even though there are several reports of treatment for mycoplasma, there is a lack of study on the contamination of K562 cell line by *mycoplasma*. In this context, the present study got its importance; here cocktails of streptomycin and penicillin antibiotics as well as individual gentamycin, tetracycline and ciprofloxacin were used to study the extent of contamination by mycoplasma. The observations of the present study show that the bacterial growth was higher in the cell-free medium as compared to that of the medium incubated with the cells. Furthermore, the study confirmed the enhanced antibacterial activity of gentamicin and tetracycline against contaminated K562 cell lines. At the same time, the physiological activity of the *Mycoplasma* bacteria was higher in the case of cells treated with the ciprofloxacin and the cocktail of penicillin and streptomycin.

Keywords: Antibiotics; K562 cell line; *Mycoplasma* contamination; Cell culture

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Introduction

Mycoplasma contamination is one of the major common contaminants [1]. There are two types of contaminations which is chemical and biological. The common cause of most of the biological contaminants are bacteria, fungi, yeast and viruses. Among the two types of chemical and biological contamination, *Mycoplasma* contamination will come under biological contamination [2]. This contamination may also result in a change in pH (<7), which in turn leads to cell death [3]. This *Mycoplasma* is a genus of bacteria that comprises the largest group of species [4] and it was isolated for the first time from a contaminated cell culture in 1956 [5,6]. They can be parasitic and saprotrophic and therefore, they can live on the surface of eukaryotic host cells [7,8]. They lack a cell wall, and so they are unaffected by the antibiotics which target the cell wall synthesis [1,9]. The morphology of their flexibility and small in size range from 0.2 to 0.8 µm is the possibility to pass through anti-bacteriological filters (0.22 µm-0.45 µm diameter pore size) which facilitates the contamination of media and cell cultures [9,10].

The K562 is a blast cell of a chronic myeloid leukemia patient and this cell line has been used as a model system for numerous studies such as molecular understanding and therapeutic development. While culturing the cells, the contamination causes a decline in the survival rate of these K562 cells and so the proliferation is inhibited. This type of contamination can be ascended from different ways such as the operator, reagents, laboratory environment and culture of multiple cell lines in the laboratory. Mostly this risk can be reduced by introducing different diagnostic and therapeutic possibilities [9,11-13]. Several studies elucidate the possibility of different antibiotics (plasmocin, gentamycin, tetracycline, neomycin, macrolids, erythromycin, quinolones, kanamycin, enrofloxacin etc.) to eliminate the contamination from the animal cell lines [14-16]. Since there were few studies of mycoplasmic contamination in the K562 cell line, it is necessary to investigate the contamination on the K562 cell line and control

the risk. In this aspect, our work got its importance, and here we try to determine the anti-mycoplasma bacterial effect on K562 cell line and on cell-free medium using four types of antibiotics which is easily available and affordable.

Materials and Methods

Cell culture

The human chronic myeloid leukemia cell line K562 blasts were obtained from National Centre for Cell Science (Pune, Maharashtra, India). The cells were cultured in RPMI medium (RPMI, Thermo Fisher Scientific) supplemented with 10% Fetal Bovine Serum (FBS, Gibco, Thermo Fisher Scientific Canada, USA), 10000 U/mL penicillin and 10000 µg/mL streptomycin (Gibco, Thermo Fisher Scientific (Canada, USA)) in a humidified atmosphere of 95% air with 5% CO_2 incubator at 37°C.

Cell viability test (MTT)

The cells were seeded in 96 well culture plates having 100 µL volumes in each well contains approximately 5000 cells/well and incubated overnight at 37°C in the CO_2 incubator. Then 100 µL volumes Imatinib (STI571) in each well treated with different concentrations (1 µM, 0.8 µM, 0.6 µM, 0.4 µM, 0.2 µM and 0.05 µM per mL) at 24 and 48 hours as triplicates. The cells without treatment are used as the control. After incubation for the specific time interval, remove media and add 100 µL MTT reagent. And then incubated for 2 hours in a CO_2 incubator at 37°C. After incubation, 100 µL of detergent was directly added to the MTT reagent in the 96 well plates and again incubated for 3 hours in a CO_2 incubator at 37°C. After incubation, read OD at 570 nm [17] and 540 nm [18] wavelength in a multiskan spectrum. The %cell viability is calculated as the (OD of treated/OD of control) × 100

Treatment of antibiotics

The culture has been kept under biosafety hood level 2 and 95% air with a 5% CO_2 incubator at 37°C for 24 hours. Detected the rate of contamination and a direct method had been used to identify the contamination in the K562 cell line. Antibiotics of the cocktail of 10000 U/mL penicillin and 10000 µg/mL of streptomycin (Gibco, Thermo Fisher Scientific) and other antibiotics (Ciprofloxacin, Gentamycin, Tetracycline) were bought from a local medical shop for lab work. The 3 mL culture of a sample with RPMI medium was treated with 50 µL antibiotics and incubated for 24 hours. The concentrations were 0.5 mg/mL in the cocktail of each penicillin and streptomycin, and 0.1 mg/mL, 2 mg/mL, 4 mg/mL for ciprofloxacin, gentamycin, tetracycline respectively. Both the contaminated K562 cell lines and the cell free medium were treated with the cocktails of penicillin and streptomycin antibiotics as well as the individual ciprofloxacin, gentamycin, tetracycline. The surface morphology of the treated cells was studied using Leica inverted phase-contrast microscope.

Results and Discussion

Initially the present study confirmed that the viability of the K562 cell line were 92.44 (540 nm)/91.55 (570 nm) to 67.56 (540 nm)/68.06 (570 nm) percentage at 1 µM/mL concentration of imatinib (treated as a drug for chronic myeloid leukemia patients) in 24 and 48 hours treatment (**Table 1**). The imatinib drug concentration was used to know about the gradient level of the cell viability using different concentrations of 1 µM, 0.8 µM, 0.6 µM, 0.4 µM, 0.2 µM and 0.05 µM per mL and two (540 nm and 570 nm) wavelengths have been used for the reading, which shows a few differences only; however, the viability of the cells was normal at the initial stage of study. The study of Guangyu, et al. used, 0.025 µM, 0.05 µM, 0.1 µM, 0.2 µM, 0.4 µM, 0.6 µM and 0.8 µM imatinib concentrations for the cell viability (MTT assay) study in the K562 cell line [18]. Drexler, et al. reported the possibility of cross-contamination occurs in various new cell lines. The leukemic cell lines of CCRF-CEM, HL-60, JURKAT, K-562 and U-937 were used to prove the proper derivation of new cell lines [19]. Fieke, et al. explains the *Mycoplasma* infection alters the protein expression levels in leukemic cell lines of AML (Kasumi-1, HL-60, OCIAML3), ALL (Jurkat, REH), CML (KBM5) and lymphoma (Raji) [20]. In this context, the continuous investigation of *Mycoplasma* contamination of various cell lines, especially K562 cell line, got its importance.

The cause of contamination on K562 cell line culture was found as *Mycoplasma* by the simplest and direct method with the help of an inverted phase-contrast microscope. McGarrity explains the study of the United States estimated that at least 15% of all cell cultures are contaminated with *Mycoplasma* [21]. Therefore the *Mycoplasma* is one of the common contaminants in the cell culture (**Figure 1**).

The contamination rate was checked in an incubator and biosafety hood level 2 by keeping the culture for 24 hours for the valuation. The result displays a comparatively high contamination rate in the incubator as compared to the biosafety hood level 2. This may be due to mycoplasma's ease of survival in the incubator's environment (humidified atmosphere of 95% air with 5% CO_2 incubator at 37°C) (**Figures 2**). The major source of *Mycoplasma* bacterial contamination found in the newborn bovine serum

Table 1: Cell viability test (MTT assay) on K562 cell line, the imatinib (treated as a drug for chronic myeloid leukemia patients) drug concentration used for to know about the gradient level of cell viability.

Sl. No	Concentration (µM/mL)	Wave length (nm)	24 hrs (%)	48 hrs (%)
1	1	540	92.44	67.56
		570	91.55	68.06
2	0.8	540	102.93	86.52
		570	102.84	87.07
3	0.6	540	100.69	79.84
		570	101.25	78.78
4	0.4	540	104.37	96.04
		570	104.87	94.41
5	0.2	540	72.08	76.49
		570	70.21	75.04
6	0.05	540	132.04	122.87
		570	136.27	162.91

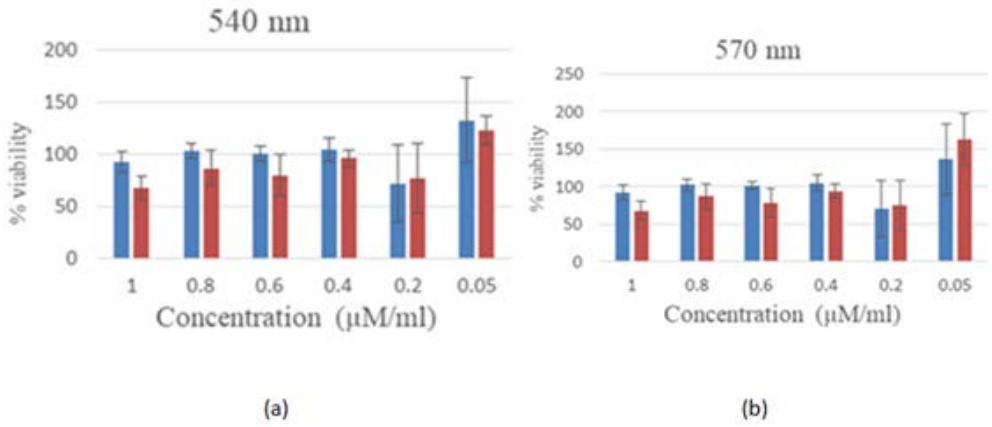

Figure 1 Cell viability test (MTT assay) on K562 cell line, the imatinib (treated as a drug for chronic myeloid leukemia patients) drug concentration used for to know about the gradient level of cell viability. a) The reading showed in the graph 540 nm wavelength; b) 570 nm wavelength.

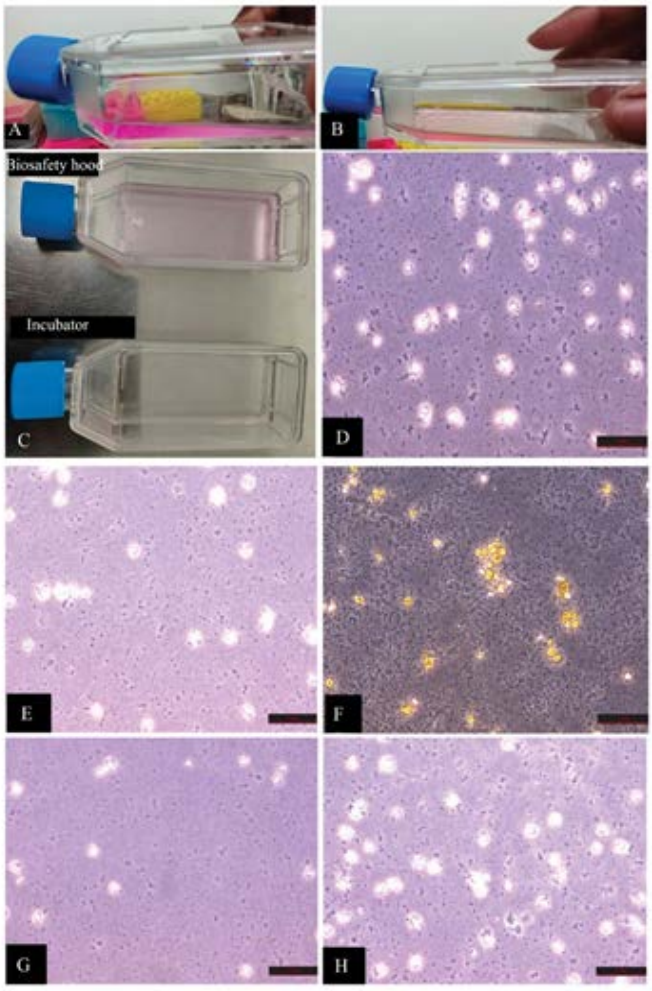

Figure 2 Culture and antibiotic treatment of mycoplasmic bacterial contaminated K562 cell line at 24 hours incubation. (A) The culture of K562 cell line in biosafety hood level 2 shows no colour change; (B) The culture of the K562 cell line in the incubator shows the colour change;(C) Differentiating the contamination from biosafety hood level 2 to incubator by the primary identification and isolation of colour change in the medium; (D-H) Antibiotics treated on mycoplasmic bacterial contaminated K562 cell line photomicrograph documented by Leica inverted phase-contrast microscope (D) Ciprofloxacin (E) Gentamycin (F) Tetracycline (G) Penicillin and streptomycin cocktail (H) Control (untreated cells).

obtained from commercial suppliers [22]. *Mycoplasma* infected cell cultures can cause further spreading by the ease of droplet formation during sample handling [9].

We have used four types of antibiotics (streptomycin and penicillin cocktail, gentamycin, tetracycline and ciprofloxacin) to treat *Mycoplasma* contaminated K562 cell lines and cell-free medium as well. It was found that the bacterial growth was higher in the cell-free medium than the medium with cells because the bacteria can grow freely in the cell free medium, despite the bacteria have to compete with the cells to grow and there will be a space consumption by the cells may also possible in the medium with cells and significantly, the cells are getting weak and decreasing their proliferation rate due to the activity of mycoplasma. According to Mirabelli, et al. normally, the mycoplasmas are resistant to the common antibiotics which inhibit cell wall synthesis or protein biosynthesis [23]. The study of Drexler and Uphoff explains, the treatment efficiency of antibiotics on mycoplasma-positive cell lines with either BM-Cyclin, sparfloxacin, enrofloxacin, or ciprofloxacin resulting the culture were either cured (66%-85%) or remained as contaminated (7%-24%; due to resistance) or died (4%-11%; due to cytotoxicity). Among these, the BM-Cyclin shows most of the growth-inhibiting effect and which can be either cytostatic or cytotoxic but after the one week treatment, they will be returning as normal cell growth [9]. The antibiotics of plasmocin, tetracycline and quinolone, which are effective against *Mycoplasma* and the treatment of antibiotics on mycoplasma, shows 80% were resistant to gentamycin, 98% to erythromycin, 73% to kanamycin, 15% to ciprofloxacin, 28% to lincomycin and 21% to tylosin [12,14-16].

Conclusion

The observation of the present study reveals the physical movement and the activity of *Mycoplasma* was higher in control (untreated cells) than antibiotic-treated cells. In this, the mycoplasma's physical movement and activity were higher in the ciprofloxacin and the cocktail of penicillin and streptomycin treated cells than the tetracycline (blocks *Mycoplasma* DNA) and gentamicin treated cells. The population of *Mycoplasma* was high in the tetracycline treated plate but, it was shrunken and the physical movement/activity was very poor than the other antibiotics treated plates. Hence, the present study proves that gentamicin and tetracycline show higher antibacterial activity on *Mycoplasma* contaminated K562 cell line than other antibiotics used in this study. These antibiotics may can be treated on other cell line contaminations as well. The uniform concentration of treatment also a possibility to find out the best one to elute the *Mycoplasma* contamination in the mammalian cell line culture.

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