ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Research Article

Antibiotic Treatment for *Mycoplasma* Contamination in *Plasmodium falciparum* Cultures

Suman Tamang and Vineeta Singh*

Parasite and Host Biology Group, ICMR-National Institute of Malaria Research (NIMR), Dwarka Sector 8, New Delhi, India

***Corresponding Author:** Vineeta Singh, Parasite and Host Biology Group, ICMR-National Institute of Malaria Research (NIMR), Dwarka Sector 8, New Delhi, India.

Received: January 24, 2023; **Published:** March 27, 2023

Abstract

Mycoplasmas, the simplest and smallest living prokaryotes are the most common contaminants that pose a serious impediment in the long-term maintenance of *Plasmodium falciparum (Pf)* cultures. The comparative efficacy of two antibiotics - *Mycoplasma* Removal Agent (MRA) and ciprofloxacin at different concentrations for eliminating *Mycoplasma* contamination in *Pf* cultures was determined. *Mycoplasma* contaminated and uncontaminated *Pf* cultures were maintained for 12 days and Giemsa-stained thin smears were prepared daily to monitor parasite growth. The presence of *Mycoplasma* was ascertained daily by 16S rRNA PCR kit. We found that MRA successfully eliminated *Mycoplasma* from contaminated culture at both 0.5 (7-day treatment) and 1 µg/mL (5-day treatment) concentrations, without affecting parasite growth but treatment with three different concentrations of ciprofloxacin (5, 10 and 20 µg/mL) showed an anti-plasmodial activity limiting its usefulness in *Pf* cultures.

Keywords: *Plasmodium falciparum; Mycoplasma Removal Agent (MRA); Ciprofloxacin; Mycoplasma; Culture Contamination*

Introduction

Mycoplasmas are the smallest and simplest self-replicating prokaryotes belonging to class *Mollicutes* and are the most worrisome contaminants of cell cultures [1-3]. As *Mycoplasma* lack cell wall, they are typically resistant to all antimicrobials targeting cell wall like fosfomycin, glycopeptides, or β-lactam antibiotics, which are routinely used against most bacterial contamination of cell cultures [4-6]. Moreover, *Mycoplasmas* are also intrinsically resistant to the first-generation quinolones, sulfonamides, polymyxin, rifampicin and trimethoprim, making its eradication an arduous job [6-8]. *Mycoplasma* contamination has been found to have a multitude of detrimental effects on physiology and metabolism of the infected cell cultures, leading to impaired growth, proteolytic degradation, inhibition of enzymatic activity, chromosomal abnormalities, severe cytopathic effects, disrupted cytokine production and altered gene expression, leading to significant problems in research and diagnosis [9-11].

Presence of various foreign contaminants adversely affects the long-term cultivation of *Pf*, causing roadblocks in malaria research aimed at vaccine/drug development. Turrini., *et al*. in 1997 reported *Mycoplasma* contamination in *Pf* cultures, which posed a serious hindrance to long-term *Pf* cultivation [10,12-15]. Because of their extremely small size (0.3 - 0.8 µm in diameter) [2] *Mycoplasmas* cannot be

identified easily in smears of malaria cultures using routine Giemsa or Acridine orange stains. The lack of cell wall provides *Mycoplasma* with elasticity and thus can easily pass through 0.1 µm filters commonly used to filter culture reagents [10,16]. Although it is well established that *Mycoplasmas* do not grow on human erythrocytes, but they are known to attach to glycolipids and receptors consisting of sialic acids [13]. Unlike other common biological contaminants like fungi and bacteria, *Mycoplasma* growth does not usually result in turbidness of culture [17] and low levels of contamination can go undetected for very long periods in *Pf* cultures [13].

Macrolides, tetracyclines and fluoroquinolones are the most effective three major classes of antibiotics commonly used to eliminate *Mycoplasma* contamination in cell cultures [5,6,18], but to date very few studies have been conducted to determine the utility of these drugs for eradicating *Mycoplasma* contamination in *Pf* cultures as most antibiotics possess anti-plasmodial activity [19-21]. To date only one drug *Mycoplasma* Removal Agent (MRA), a 4-oxo-quinoline-3-carboxylic acid derivative has been found to successfully eliminate *Mycoplasma* from contaminated *Pf* cultures at 0.5 µg/mL concentration, without showing anti-plasmodial activity [13]. Ciprofloxacin, a fluoroquinolone antibiotic is one of the most effective and often used antibiotic for *Mycoplasma* decontamination in different cell lines [22-25], but its potential in *Mycoplasma* contaminated *Pf* cultures has never been fully explored. In light of these information, the present study was carried out to determine the comparative efficacy of two drugs: MRA and ciprofloxacin, at three different concentrations each, for eliminating *Mycoplasma* contamination from *Pf* cultures, as it is of outmost importance for researchers.

Materials and Methods

Samples

In the present study, *Pf* field isolate 'Mew-40' collected in the year 2018 from Mewat region (Haryana, India) was used along with *Pf* reference strain 3D7 culture as a control for comparing growth profile. One vial of sample in continuous culture was found to be *Mycoplasma* contaminated (source unknown) while another vial of the same sample was free of contamination.

Drug concentrations

Three different concentrations of MRA (MP Biomedicals, Cat. No. 093050044) viz. 0.25, 0.5, 1 µg/mL, supplemented in RPMI culture media were used to culture *Mycoplasma* contaminated samples (Mew-40) along with three controls without MRA viz. C1 (*Mycoplasma* contaminated culture), C2 (contamination free culture) and C3 (3D7 culture with no contamination). Similarly, three different concentrations of ciprofloxacin (Sigma-Aldrich, ID-17850) viz. 5, 10, 20 µg/mL supplemented in RPMI culture media were used along with three different controls without ciprofloxacin viz. C4 - C6, were C4 and C5 were *Mycoplasma* contaminated and contamination-free cultures respectively, whereas C6 was 3D7 culture with no contamination. Experiments were performed in duplicates independently.

Parasite culture

Mycoplasma contaminated and uncontaminated *Pf* cultures were maintained in RPMI 1640 culture media supplemented with 5% human serum, gentamicin (0.01 mg/ml), 25 mM HEPES buffer, 25 mM $NaHCO_3$ and maintained in 5% CO_2 with incubation at 37°C [26]. As *Mycoplasma* grows at a slow rate where recurrence is a major challenge thus; to ascertain its removal, cultures were maintained for 12 days (day 0 - day 11). The spent media was replaced every 24 hrs with fresh media containing antibiotics (MRA/ciprofloxacin) of respective concentrations as well as media without antibiotics for control groups. About 250 µl of culture suspension was collected daily from each sample (except 3D7 cultures) at the same time during media replacement. Fresh human RBCs were added to cultures on days 2, 5 and 8 (Figure 1). To monitor the level of parasitemia, thin smear slides were prepared daily and Giemsa stained for microscopy. DNA extraction from the culture suspension was carried out daily for PCR detection of *Mycoplasma*. Once a culture tested negative for *Mycoplasma* contamination, antibiotic treatment was withdrawn and maintained further.

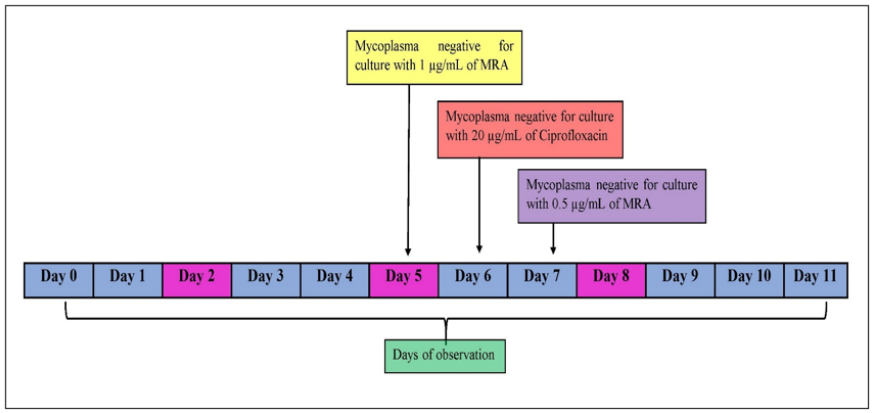


Figure 1: *Systematic representation of experiment timeline showing days in which antibiotic treated cultures tested negative for the presence of Mycoplasma.* ■ *Days on which fresh human RBCs are added to the cultures.*

Microscopy

The thin smear slides were air dried, fixed in methanol for 10 seconds and stained using Giemsa stain. The slides were observed under the light microscope (100X, immersion oil), 20 fields were scanned as described by WHOs malaria microscopy standard operating procedure (MM-SOP-09) and percent parasitemia was calculated and plotted [27]. To have an elementary understanding of the effect of *Mycoplasma* contamination on parasites morphology, slides were prepared daily for light microscopic examination from contaminated and uncontaminated cultures.

Isolation of nucleic acid and *Mycoplasma* detection

DNA from collected culture suspensions was isolated using QIAamp DNA Mini Kit (Qiagen) and used for *Mycoplasma* detection by EZdetect PCR Kit (Himedia) based on amplification of 16S rRNA which is highly conserved among various *Mycoplasma* spp [28]. The detection spectrum of kit includes seven major spp. *M. fermentans*, *M. arginini*, *M. hominis*, *M. orale*, *M. bovis*, *M. hyorhinis* and *A. laidlawii*, out of which five spp. excluding *M. hominis* and *M. bovis* are known to account for more than 95% of *Mycoplasma* contaminations in cell cultures [29].

Results and Discussion

Morphological examination

Upon microscopic examination of Giemsa-stained thin smears of *Mycoplasma* contaminated cultures (C1) under oil immersion (100X), we observed underdeveloped parasite stages; disintegrated nucleus and cytoplasm; abnormal shaped parasites, suggesting unhealthy/stressed state of parasites (Figure 2). These effects might be due to utilization of key components from culture medium by *Mycoplasma* making it unavailable for malaria parasites along with production of various metabolic by-products of *Mycoplasma* origin that may be toxic to malaria parasites. In agreement to observation made by Agarwal., *et al*. 2013 [14] we also noticed that color of *Mycoplasma* contaminated *Pf* culture was darker (dark brown/black) in colour as compared to uncontaminated cultures (Figure 2).

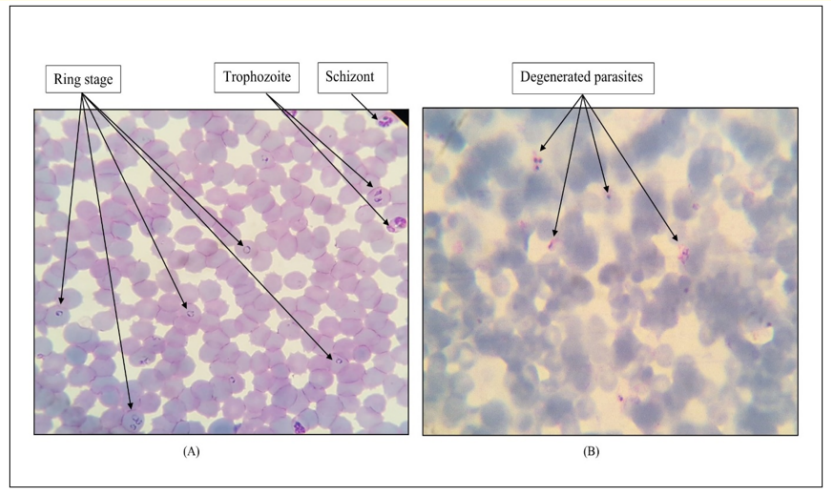


Figure 2: *Morphological examination of Mycoplasma contaminated (B) and contamination free cultures (A). (A): Healthy parasites in Mycoplasma free culture (C2) showing different erythrocytic stages. (B): Disintegrated parasites in Mycoplasma contaminated culture (C1).*

Parasitemia and *Mycoplasma* detection

The growth profile of *Mycoplasma* contaminated and uncontaminated *Pf* cultures observed during MRA and ciprofloxacin treatment along with controls till day 11 is depicted in figure 3 and 4, respectively. The drop in percent parasitemia readings at day 3, 6 and 9 was due to dilution of running culture with fresh human erythrocytes at day 2, 5 and 8.

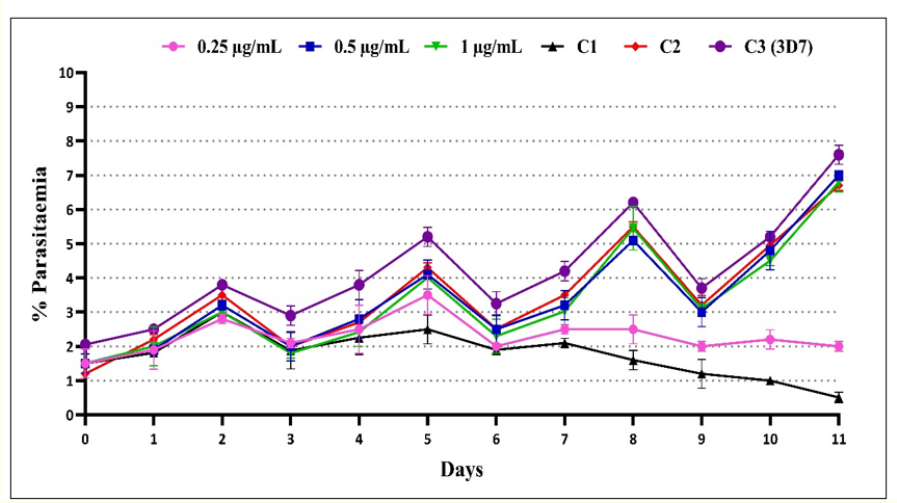


Figure 3: *Growth profile of Mycoplasma contaminated Pf cultures during treatment with different concentrations of MRA along with controls (C1, C2 and C3). C1: Mycoplasma contaminated 'Mew-40' culture, C2: 'Mew-40' culture without Mycoplasma contamination, and C3: 3D7 culture without Mycoplasma contamination.*

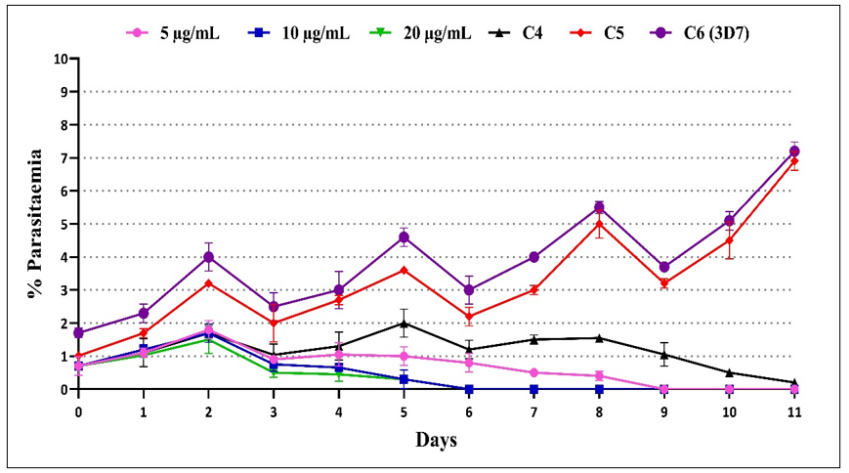
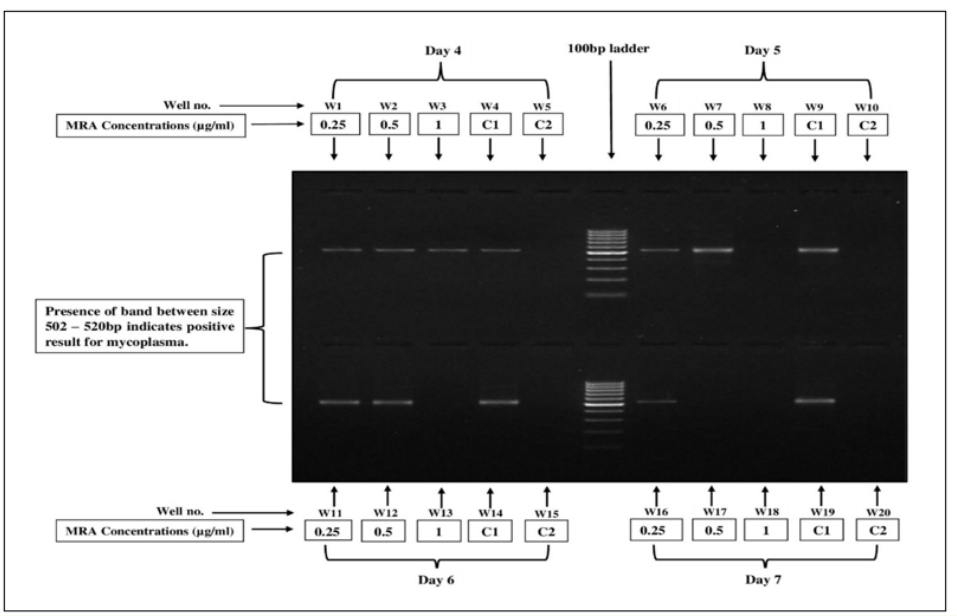


Figure 4: *Growth profile of Mycoplasma contaminated Pf cultures during treatment with different concentrations of ciprofloxacin along with controls (C4, C5 and C6). C4: Mycoplasma contaminated 'Mew-40' culture, C5: 'Mew-40' culture with no Mycoplasma contamination, and C6: 3D7 culture with no Mycoplasma contamination.*

It is clearly evident from figure 3, that culture treated with 0.5 and 1 µg/mL of MRA showed normal growth with continuous rise in percent parasitemia, similar to C2 and C3. In contrast, C1 culture showed gradual increase in percent parasitemia till day 3 but showed a downhill growth until day 11 and this decrease in parasitemia may be due to increase in *Mycoplasma* population as culture continued. In the cultures treated with 0.25 µg/mL of MRA, initial rise in parasitemia till day 5 was seen after which parasitemia started to decline moderately. Parasitemia at day 0 was same i.e. 1.5% for cultures with three different concentrations of MRA along with C1 but in C2 parasitemia was slightly lower i.e. 1.2% and for C3 starting parasitemia was 2%. The PCR detection of *Mycoplasma* showed that culture treated with 0.5 and 1 µg/mL of MRA tested negative for *Mycoplasma* on day 7 and 5 respectively, whereas the culture treated with 0.25 µg/mL of MRA along with C1 were positive for *Mycoplasma* presence till day 11. Results from day 4 to 7 during MRA treatment is shown in supplementary figure 1.

The absence of *Mycoplasma* contamination starting on day 5 and 7 for culture treated with 1 and 0.5 µg/mL of MRA respectively (W8 and W17 respectively, supplementary figure 1) till day 11 of culture shows successful elimination of *Mycoplasma* contamination by MRA without harming malaria parasites with no re-emergence of *Mycoplasma*. In agreement with results obtained by Rowe., *et al*. 1998 and Singh., *et al*. 2008, 7-day MRA treatment at 0.5 µg/mL concentration was effective in eradicating *Mycoplasma* contamination without affecting the growth of malaria parasites. Similarly, 1 µg/mL concentration not investigated in previous reports was found to be more effective in eliminating *Mycoplasma* contamination, as only 5-day treatment resulted in *Mycoplasma* free *Pf* culture without any re-emergence. In contrast, culture treated with 0.25 µg/mL of MRA showed no improvement in percent parasitemia after day 5, following which gradual decrease in parasite population was observed, showing inability of MRA to cleanse *Mycoplasma* contamination at this concentration. Decrease in percent parasitemia for both C1 and culture treated with 0.25 µg/mL of MRA may be attributed to increase in *Mycoplasma* population in culture leading to utilization of key medium components by *Mycoplasma* itself and making it unavailable for malaria parasites. As *Mycoplasma* contamination and its production of various metabolites have been shown to exert various detrimental effects in

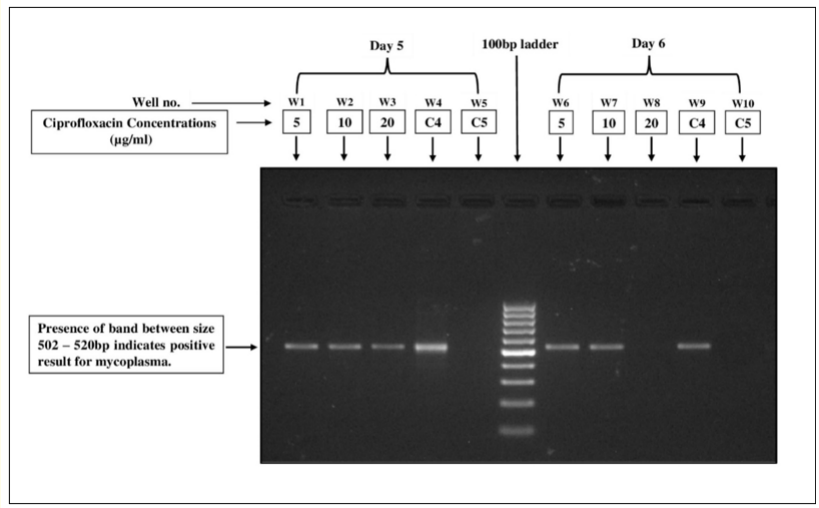


Supplementary Figure 1: *PCR detection of Mycoplasma in MRA treated cultures from day 4 to 7. Absence of DNA bands on well no. 8 (W8) and 17 (W17) indicates the successful clearance of contamination on day 5 and 7 for culture treated with 1 and 0.5 µg/mL concentrations of MRA respectively.*

infected cell cultures, which ranges from proteolytic degradation/inhibition of enzymatic activity to altered gene expressions [9-11,30]. Decrease in percent parasitemia for culture treated with 0.25 µg/mL and C1 may be due to myriads of detrimental effects *Mycoplasma* contamination had on physiology and metabolism of malaria parasites [3,10,17,31].

Growth profile of ciprofloxacin treated parasite cultures along with controls (Figure 4) revealed, that no parasites were seen after day 5 in cultures treated with both 10 and 20 µg/mL concentrations of the drug. Similarly, after day 8 no parasites survived in culture treated with 5 µg/mL concentration of the drug. This finding suggests that fluoroquinolone antibiotic ciprofloxacin does exhibit an anti-plasmodial activity which makes it less useful for eliminating/controlling *Mycoplasma* contamination in *Pf* cultures at above mentioned concentrations. As expected, untreated C4 showed some rise in percent parasitemia until day 5, after which there was a gradual decline in parasitemia which may be due to increase in the population of *Mycoplasma* in culture. The other controls viz. C5 and C6 showed normal parasite growth reaching up to 6.9 and 7.2 percent of parasitemia respectively on day 11. The initial starting parasitemia (day 0) was same i.e. 0.7% for cultures treated with 3 different ciprofloxacin concentration and C4. For C5 and C6 it was 1 and 1.7% respectively. The PCR detection of *Mycoplasma* for culture treated with 20 µg/mL of drug showed negative result on day 6 (W8) indicating successful elimination of *Mycoplasma* from culture but no parasites were seen on the same day (Supplementary figure 2).

These findings suggest that although ciprofloxacin can successfully eliminate *Mycoplasma* from *Pf* culture at 20 µg/mL concentration after 6-day treatment, but its usefulness is limited by the fact that it possesses anti-plasmodial activity. Although ciprofloxacin is one of the most effective and widely used antibiotic for *Mycoplasma* eradication in various cell lines [22-25,32-34], it has been shown to pos-



Supplementary Figure 2: *PCR detection of Mycoplasma in ciprofloxacin treated cultures for days 5 and 6. Absence of DNA bands on well no. 8 (W8) indicates the successful clearance of contamination on day 6 by 20 µg/mL concentration of MRA respectively.*

sess some level of reversible cytotoxic effect as reported previously [23,35]. In our study no reversal in cytotoxic effect was observed as parasite did not gain back its normal growth upon withdrawal of the drug. Since the removal of *Mycoplasma* contamination is not the only objective while treating *Mycoplasma* contaminated *Pf* cultures, it is also important that the drug used for *Mycoplasma* removal should not have an adverse effect on *Pf* survival. Hence, ciprofloxacin is not recommended because of its cytotoxic effect towards malaria parasites.

Mycoplasma contamination of *Pf* cultures, aptly referred to by Turrini and colleagues as "case of parasite parasitism", poses a major setback in our way to understanding *Plasmodium* biology, as laboratory cultures help in carrying basic biological research [12]. *Mycoplasma* produces a whole range of enzymes and metabolites for its survival which can be erroneously attributed to *Pf* itself and thus can lead to false experimental results [12]. *Mycoplasma* due to its limited biosynthetic capability also makes use of various hosts enzymes and metabolites, simultaneously, it depletes the essential nutrients from media affecting the metabolism and functioning of infected host cells [9-11,30]. Moreover, similarity in AT content (61 - 76%) between two parasites may pose a further challenge for molecular studies [36]. Although *Mycoplasma* does not proliferate in liquid nitrogen, they are able to persist and contaminate cell cultures stored in liquid nitrogen and thus may pose immense problem in using archived samples derived from parasite banks [12,37]. It has been estimated that the rate of *Mycoplasmas* contamination in banked cell lines range between 15 - 35% [9]. Trypsinization seems to be another interesting stage-unspecific alternative for the problem, as they have been found to effectively eliminate *Mycoplasma* contamination in mixed culture (culture containing ring, trophozoite and schizont stages) [14,38]. However, it adds up an additional step to the *Pf* culture process, when compared to MRA treatment. Damaged induced by centrifugation during trypsinization process to RBC and parasite would result in reduced parasitemia leading to cell death [39,40]. Study by Malave-Ramos., *et al*. 2022 recently showed a relatively low-cost fluoroquinolone antibiotic sparfloxacin to be effective in clearing MRA sensitive and resistance *Mycoplasmas* from *Pf* culture, however authors have not explored the recurrence of *Mycoplasma* beyond 8 days in sparfloxacin treated cultures, while recurrence of *Mycoplasma* in a treated culture is a major issue while dealing with a *Mycoplasma* [14,41]. It has been noted that the incidence of *Mycoplasma* contamination is more

frequent in the continuous cultures as compared to cultures that are in early passage [9], so *Mycoplasma* contamination of *Pf* culture is a major hurdle in continuous *Pf* culture. Our results suggest MRA can effectively eradicate *Mycoplasma* contamination from contaminated *Pf* culture and does not possess any cytotoxicity towards the survival of malaria parasites.

Conclusion

Our current findings suggest that MRA can be effectively used to eliminate *Mycoplasma* contamination in *Pf* culture in two different combinations i.e. 0.5 µg/mL (7-day treatment) and 1 µg/mL (5-day treatment) concentrations. However, close and periodic monitoring of *Mycoplasma* contamination is necessary to check *Mycoplasmas* reappearance which may pose obstacle in long-term cultivation of *Pf*. On the other hand, ciprofloxacin is not recommended for eradicating *Mycoplasma* contamination in *Pf* cultures because of its anti-plasmodial activity.

Acknowledgements

We would like to thank the Indian Council of Medical Research (ICMR)-NIMR for providing the infrastructure to carry out this work. We also duly acknowledge the support of Mrs. Sangeeta Arora for technical help.

Conflict of Interest

The authors declare that they have no competing interests.

Bibliography

1. Olarerin-George AO and Hogenesch JB. "Assessing the prevalence of *mycoplasma* contamination in cell culture via a survey of NCBI's RNA-seq archive". *Nucleic Acids Research* 43 (2015): 2535-2542.
2. Rottem S and Barile MF. "Beware of *mycoplasmas*". *Trends in Biotechnology* 11 (1993): 143-151.
3. Armstrong SE., *et al*. "The scope of *mycoplasma* contamination within the biopharmaceutical industry". *Biologicals* 38 (2010): 211-213.
4. Rottem S., *et al*. "Contamination of Tissue Cultures by *Mycoplasmas*". *Biomedical Tissue Culture* (2012).
5. Chernova OA., *et al*. "*Mycoplasmas* and Their Antibiotic Resistance: The Problems and Prospects in Controlling Infections". *Acta Naturae* 8 (2016): 24.
6. Gautier-Bouchardon Av. "Antimicrobial Resistance in *Mycoplasma* spp". *Microbiology Spectrum* (2018): 6.
7. Visser IJR., *et al*. "Failure of antibiotics gentamycin, tylosin, lincomycin and spectinomycin to eliminate *Mycoplasma bovis* in artificially infected frozen bovine semen". *Theriogenology* 51 (1999): 689-697.
8. Schultz KK., *et al*. "Optimization of an antibiotic sensitivity assay for *Mycoplasma hyosynoviae* and susceptibility profiles of field isolates from 1997 to 2011". *Veterinary Microbiology* 158 (2012): 104-108.
9. Drexler HG and Uphoff CC. "*Mycoplasma* contamination of cell cultures: Incidence, sources, effects, detection, elimination, prevention". *Cytotechnology* 39 (2002): 75-90.
10. Rowe JA., *et al*. "Implications of *mycoplasma* contamination in *Plasmodium falciparum* cultures and methods for its detection and eradication". *Molecular and Biochemical Parasitology* (1998): 92.

11. Rottem S. "Interaction of *mycoplasma*s with host cells*". Physiological Reviews* 83 (2003): 417-432.

12. Turrini F., *et al*. "*Mycoplasma* contamination of *Plasmodium* cultures - a case of parasite parasitism". *Parasitology Today* 13 (1997): 367-368.

13. Singh S., *et al*. "Treatment and control of *mycoplasma* contamination in *Plasmodium falciparum* culture". *Parasitology Research* 104 (2008): 181-184.

14. Agarwal P., *et al*. "Management of *mycoplasma* contamination in *in vitro* culture of *Plasmodium falciparum* without antibiotic treatment - a preliminary report". *Microbiological Research* 164 (2013): 211-215.

15. Stanisic DI., *et al*. "Development of cultured *Plasmodium falciparum* blood-stage malaria cell banks for early phase *in vivo* clinical trial assessment of anti-malaria drugs and vaccines". *Malaria Journal* (2015): 14.

16. Pall Corporation. *Mycoplasma* Reduction - Laboratory | Pall Corporation n.d (2022).

17. Rawadi G and Dussurget O. "Advances in PCR-based detection of *mycoplasma*s contaminating cell cultures". *PCR Techniques and Applications* 4 (1995): 199-208.

18. Uphoff CC and Drexler HG. "Eradication of *Mycoplasma* contaminations from cell cultures". *Current Protocols in Molecular Biology* (2014): 106.

19. Geary TG and Jensen JB. "Effects of Antibiotics on *Plasmodium Falciparum in Vitro*". *The American Journal of Tropical Medicine and Hygiene* 32 (1983): 221-225.

20. Pradines B., *et al*. "In Vitro Activities of Antibiotics against *Plasmodium falciparum* Are Inhibited by Iron". *Antimicrobial Agents and Chemotherapy* 45 (2001): 1746.

21. Gaillard T., *et al*. "Antibiotics in malaria therapy: which antibiotics except tetracyclines and macrolides may be used against malaria?" *Malaria Journal* 15 (2016): 1-10.

22. Uphoff CC., *et al*. "Elimination of *mycoplasma* from leukemia-lymphoma cell lines using antibiotics". *Leukemia* 16 (2002): 284-288.

23. Romorini L., *et al*. "Effect of antibiotics against *Mycoplasma* sp. on human embryonic stem cells undifferentiated status, pluripotency, cell viability and growth". *PLoS One* (2013): 8.

24. Taylor-Robinson D and Bébéar C. "Antibiotic susceptibilities of *mycoplasma*s and treatment of *mycoplasma*l infections". *Journal of Antimicrobial Chemotherapy* 40 (1997): 622-630.

25. Drexler HG., *et al*. "Treatment of *mycoplasma* contamination in a large panel of cell cultures". *In Vitro Cellular and Developmental Biology 30A* (1994): 344-347.

26. Trager W and Jensen JB. "Human malaria parasites in continuous culture". *Science* 193 (1976): 673-675.

27. World Health Organization. Malaria parasite counting. Malaria Microscopy Standard Operating Procedure (2016).

28. HiMedia-EZdetect TM. "Animal Cell Culture | *Mycoplasma* Detection and Elimination Platform | EZdetect TM *Mycoplasma* Detection Kits n.d (2016).

29. Harasawa R., *et al*. "Detection and tentative identification of dominant *mycoplasma* species in cell cultures by restriction analysis of the 16S-23S rRNA intergenic spacer regions". *Microbiological Research* 144 (1993): 489-493.

30. Sasaki T., *et al*. "Inhibition of growth of mammalian cell cultures by extracts of arginine-utilizing *mycoplasmas". In Vitro* 20 (1984): 369-375.

31. Miller CJ., *et al*. "*Mycoplasma* infection significantly alters microarray gene expression profiles". *Biotechniques* 35 (2003): 812-814.

32. Somasundaram C., *et al*. "Use of ciprofloxacin and BM-Cyclin in *mycoplasma* decontamination". *In Vitro Cellular and Developmental Biology 28A* (1992): 708-710.

33. Schmitt K., *et al*. "A safe and efficient method for elimination of cell culture *mycoplasma*s using ciprofloxacin". *The Journal of Immunological Methods* 109 (1988): 17-25.

34. Mowles JM. "The use of ciprofloxacin for the elimination of *mycoplasma* from naturally infected cell lines". *Cytotechnology* 1 (1988): 355-358.

35. Mahmoudi N., *et al*. "*In vitro* activities of 25 quinolones and fluoroquinolones against liver and blood stage Plasmodium spp". *Antimicrobial Agents and Chemotherapy* 47 (2003): 2636-2639.

36. Bové JM. "Molecular features of mollicutes". *Clinical Infectious Diseases* 17.1 (1993): S10-S31.

37. Nikfarjam L and Farzaneh P. "Prevention and Detection of *Mycoplasma* Contamination in Cell Culture". *Cell Journal* 13 (2012): 203.

38. Razin S., *et al*. "Adhesion of *mycoplasma*s to eukaryotic cells". *Ciba Foundation Symposium* 80 (1981): 98-118.

39. Peterson BW., *et al*. "Bacterial Cell Surface Damage Due to Centrifugal Compaction". *Applied and Environmental Microbiology* 78 (2012): 120.

40. Kiss F., *et al*. "The effect of centrifugation at various g force levels on rheological properties of rat, dog, pig and human red blood cells". *Clinical Hemorheology and Microcirculation* 62 (2016): 215-227.

41. Malave-Ramos DR., *et al*. "Safe, effective, and inexpensive clearance of *mycoplasma* contamination from cultures of apicomplexan parasites with Sparfloxacin". *Bio Rxiv* (2022): 505931.

Volume 19 Issue 4 April 2023