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ORIGINAL RESEARCH

Efficiency of Plasmocin™ on various mammalian cell lines infected by mollicutes in comparison with commonly used antibiotics in cell culture: a local experience

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Abstract Mycoplasma contamination is a deleterious event for cell culture laboratories. Plasmocin™ is used to prevent and eradicate mycoplasma infections from cell. In this study, 80 different mammalian cell lines from various sources; human, monkey, mice, hamster and rat were used to study and evaluate plasmocin™ efficiency and compare it to commonly used antibiotics such as BM-cyclin, ciprofloxacin and mycoplasma removal agent (MRA). It was shown that mycoplasma infections were eradicated by plasmocin™, BM-cyclin, ciprofloxacin and MRA in 65%, 66.25%, 20%, and 31.25%, respectively, of infected cell cultures. However, re-infection with mycoplasmas after the period of 4 months occurred in 10–80% of the studied cell lines. Cell cytotoxicity and culture death was observed in 25, 17.5 and 10% of the treated cells, for plasmocin™, BM-cyclin and MRA, respectively. In this study, Plasmocin™ showed strong ability to eradicate mollicutes from our cell lines with minimal percentage of regrowth. However, due to its high cell cytotoxicity it should be used with caution especially when dealing with expensive or hard-to-obtain cell lines. Amongst the antibiotics tested, BM-cyclin was shown to remove mycoplasma with the highest efficiency.

Keywords Mycoplasma · Plasmocin · Treatment · Cell line · Cytotoxicity

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Introduction

Human and animal continuous cell lines are precious and indispensable tools for both biotechnological and biomedical research. In this respect, mycoplasma contamination is a deleterious event for a cell culture laboratory resulting in the production of false data or, in the worst cases, in the loss of cell culture itself (Mariotti et al. 2008). It is well-known that mycoplasma can have adverse effects on cell cultures such as altered levels of protein and of RNA/DNA synthesis, induction of chromosomal aberrations, changes in cell membrane composition and modification of cellular morphology (Drexler et al. 2002; Drexler and Uphoff 2002). It has been highly advised to autoclave and discard infected cultures to minimize the risk of infection transmission to other clean cultures and eradication strategies in a separate quarantine laboratory should be considered as a last resort, if a valuable cell line or primary cell culture

is not replaceable (Molla Kazemiha et al. 2009). For eradication purposes, three classes of antibiotics, i.e., tetracyclines, macrolides and quinolones, have been shown to be highly effective against mycoplasmas, both in human/veterinary medicine and in cell culture (Singh et al. 2008; Uphoff and Drexler 2005). Since each antibiotic has its own activity and might not completely treat all the mycoplasma contaminants present in a culture, using a combination of antibiotics have attracted a lot of attentions. To this end, Plasmocin (Macrolid), have been used to prevent and eradicate mycoplasma infections from cell lines. The use of Plasmocin for eliminating mycoplasma from particular cell lines has been studied previously (Bronckaers et al. 2008; Singh et al. 2008; Mnif et al. 2007). There are also some studies on the efficacy of commonly used antibiotics such as Ciprofloxacine (Ridgway et al. 1984; Mowles 1988), BM-Cycline (Shin et al. 2003), and Mycoplasma removal agent (Drexler 1994; Nakai et al. 2000; Souza et al. 2007). However, few studies have reported on the comparison of these four antibiotics (Plasmocin, Ciprofloxacine, BM-Cycline and Mycoplasma removal agent) on the mycoplasma eradication from different cell lines. Previously, we reported the types of contaminants of our cell cultures and eradication strategies using three commonly used antibiotics (Molla Kazemiha et al. 2009). In this study, we aimed to compare the efficacy of Plasmocin™ against three previously studied antibiotics; BM-Cycline, Ciprofloxacine and MRA for decontamination of various mammalian cell lines.

Materials and methods

Cell cultures

A total of 80 different cell types (Table 1) from the National Cell Bank of Iran (Pasteur Institute of Iran, Tehran, Iran) were analyzed in this study. Cell lines were grown at 37 °C in a humidified atmosphere of air containing 5% CO_2. The basic growth media were supplemented with 10–20% fetal bovine serum (Sigma, Deisenhofen, Germany). For growth factor-dependent cell lines, specific growth factors or conditioned media containing growth factors were added (Drexler et al. 2001). Fetal Bovine Serum (FBS, Cat No: 10270-106), Roswell Park Memorial Institute medium (RPMI, Cat No: 31800-022), Dulbecco's Modified Eagle Medium High Glucose (DMEM, Cat No: 12100-046), F12 nutrient mixture (Hams'F12, Cat No: 21700-075), Non Essential Amino Acid (NEAA, Cat No: 11140-050), Penicillin/Streptomycin (Cat No: 15070-063), Horse serum (H.S, Cat No: 16050-130), Trypsin–EDTA (Cat No: 25300) were supplied by Gibco/Invitrogen company. Oxalate, Pyruvate, and Insulin (OPI, Cat No: O 5003), Bovine Insulin (Cat No: I 6634), Human Insulin (Cat No: I 9278), Epidermal Growth Factor (EGF, Cat No: E 9644), Fibroblast Growth Factor—from bovine pituitary (bFGF, Cat No: F 5392), and Human Endothelial Cell Growth Factor (Cat No: E 9640) were purchased from Sigma. All supplements, such as serum, conditioned media and trypsin were mycoplasma negative as indicated by the suppliers. For detection of mycoplasma, the cell lines were cultured initially for at least 1 week after thawing, and samples were taken after a culture period of at least 2 days without medium exchange. No antibiotics were added to the cultures.

Detection of mollicutes

The mycoplasma contamination status in 80 cell lines was determined using PCR-based method as described by Molla kazemiha et al. (2009). Table 2 shows the primer sequences with melting temperature (Tm) and guanine-cytosine content (GC %). All primers were obtained from CinnaGene (Iran) company.

Previous studied cell lines (Molla kazemiha et al. 2009) were treated with Plasmocin™ (Invivogen, USA, cat. No 04J05-SV) and the Plasmocin™ efficacy compared with previously used antibiotics, BM-Cyclin (Roche, Mannheim, Germany, cat. No.799050), Ciprofloxacin (ICN, USA, cat. No. 199020) and MRA (Serotec,UK, cat. No, Buf035). Moreover, another 40 new cell lines were screened for their mycoplasma contamination status and treated with above mentioned four antibiotics. The effects of each antibiotic on culture death as well as eradication and regrowth of mycoplasma were evaluated. Samples were treated with the antibiotics with doses and duration as stated below:

1. Plasmocin™ was added for 14 days at a final concentration of 25 μg/mL.
2. BM-Cyclin I (10 μg/mL) and BM-Cyclin II (5 μg/mL) were used in three alternating cycle of 3 and 4 days, respectively.

Table 1 PCR results of mycoplasma detection in 80 cell lines

No[a]	Cell line name	Cell type	NCBI Code	Mar	Mfe	Mor	Mhy	Ala	Msa	Mpi	Mho	Mge	Uur
1	MCF-7	Human breast Adeno carcinoma	C135	−	−	−	+	−	−	−	−	−	−
2	T-47D	Human breast ductal carcinoma	C203	−	+	−	−	−	−	−	−	−	−
3	RAJI	Human Burkitt's lymphoma	C127	−	+	−	−	−	−	−	−	−	−
4	NSO	Mouse myeloma	C142	−	+	−	−	−	−	−	−	−	−
5	HEP G2	Human hepatocyte carcinoma	C158	−	−	−	+	−	−	−	−	−	−
6	A-431	Human squamous carcinoma	C204	+	−	−	+	−	−	−	−	−	−
7	HFFF-PI6	Human fetal foreskin fibroblast	C170	−	−	+	−	−	−	−	−	−	−
8	Hep2	Human Larynx carcinoma	C144	−	−	−	+	−	−	−	−	−	−
9	MRC-5	Human foetal lung fibroblast	C125	+	−	−	−	−	−	−	−	−	−
10	Saos-2	Human osteogenic sarcoma	C453	+	+	−	+	−	−	−	−	−	−
11	McCoy	Synovial tissue fibroblast	C123	+	−	−	−	−	−	−	−	−	−
12	CCRF-CEM	Human Acute lymphoblastic leukemia	C105	−	+	−	−	−	−	−	−	−	−
13	PANC-1	Human pancreas duct epithelial carcinoma	C556	+	+	−	+	−	−	−	−	−	−
14	B95-8	Marmoset EBV transformed lymphocytes	C110	+	+	−	−	−	−	−	−	−	−
15	CHO	Chinese hamster ovary	C111	+	−	−	−	−	−	−	−	−	−
16	DAUDI	Human Burkitt's lymphoma	C112	−	−	+	−	−	−	−	−	−	−
17	BT-474	Human breast ductal carcinoma	C435	+	−	−	−	−	−	−	−	−	−
18	JIYOYE	Human Burkitt's lymphoma	C117	−	−	+	−	−	−	−	−	−	−
19	MDA-MB-468	Human breast adenocarcinoma	C208	+	+	−	+	−	−	−	−	−	−
20	HUT-78	Human cutaneus T cell lymphoma	C185	+	+	−	−	+	−	−	−	−	−
21	J774A.1	Mouse monocyte/macrophage	C483	+	−	−	+	−	−	−	−	−	−
22	LNCap-FGC-10	Human prostate cancer	C439	−	−	−	+	−	−	−	−	−	−
23	F3B6	Human × mouse heterohybridoma	C197	+	−	+	−	−	−	−	−	−	−
24	SP2/0-Ag14	Mouse myeloma	C129	−	+	−	+	−	−	−	−	−	−
25	Vero	African Green Monkey Kidney	C101	+	+	−	+	−	−	−	−	−	−
26	K562	Human CML	C122	−	−	−	−	+	−	−	−	−	−
27	WEHI-164	Mouse BALB/c fibrosarcoma	C200	−	−	−	+	−	−	−	−	−	−
28	BCL1 clone 5B1b	Mouse lymphoma	C551	−	−	−	−	+	−	−	−	−	−
29	SK-N-MC	Human neuroblastoma	C535	−	+	−	−	−	−	−	−	−	−
30	BW5147	Mouse thymoma	C542	−	−	−	+	−	−	−	−	−	−
31	STO	Mouse SIM fetal fibroblast	C537	−	−	−	+	−	−	−	−	−	−
32	CT26	Mouse colon carcinoma	C532	−	−	−	+	−	−	−	−	−	−
33	THP-1	Human acute Monocytic leukemia	C563	−	−	+	−	−	−	−	−	−	−
34	PC12	Rat adrenal fibroblast pheochromocytoma	C153	−	−	−	+	−	−	−	−	−	−
35	Seraphina	Human Burkitt's lymphoma	C102	−	+	−	−	−	−	−	−	−	−

Table 1 continued

No[a]	Cell line name	Cell type	NCBI Code	Mar	Mfe	Mor	Mhy	Ala	Msa	Mpi	Mho	Mge	Uur
36	LCL-PI 12	Human EBV transformed cord blood B cell	C178	−	+	−	−	−	−	−	−	−	−
37	HGF3-PI 53	Human gingival fibroblast	C502	−	−	−	+	−	−	−	−	−	−
38	HSF-PI 17	Human skin fibroblast	C193	−	+	−	−	−	−	−	−	−	−
39	B65	Rat nervous tissue neuronal tumor	C134	+	−	−	−	−	−	−	−	−	−
40	P3X63Ag8.653	Mouse-myeloma	C109	+	−	−	−	−	−	−	−	−	−
41	Hela	Human cervix carcinoma	C115	+	+	−	+	−	−	−	−	−	−
42	U937	Human Histiocytic lymphoma	C130	−	+	−	−	−	−	−	−	−	−
43	Hek293	Human embryonic kidney cells	C497	−	−	−	+	−	−	−	−	−	−
44	HL60	Human promyelocytic leukemia	C217	+	−	−	−	−	+	−	−	−	−
45	SKBR3	Human breast adenocarcinoma	C207	−	−	+	+	−	−	−	−	−	−
46	Peer	Human acute T cell lymphoblastic leukemia	C511	−	−	−	−	+	−	−	−	−	−
47	HL60/mix 1	Human acute promyelocytic leukemia	C553	+	−	−	−	−	−	+	−	−	−
48	HPA/ALL	Human T cell acute lymphoblastic leukemia	C213	−	+	−	−	−	−	−	−	−	−
49	MG63	Human osteosarcoma	C555	+	−	−	−	−	−	−	−	−	−
50	LB3.1 (HB-298)	Mouse anti human HLA-DR alpha chain hybridoma	H195	+	−	−	+	−	−	−	+	−	−
51	NIH3T3	Mouse Swiss embryo fibroblast	C156	−	−	−	+	−	−	−	−	−	−
52	Caco2	Human colon adenocarcinoma	C139	+	−	−	+	−	−	−	−	−	−
53	CoR-L-105	Human lung adenocarcinoma	C113	−	−	+	−	−	−	−	−	−	−
54	L929	Mouse connective tissue fibroblast	C161	−	+	−	−	−	−	−	−	−	−
55	A3.6B10 (HB-12318)	Mouse anti human CTLA-4 (CD152) hybridoma	H196	+	−	−	+	−	−	−	+	−	−
56	Nalm6	Pre B cell leukemia	C212	−	+	−	−	−	−	−	−	−	−
57	KG1	Human Caucasian bone marrow myeloid leukemia	C119	−	−	−	−	−	+	−	−	−	−
58	T45	Human T cell acute lymphoblastic leukemia	C180	−	−	−	−	−	−	+	−	−	−
59	PC3	Human prostate adenocarcinoma	C427	−	−	−	+	−	−	−	−	−	−
60	MDA-MB231	Human breast adenocarcinoma	C578	+	−	−	−	−	−	−	−	−	−
61	CHO DG-44	Dihydrofolate reductase-deficient CHO	C576	+	−	−	−	−	−	−	−	−	−
62	PC12 (Suspension)	Rat adrenal pheochromocytoma	C189	−	−	−	+	−	−	−	−	−	−
63	G28	Mouse anti human CD40 hybridoma	H150	+	−	−	+	−	+	−	−	−	−
64	NS1	Mouse myeloma	C522	−	−	−	+	−	−	−	−	−	−
65	MDBK	Bovine normal kidney epithelial cells	C500	+	−	−	+	−	−	−	−	−	−
66	Rael	Human Burkitt's lymphoma	C186	−	−	+	−	−	−	−	−	−	−
67	DFW	Human melanoma	C496	−	+	−	−	−	−	−	−	−	−

Table 1 continued

No[a]	Cell line name	Cell type	NCBI Code	Mar	Mfe	Mor	Mhy	Ala	Msa	Mpi	Mho	Mge	Uur
68	LCL PI7	Human EBV-transformed peripheral blood B cells	C175	−	+	−	−	−	−	−	−	−	−
69	B16F10	Mouse melanoma	C540	+	+	−	+	−	−	−	−	−	−
70	ASPC1	Human pancreas adenocarcinoma	C558	−	−	−	+	−	−	+	−	−	−
71	HT29	Human colon adenocarcinoma	C466	−	−	−	+	−	−	−	−	−	−
72	LS180	Human colon adenocarcinoma	C508	+	−	−	−	−	−	−	−	−	−
73	A2780S	Human ovarian carcinoma (sensitive to Cisplatin)	C461	−	−	−	+	−	−	−	−	−	−
74	MOLT-17	Human T cell leukemia	C516	−	+	−	−	−	−	−	−	−	−
75	CAOV-4	Human ovary adenocarcinoma	C595	−	+	−	−	−	−	−	−	+	−
76	DND-41	Human T cell acute lymphoblastic leukemia	C183	−	−	+	−	−	−	−	−	−	−
77	RPMI 8402	Human T cell acute lymphoblastic leukemia	C184	−	+	−	−	−	−	−	−	−	−
78	LL/2(LLC1)	Mouse Lewis lung carcinoma	C587	+	−	−	+	+	−	−	−	−	−
79	BHK21-2PCLone13	Baby hamster kidney from Syrian hamster (suspension culture)	C108	+	+	−	−	−	−	−	−	−	−
80	HFIF PI4	Human fetal liver fibroblast	C168	−	+	−	+	−	−	−	−	−	+

[a] Cell lines 1–40 (Molla Kazemiha et al. 2009), Cell lines 41–80 (Present study)

Mar, *M. arginini*; Mfe, *M. fermentans*; Mor, *M. orale*; Mhy, *M. hyorhinis*; Ala, *A. laidlawii*; Msa, *M. salivarium*; Mpi, *M. pirum*; Mho, *M. hominis*; Mge, *M. genitalium*; Uur, *U. urealyticum*; (−), non-detected mycoplasma species; (+), detected species-specific mycoplasma; NCBI, National Cell Bank of Iran

3. Ciprofloxacin was used for 14 days at10 μg/mL.
4. MRA was added to the culture medium for 10 days at a final concentration of 0.5 μg/mL.

The concentration of Plasmocin, BM cyclin and MRA were chosen according to the manufacturer's instructions. Ciprofloxacin concentration was specified according to the published report (Fleckenstein and Drexler 1996). Following the treatment with these reagents, cells were cultured in antibiotic-free medium (also without penicillin, streptomycin or other commonly used antibiotics) for at least another 2 weeks prior to testing for residual mycoplasmal contamination. All cured cultures were retested for regrowth of mycoplasmas for up to 4 months following the treatment.

Statistical analysis

All statistical analyses were performed with SPSS 16.0 (IBM® SPSS® Statistics, USA). Since the data were ordinal and non-normally distributed, non-parametric Kruskal–Wallis analysis was used for comparison of the 4 groups, and two-by-two comparisons were made using the Mann–Whitney U test. Differences at $P < 0.05$ were considered to be statistically significant.

Results

Determination of mycoplasma contamination status

Table 1 summarizes types of mollicutes in each cell lines determined by PCR-based method. It can be seen that 55/80 (68.75%) of the infected cell cultures contained one mycoplasma species. Other samples were infected with two 13/80 (16.25%) or three 12/80 (15%) different species. *M. hyorhinis* was detected in 35/80 (43.75%) of the studied samples, *M. arginini* in 30/80 (37.5%), *M. fermentans* in 28/80 (35%), *M. orale* in 9/80 (11.25%), *A. laidlawii* in 5/80 (6.25%), *M. salivarium* and *M. pirum* in 3/80

Table 2 The sequences of oligonucleotide primers used for detection of mycoplasmas

Primers of species-specific mycoplasmas	Primer sequence	Tm	GC	Amplicon size
Universal primer	S: GTG GGG AGG AAA YAG GAT TAG A	53–54.8	45–50	425 bp
	AS: GGC ATG ATG ATT TGA CGT CRT	50.5–52.4	45–48	
M. Arginini	S: TGA TCA TTA GTC GGT GGA GAG TTC	55.7	46	326 bp
	AS: TAT CTC TAG AGT CCT CGA CAT GAC TC	58	46	
M. Orale	S: TGA TCA TTA GTC GGT GGA AAA CTA	52.3	38	325 bp
	AS: TAT CTC TAG AGT CCT CGA CAT GAC TC	58	46	
M. Hyorhinis	S: CGA TGA TCA TTA GTT GGT GGA ATA AAT	53.7	33	334 bp
	AS: AGG CAG TAT CTC TAG AGT CCT TAA CTT A	57	39	
M. Fermentans	S: TGA TCA TTA GCT GAT GGG GAA CT	53.5	43	324 bp
	AS: TCT CTT AGA GTC CTC AAC TAA ATG	52.3	38	
M. Genitalium	S: ATA GAT ACT AGC TGT CGG AGC GAT	55.7	46	335 bp
	AS: CCA ATT TAC ATT AGC AGT CTC GTT AA	53.2	35	
A. Laidlawii	S: GAT GAG AAC TAA GTG TTG GCC ATA A	54.4	40	300 bp
	AS: CGC TAG AGT CCC CAA CTT AAT GA	55.3	48	
M. Hominis	S: ATC ATT AGT CGG TGG AGA ATC A	55.1	41	301 bp
	AS: GCA GTA TCT CTA CTA GAG TCC TCA ACT TAAT	59.1	39	
M. Pirum	S: TGG ATG TTA GAT GTC GGG GTA AA	53.5	43	324 bp
	AS: GTT GGC AGT ATC GCT AGA CAA A	56.7	41	
M. Pneumoniae	S: GAT ACT AGC TGT CGG GGC GAT	56.3	57	329 bp
	AS: AAT TTG CAT TAG TAG CAG TCT CGC TAG	56.7	41	
M. Salivarium	S: GAT CAT TAG TCG GCA GAG AAC TCG	57.4	50	324 bp
	AS: TAT CTC TAG AGT CCT CGA CAT GAC TC	58	46	
U. Urealyticum	S: CAT CAT TAA ATG TCG GCT CGA A	51.1	41	323 bp
	AS: CGG TAG CAG TAT CGC TAG AAA AGC	57.4	50	

(3.75%), *M. hominis* in 2/80 (2.5%), *M. genitalium* and *U. urealyticum* in 1/80 (1.25%).

Eradication of mycoplasmal contamination

The results obtained from eradication experiments (mycoplasma removal) are summarized in Fig. 1 and Table 3. Mycoplasma infections were eliminated by BM-Cyclin, Plasmocin™, MRA and Ciprofloxacin and in 66.25, 65, 31.25 and 20% of the infected cell cultures, respectively. Furthermore, the decontamination was confirmed by PCR, as no mycoplasma was detected in treated cultures 14 days after completion of antibiotic treatment (Fleckenstein and Drexler 1996). Mycoplasma regrowth was observed in 10, 16.25, 80 and 58.75% of the cured cell lines 4 months after treatment with Plasmocin™, BM-cyclin, ciprofloxacin and MRA, respectively. In spite of the absence of Plasmocin™—targets in eukaryotic cells,

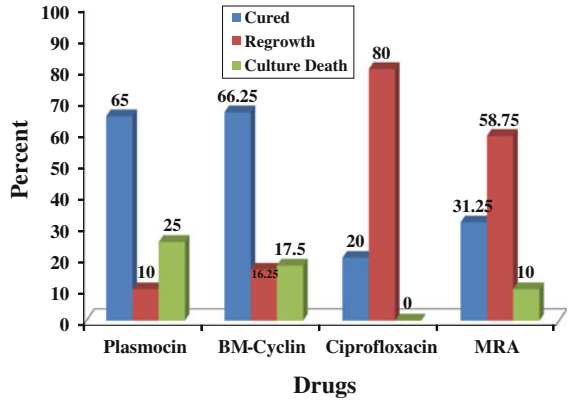

Fig. 1 Results of the treatment of mycoplasma-positive cell cultures with four antibiotics including Plasmocin, BM-cyclin, Ciprofloxacin and MRA

the highest level (25%) of cell cytotoxicity was observed among Plasmocin™ treated cell lines. While BM-cyclin, ciprofloxacin and MRA were

Table 3 The results of antibiotic treatment of each cell line

No*	Cell line name	Cell type	NCBI code	Pla	BM	MRA	Cip	Curing antibiotic	Mycoplasma species
1	MCF-7	Human breast Adeno carcinoma	C135	O	O	O	Δ	Pla, BM, MRA	Mhy
2	T-47D	Human breast ductal carcinoma	C203	O	O	Δ	Δ	Pla, BM	Mfe
3	RAJI	Human Burkitt's lymphoma	C127	O	X	X	Δ	Pla	Mfe
4	NSO	Mouse myeloma	C142	O	X	O	O	Pla, MRA, Cip	Mfe
5	HEP G2	Human hepatocyte carcinoma	C158	O	O	X	Δ	Pla, BM	Mhy
6	A-431	Human squamous carcinoma	C204	O	Δ	Δ	Δ	Pla	Mar, Mhy
7	HFFF-PI6	Human fetal foreskin fibroblast	C170	X	O	Δ	Δ	BM	Mor
8	Hep2	Human Larynx carcinoma	C144	O	O	Δ	Δ	Pla, BM	Mhy
9	MRC-5	Human foetal lung fibroblast	C125	X	O	O	O	BM, MRA, Cip	Mar
10	Saos-2	Human osteogenic sarcoma	C453	O	Δ	Δ	Δ	Pla	Mar, Mfe, Mhy
11	McCoy	Synovial tissue fibroblast	C123	O	O	Δ	Δ	Pla, BM	Mar
12	CCRF-CEM	Human Acute lymphoblastic leukemia	C105	X	O	O	O	BM, MRA, Cip	Mfe
13	PANC-1	Human pancreas duct epithelial carcinoma	C556	O	O	Δ	Δ	Pla, BM	Mar, Mfe, Mhy
14	B95-8	Marmoset EBV transformed lymphocytes	C110	Δ	O	Δ	Δ	BM	Mar, Mfe
15	CHO	Chinese hamster ovary	C111	O	O	O	O	Pla, BM, MRA, Cip	Mar
16	DAUDI	Human Burkitt's lymphoma	C112	X	O	Δ	Δ	BM	Mor
17	BT-474	Human breast ductal carcinoma	C435	O	O	O	Δ	Pla, BM, MRA	Mar
18	JIYOYE	Human Burkitt's lymphoma	C117	O	X	O	Δ	Pla, MRA	Mor
19	MDA-MB-468	Human breast adenocarcinoma	C208	O	Δ	Δ	Δ	Pla	Mar, Mfe, Mhy
20	HUT-78	Human cutaneus T cell lymphoma	C185	O	O	Δ	Δ	Pla, BM	Mar, Mfe, Ala
21	J774A.1	Mouse monocyte/macrophage	C483	Δ	Δ	Δ	Δ		Mar, Mhy
22	LNCap-FGC-10	Human prostate cancer	C439	O	O	Δ	Δ	Pla, BM	Mhy
23	F3B6	Human × mouse heterohybridoma	C197	X	X	Δ	Δ		Mar, Mor
24	SP2/0-Ag14	Mouse myeloma	C129	Δ	O	Δ	Δ	BM	Mfe, Mhy
25	Vero	African Green Monkey Kidney	C101	O	O	Δ	Δ	Pla, BM	Mar, Mfe, Mhy
26	K562	Human CML	C122	O	O	Δ	Δ	Pla, BM	Ala
27	WEHI-164	Mouse BALB/c fibrosarcoma	C200	O	O	Δ	Δ	Pla, BM	Mhy
28	BCL1 clone 5B1b	Mouse lymphoma	C551	X	X	O	O	MRA, Cip	Ala
29	SK-N-MC	Human neuroblastoma	C535	O	Δ	Δ	Δ	Pla	Mfe
30	BW5147	Mouse thymoma	C542	X	O	X	Δ	BM	Mhy
31	STO	Mouse SIM fetal fibroblast	C537	Δ	O	Δ	Δ	BM	Mhy
32	CT26	Mouse colon carcinoma	C532	Δ	O	Δ	Δ	BM	Mhy
33	THP-1	Human acute Monocytic leukemia	C563	O	O	Δ	Δ	Pla, BM	Mor

Table 3 continued

No*	Cell line name	Cell type	NCBI code	Pla	BM	MRA	Cip	Curing antibiotic	Mycoplasma species
34	PC12	Rat adrenal fibroblast pheochromocytoma	C153	Δ	O	Δ	Δ	BM	Mhy
35	Seraphina	Human Burkitt's lymphoma	C102	O	O	O	O	Pla, BM, MRA, Cip	Mfe
36	LCL-PI 12	Human EBV transformed cord blood B cell	C178	O	O	O	O	Pla, BM, MRA, Cip	Mfe
37	HGF3-PI 53	Human gingival fibroblast	C502	X	O	Δ	Δ	BM	Mhy
38	HSF-PI 17	Human skin fibroblast	C193	X	O	X	Δ	BM	Mfe
39	B65	Rat nervous tissue neuronal tumor	C134	X	X	O	Δ	MRA	Mar
40	P3X63Ag8.653	Mouse-myeloma	C109	X	X	Δ	Δ		Mar
41	Hela	Human cervix carcinoma	C115	O	Δ	Δ	Δ	Pla	Mar, Mfe, Mhy
42	U937	Human Histiocytic lymphoma	C130	O	O	Δ	Δ	Pla, BM	Mfe
43	Hek293	Human embryonic kidney cells	C497	Δ	O	Δ	Δ	BM	Mhy
44	HL60	Human promyelocytic leukemia	C217	O	O	Δ	Δ	Pla, BM	Mar, Msa
45	SKBR3	Human breast adenocarcinoma	C207	O	Δ	Δ	Δ	Pla	Mor, Mhy
46	Peer	Human acute T cell lymphoblastic leukemia	C511	X	O	X	O	BM, Cip	Ala
47	HL60/mix 1	Human acute promyelocytic leukemia	C553	X	O	Δ	Δ	BM	Mar, Mpi
48	HPA/ALL	Human T cell acute lymphoblastic leukemia	C213	X	X	X	O	Cip	Mfe
49	MG63	Human osteosarcoma	C555	O	O	O	Δ	Pla, BM, MRA	Mar
50	LB3.1 (HB-298)	Mouse anti human HLA-DR alpha chain hybridoma	H195	O	Δ	Δ	Δ	Pla	Mar, Mhy, Mho
51	NIH3T3	Mouse Swiss embryo fibroblast	C156	O	O	O	Δ	Pla, BM, MRA	Mhy
52	Caco2	Human colon adenocarcinoma	C139	O	Δ	Δ	Δ	Pla	Mar, Mhy
53	CoR-L-105	Human lung adenocarcinoma	C113	O	O	Δ	Δ	Pla, BM	Mor
54	L929	Mouse connective tissue fibroblast	C161	O	O	Δ	Δ	Pla, BM	Mfe
55	A3.6B10 (HB-12318)	Mouse anti human CTLA-4 (CD152) hybridoma	H196	O	Δ	Δ	Δ	Pla	Mar, Mhy, Mho
56	Nalm6	Pre B cell leukemia	C212	O	O	O	O	Pla, BM, MRA, Cip	Mfe
57	KG1	Human Caucasian bone marrow myeloid leukemia	**C119**	O	O	O	O	Pla, BM, MRA, Cip	Msa
58	T45	Human T cell acute lymphoblastic leukemia	C180	X	X	O	Δ	MRA	Mpi
59	PC3	Human prostate adenocarcinoma	C427	O	O	Δ	Δ	Pla, BM	Mhy
60	MDA-MB231	Human breast adenocarcinoma	C578	O	O	Δ	Δ	Pla, BM	Mar
61	CHO DG-44	Dihydrofolate reductase-deficient CHO	C576	O	O	O	O	Pla, BM, MRA, Cip	Mar
62	PC12 (Suspension)	Rat adrenal pheochromocytoma	C189	X	Δ	O	Δ	MRA	Mhy
63	G28	Mouse anti human CD40 hybridoma	H150	Δ	X	O	Δ	MRA	Mar, Mhy, Msa
64	NS1	Mouse myeloma	C522	O	O	O	O	Pla, BM, MRA, Cip	Mhy

Table 3 continued

No*	Cell line name	Cell type	NCBI code	Pla	BM	MRA	Cip	Curing antibiotic	Mycoplasma species
65	MDBK	Bovine normal kidney epithelial cells	C500	O	X	Δ	Δ	Pla	Mar, Mhy
66	Rael	Human Burkitt's lymphoma	C186	O	X	Δ	Δ	Pla	Mor
67	DFW	Human melanoma	C496	O	O	O	O	Pla, BM, MRA, Cip	Mfe
68	LCL PI7	Human EBV-transformed peripheral blood B cells	C175	O	O	O	Δ	Pla, BM, MRA	Mfe
69	B16F10	Mouse melanoma	C540	O	Δ	Δ	Δ	Pla	Mar, Mfe, Mhy
70	ASPC1	Human pancreas adenocarcinoma	C558	O	O	Δ	Δ	Pla, BM	Mhy, Mpi
71	HT29	Human colon adenocarcinoma	C466	O	O	Δ	Δ	Pla, BM	Mhy
72	LS180	Human colon adenocarcinoma	C508	X	O	O	Δ	BM, MRA	Mar
73	A2780S	Human ovarian carcinoma (sensitive to Cisplatin)	C461	O	X	O	Δ	Pla, MRA	Mhy
74	MOLT-17	Human T cell leukemia	C516	O	O	O	O	Pla, BM, MRA, Cip	Mfe
75	CAOV-4	Human ovary adenocarcinoma	C595	O	O	Δ	Δ	Pla, BM	Mfe,Mge
76	DND-41	Human T cell acute lymphoblastic leukemia	C183	X	O	X	Δ	BM	Mor
77	RPMI 8402	Human T cell acute lymphoblastic leukemia	C184	O	X	X	O	Pla, Cip	Mfe
78	LL/2(LLC1)	Mouse Lewis lung carcinoma	C587	O	Δ	Δ	Δ	Pla	Mar, Mhy, Ala
79	BHK21-2PCLone13	Baby hamster kidney from Syrian hamster (suspension culture)	C108	X	O	Δ	Δ	BM	Mar, Mfe
80	HFIF PI4	Human fetal liver fibroblast	C168	X	O	Δ	Δ	BM	Mfe, Mhy, Uur

O, cured; Δ, regrowth; X, culture death; Pla, Plasmocin™; BM, BM-cyclin; Cip, Ciprofloxacin; NCBI, National Cell Bank of Iran; Mar, *M. arginini*; Mfe, *M. fermentans*; Mor, *M. orale*; Mhy, *M. hyorhinis*; Ala, *A. laidlawii*; Msa, *M. salivarium*; Mpi, *M. pirum*; Mho, *M. hominis*; Mge, *M. genitalium*; Uur, *U. urealyticum*; (−), non-detected mycoplasma species; (+), detected species-specific mycoplasma

cytotoxic up to 17.5, 0, and 10% of the studied cell lines (Fig. 1). Notably, except in one case, regrowth problem for BM-cyclin treated cell lines could be solved by its replacement with Plasmocin™ and vice versa. The statistical results of two-by-two comparisons are indicated in Table 4 with significant differences between four groups but not between the Plasmocin™/BM-cyclin and MRA/Ciprofloxacin.

Table 4 Statistical results of two-by-two comparisons

Antibiotic 1	Antibiotic 2	*P* value
Plasmocin™	BM-cyclin	0.643
Plasmocin™	MRA	0.017
Plasmocin™	Ciprofloxacin	0.003
BM-cyclin	MRA	0.002
BM-cyclin	Ciprofloxacin	0.000
MRA	Ciprofloxacin	0.660

Discussion

Methods of elimination should ideally be simple, easy, rapid, efficient, reliable and inexpensive and have minimal effect on the eukaryotic cells. However, there is clearly not a single method available that is both 100% effective and fulfills all the ideal requirements. To this end, administration of antibiotics is the most common and efficient approach (Drexler and Uphoff 2002). Obviously it is important to know the efficacy of the antibiotics for the

eradication of mycoplasma in cultures, as well as the practicality of the approach, and the potential side-effects on the eukaryotic cells (Uphoff et al. 2002). The antibiotic effectiveness for eradication of each strain of mycoplasma is related to several parameters including cell types, cell species (human or animal) and severity of infection. In addition, some cell types may be infected with several species making it difficult to draw an accurate conclusion.

The mechanism of action of each antibiotic is different. Plasmocin (Macrolid and quinolone) acts on the protein machinery and also on DNA replication by interfering with ribosomal translation and replication fork, respectively. BM-Cyclin binds to the 30S and 50S ribosomal subunits and inhibits protein synthesis. According to the manufacturer's information, the bactericidal components of BM cyclin (Roche) are composed of pleuromutilin and tetracycline while Plasmocin^{TM} (InvivoGen) is composed of a macrolide and a quinolone. On the other hand, MRA and Ciprofloxacin are members of the quinolones family inhibiting bacterial DNA gyrase and replication of DNA (Helgason and Miller 2005; Uphoff and Drexler 2004, 2011).

Plasmocin^{TM} and BM-Cyclin were efficient in removing mollicutes and curing 65 and 66.25% of our cell lines, respectively. While, Ciprofloxacin and MRA were considerably less efficient as they cured only 20 and 31.25% of our cell lines, respectively. The high efficiency of Plasmocin and BM-Cyclin were not surprising as they are composed of two types of bactericidal agents (www.plasmocin.com, Uphoff et al. 2002). Zakharova et al. (2010) showed that Plasmocin can effectively remove mycoplasma contamination in treatment of chronic mycoplasma infections.

Notably, Plasmocin^{TM} and BM-Cyclin were highly efficient especially in curing of *M. orale*, *M. hyorhinis* and *M. arginini* infected cell lines which were resistant to other antibiotics used in this study. Fleckenstein and Drexler (1996) also reported these strains as resistant strains. For instance, we observed high level of ciprofloxacin as well as MRA resistance/regrowth after treating cell lines infected with above mentioned mollicutes.

As for regrowth, Plasmocin^{TM} showed the lowest level (10%) in our experiment, while regrowth was observed in 16.25% of cell lines after treating with BM-Cyclin. On the other hand, ciprofloxacin and MRA showed striking levels of regrowth (80 and 58.75% respectively) in treated cells. It has been shown by other researchers that ciprofloxacin may increase the mycoplasma resistance to antibiotic treatment (Momynaliev et al. 2002). Interestingly, for each cell lines (except for J774 in which regrowth was observed after treating with all the studied antibiotics) for which regrowth was observed after BM-Cyclin treatment, Plasmocin^{TM} may solve the problem and vice versa.

Surprisingly, Plasmocin^{TM} showed the highest percentage (25%) of culture death, although plasmocin^{TM} targets, the prokaryotic DNA replication and protein synthesis machineries, which are totally different from those of eukaryotic cells (www.plasmocin.com). However, Because of these two combined mechanisms, the cells are more affected by the antibiotic activity and the environment is more toxic for cells. Similarly, Singh et al. (2008) lost their culture during plasmocin^{TM} treatment. BM-Cyclin showed slightly lower cytotoxic effects on the studied cell lines. Therefore, it might reduce the risk of culture loss, especially in the case of expensive or hard-to-obtain cell lines to treat with BM-Cyclin first before treating with Plasmocin.

Since the higher amount of culture death was observed during treatment with Plasmocin^{TM} or BM-cyclin, treatment with other antibiotics such as MRA or ciprofloxacin must be considered spontaneously in the case of valuable cell lines.

On the other hand, ciprofloxacin showed no cell cytotoxicity at all, while MRA caused 10% cell death during or after treatment. Although cytotoxic effect of ciprofloxacin was not detected in this study, such observation has been reported before (Fleckenstein and Drexler 1996; Kloskowski et al. 2010; Sousa and Poiares-da-Silva 2001). Although the latter two antibiotics, showed lower percentage of cell cytotoxicity, they are not recommendable as they exhibited poor results in curing and regrowth experiments.

It is well known that several parameters are changed during infection of cultured cells by mycoplasma including reduction in the number of chromosomes in the cells or difficulty in the interpretation of the results of enzymatic activity (Souza et al. 2007). On the other hand different antibiotic doses administered during antibiotic treatment may cause different effects on cultured cells. Therefore it is difficult to detect and assess the effect of antibiotic treatment on the cell cycle stages during treatment of mycoplasmal infected cells. There are few studies on the antibiotic administration

and its effect on different stages of the cell cycle. It has been reported that antibiotics may inhibit the cell proliferation and cause cell cycle arrest at the G2/M phase (Tsai et al. 2008; Koziel et al. 2010). For this reason the antibiotic concentration should be determined precisely while apoptosis induction or mitosis inhibition should be considered.

The combination of two or more antibiotics with different action mechanisms for treatment of a single cell culture makes the interpretation of the results more complicated. Based on our experiment this might increase the risk of culture death or antibiotic resistance of bacteria. For this reason we suggest that using two or more antibiotics in an alternating procedure may be suitable for a successful eradication. For example BM cyclin (inhibits the protein synthesis) and Ciprofoxacin (inhibits the DNA gyrase activity) with different action mechanisms can be used alternately during treatment period with specified intervals. It should be noted that the outcomes of cocktail effects are under investigation and will be discussed in more detail in the future publications.

In conclusion, results obtained from Plasmocin™ and BM-cyclin in treatment efficacy were almost similar. They exhibited efficient eradication ability with minimum level of regrowth. However, relatively high percentage of cell cytotoxicity for these antibiotics introduces the risk of losing cell lines during the treatment which should be considered especially when dealing with expensive or hard-to-obtain cell lines. Finally it can be concluded that, ciprofloxacin and MRA did not efficiently eradicate mollicutes from the studied cell lines. BM cycline has been chosen as the best antibiotic with the highest amount of cured cells after treatment. Although Plasmocin showed the highest amount of cell death, it can be considered as a selective antibiotic, because of the lowest rate of mycoplasma regrowth compared to the other antibiotics.

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