Screening the activity of anti-tuberculosis drugs against *M. bovis* BCG Connaught and *M. bovis* BCG Pasteur growing in J774A.1 cell line macrophages.

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A B S T R A C T

Mycobacterium bovis BCG is attenuated strain derived from *M. bovis*. It has been known to prevent childhood pulmonary and meningeal tuberculosis and to treat superficial bladder cancer as immunotherapy. Administration of BCG has been restricted to immune-deficient individuals since 2007 because of its combined complications. Which treated with antituberculosis drugs based on assumption is fully responsive to these drugs. BCG Connaught showed more susceptibility to the activity of isoniazid, ethambutol, kanamycin, ofloxacin, streptomycin, amikacin and capreomycin than BCG Pasteur growing in J774A.1 cells. While the activity of rifampicin and levofloxacin was the same between the two tested strains. The spectrum of intracellular drug action can be ordered based on a decreasing order of inhibitory activity, as following rifampin > isoniazid > ethambutol > streptomycin > kanamycin> amikacin> ofloxacin> capreomycin> levofloxacin against BCG Connaught. While rifampin > isoniazid > ethambutol > kanamycin = streptomycin > ofloxacin> amikacin> levofloxacin> capreomycin for BCG Pasteur. There is a very limited publications describe the activity of antituberculosis drugs against intracellular growing BCG strains.

Keywords: *M. bovis* BCG – anti-tuberculosis drugs - macrophages.

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1. INTRODUCTION

Tuberculosis is a serious infectious bacterial disease caused by members of mycobacterium tuberculosis complex (MTBC) which includes M. tuberculosis, M. africanum, M. bovis, M. bovis BCG, M. microti, M. canettii, M. pinnipedii, M. mungi (Thoen et al., 2009). They are facultative intracellular pathogens, where they can survive and replicate within host macrophages through several mechanisms to escape macrophage destruction ability (Xu et al., 1994). These survival mechanisms make mycobacterial infections efficient and once intracellular they are difficult to treat by conventional antibiotics that do not accumulate well in the macrophage where the pathogen is sequestered (Barrow W, 2001). Therefore, evaluation of drug activity against intracellular growing mycobacterium is one of the main steps in screening drug susceptibility against mycobacterium (Andreu et al., 2012). *M. bovis* BCG is a member of MTBC. It is attenuated strain derived from *M. bovis* (Russell et al., 2010). BCG has been known to safely prevent severe form of the disease like childhood pulmonary and meningeal tuberculosis (Roy et al., 2014) and to treat superficial bladder cancer as immunotherapy (Watts et al., 2011 and Lukacs et al., 2013). But its administration is not recommended to individuals with impaired cellular immunity since 2007 because of its combined complications (FitzGerald, 2000; Paiman et al., 2006 and Arend and van Soolingen, 2011). Which may involve life-threatening side effects including BCG sepsis (Lukacs et al., 2013). These complications combating regime depends on using antituberculosis drugs; with assumption of BCG is fully responsive to these drugs (Kolibab et al., 2011 and Fahimzad et al., 2015). To the best of authors knowledge there is a very limited literatures describe the susceptibility of antituberculosis drugs against intracellular growing BCG.

Therefore, this paper was aimed to screen the activity of nine antituberculosis drugs against two of common BCG strains growing in J774A.1 cell line macrophages.

2. MATERIAL AND METHODS:

2.1. Mycobacterial strains:

Mycobacterium bovis (ATCC® 35745™) (BCG Connaught) and Mycobacterium bovis (ATCC® 35734™) (BCG Pasteur) were obtained from American Type Culture Collection **(**ATCC, USA).

2.2. Cell culture:

According to manufacturer instructions, Mouse Macrophages J774A.1 (ATCC® TIB-67™) was maintained in 75 cm^2 flask containing 15 ml 1X Dulbecco's modification of Eagle's Medium (DMEM) (Corning Cellgro, Mediatech, Inc. USA, Catalog No. 10-013-CV) supplemented with 10% Heat in-activated Calf Serum-Iron fortified (FBS) (Sigma-Aldrich, Catalog No. 15A104) at 37°C in humidified, 5% CO_2 atmosphere until 90 % confluent.

2.3. Antimicrobial agents:

Isoniazid (INH), streptomycin (SM), ethambutol (EMB), rifampicin (RMP), pyrazinamide (PZA), amikacin (AMI), ofloxacin (OFL), levofloxacin (LEV) and capreomycin (CAP) (Sigma, USA) and kanamycin (KAN) (Fisher Biotech). All the drugs were dissolved in sterile distilled water except RMP, OFL and LEV were dissolved in methyl alcohol, 1.0 N sodium hydroxide (NaOH) and 0.1 N NaOH respectively. They were kept in aliquot at -20°C at final concentration of 10 mg/L until used (NCCLS, 2003).

2.4. Drug concentrations:

Three concentrations of each drug (Table 1) were used to treat infected macrophage cell line for two and five days. It was selected depending on previously determined MBC concentrations against BCG Connaught (data not published yet). The drug concentrations were diluted in DMEM supplemented with 1 % FBS.

2.5. MTS Cell Proliferation assay or Cytotoxicity assay (Rajasekaran et al., 2013)

To determine whether the tested drug concentrations had any cytotoxic effect on macrophage cell line, a tetrazolium compound based cell viability assay [CellTiter 96® AQueous Non-Radioactive Cell Proliferation Assay (MTS assay) (Promega Inc., USA, Catalog No. G5421) was performed on J774A.1 macrophage. MTS is a tetrazolium dye that reduced by the live cell and produce water soluble colored formazan product. The macrophages were incubated with each drug concentration, in triplicate, overnight at 37°C under 5% CO_2. The number of the viable cells were measured based on the amount of absorbance at 490 nm due to formazan product. Cell viability present was calculated using this equation:
Average treatment data of each drug/avg control formazan data (un-treated) × 100. The drug concentration was considered toxic if the cell viability below 90 %.

2.6. Processing of BCG for infection (NCCLS, 2003 and Talaue et al., 2006):

1 McFarland No. 1 standardized BCG suspension was prepared as described in M24-A of the Clinical and Laboratory Standards Institute (NCCLS, 2003). Then it was diluted (1:5) in phosphate-buffered saline (PBS) and centrifuged at 10,000 X *g* for 10 min at room temperature. The bacterial pellet was re-suspended in DMEM supplemented with 10 % FBS.

2.7. Macrophage assay (Rastogi and Blom-Potar, 1990; Talaue et al., 2006; Andreu et al., 2012 and Jhamb et al., 2014):

J774A.1 cells were seeded at a concentration of 1×10^4 cells/ well in 96-well tissue culture white plates with clear bottoms(Corning®) 24 hours prior to infection (until the cells became spindle). After washing, the cells were infected with 100 µl of prepared BCG suspension (multiplicity of infection, MOI, 1-10: 1). After 2 hours of infection at 37°C in 5% CO_2, macrophages were washed twice with HBSS, 1X (Hank's Balanced salt solution) (Cellgro®, Mediatech, Inc. USA, Catalog No. 21-021-CV) to eliminate any extracellular bacteria. finally, 300 µl of DMEM supplemented with 1% FBS, with or without drugs, was added to each well. The plates were incubated at 37°C in humidified 5% CO_2 atmosphere for up to 5 days. The media with/without drugs were changed when the media began to show acidic reaction, orange color, (usually after 2 days). The cells were lysed, after time intervals 2 and 5 days, with SDS 0.125% for 20 minutes. The cell lysate was serially diluted and plated on Middlebrook 7H10 agar (Becton Dickinson and company, USA, Catalog No. 262710) supplemented with OADC 10% (Becton Dickinson and company, USA, Catalog No. 212351) for determination of colony forming units (CFUs). The experiment was repeated three times, each in triplicate.

2.8. Statistical analysis:

Unpaired, two tailed student's *t* test was performed between the three sets of the experiment. A *p* values of ≤ 0.05 were considered significant.

3. RESULTS

3.1. Cytotoxicity assay:

The cytotoxic effect of anti-tuberculosis drugs were determined using MTS assay. Figure 1 showed that all tested concentrations of the drugs were nontoxic. Except for levofloxacin at concentration 1 µg/ml showed fair cytopathic effect (88.25 % viable cell)

3.2. Sensitivity of actively growing intracellular BCG against anti-tuberculosis drugs:

The susceptibility of actively growing intracellular *M. bovis* (ATCC® 35745™) (BCG Connaught) and *M. bovis* (ATCC® 35734™) (BCG Pasteur) in J774A.1 cells to antituberculosis drugs after 2 & 5 days' treatment. The results were expressed as Log CFUs/ml ±standard error, log reduction and present of inhibition compared with the growth of bacteria in the untreated cells (Table 2 &3). BCG Connaught showed more susceptibility to the activity of isoniazid, ethambutol, kanamycin, ofloxacin, streptomycin, amikacin and capreomycin than BCG Pasteur growing in J774A.1 cells. While the activity of rifampicin and levofloxacin was the same between the two tested strains. The spectrum of intracellular drug action can be ordered based on a decreasing order of inhibitory activity, as following rifampin > isoniazid > ethambutol > streptomycin > kanamycin> amikacin> ofloxacin> capreomycin> levofloxacin against BCG Connaught. While rifampin > isoniazid > ethambutol > kanamycin = streptomycin > ofloxacin> amikacin> levofloxacin> capreomycin for BCG Pasteur.

Table 1. Anti-tuberculosis drug concentrations used to treat infected macrophage cell line.

Drug	Concentration (µg/ml)	Drug	Concentration (µg/ml)
INH	1.00-2.00- 4.00	KAN	2.5-5.00-10.00
RMP	0.125-0.25-0.50	OFL	0.50- 1.00- 2.00
SM	1.00-2.00- 4.00	LEV	0.25-0.50- 1.00
EMB	2.00- 4.00- 8.00	CAP	1.25- 2.5- 5.00
AMI	0.50- 1.00- 2.00		

isoniazid (INH), streptomycin (SM), ethambutol (EMB), rifampicin (RMP), pyrazinamide (PZA), amikacin (AMI), ofloxacin (OFL), levofloxacin (LEV) and capreomycin (CAP) and kanamycin (KAN).

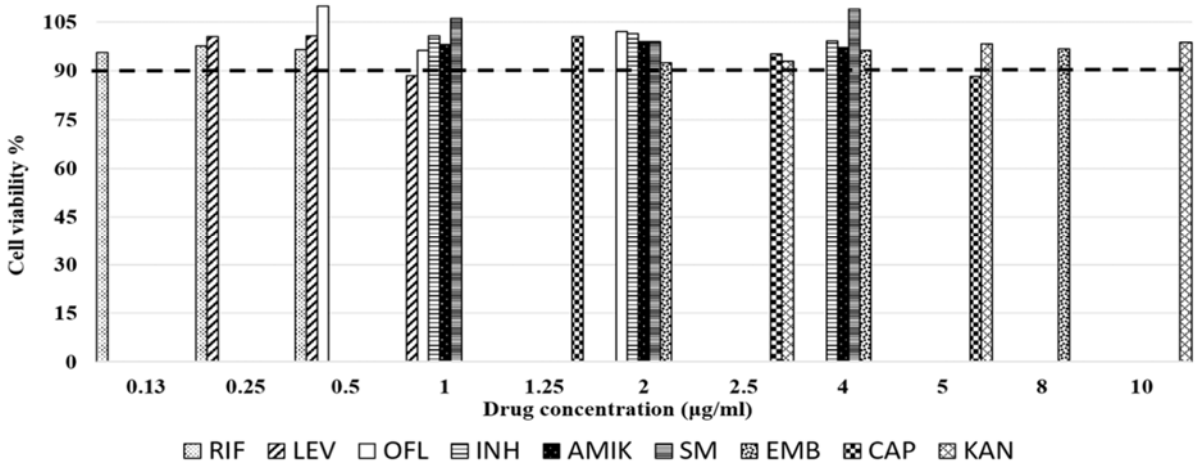

Figure 1: Cytotoxicity of anti-tuberculosis drugs on J774A.1 cells, the data represents the means of triplicate. The dotted line represented 90% of viable cell.

Table (2) susceptibility of *M. bovis* (ATCC® 35745™) (BCG Connaught) to anti-tuberculosis drugs (MOI 1-10 CFU:1 macrophage)

M. bovis (ATCC® 35745™) (BCG Connaught)										
	Two days' treatment					Five days' treatment				
Drug conc. mg/L	Control (untreated) Log CFUs/ml*	Drug (treated) Log CFUs/ml*	*P* value**	Log reduction	% of inhibition	Control (untreated) Log CFUs/ml*	Drug (treated) Log CFUs/ml*	*P* value**	Log reduction	% of inhibition
INH										
1		6.16±0.36	0.03	0.54	65.24		6.23±0.09	0.00	1.42	96.14
2		6.17±0.38	0.04	0.53	63.45	7.65±0.08	6.11±0.07	0.00	1.54	97.14
4		6.08±0.36	0.02	0.61	70.77		6.04±0.02	0.00	1.61	97.63
RIF	6.7±0.27									
0.125		6.15±0.23	0.00	0.55	73.41	7.53±0.08	6.49±0.11	0.00	1.04	90.57
0.25		5.84±0.26	0.00	0.86	86.24		5.83±0.20	0.00	1.70	97.60
0.50		5.56±0.23	0.00	1.14	93.21		5.45±0.09	0.00	2.08	99.17
SM										
1	7.05±0.08	6.84±0.13	0.05	0.18	34.89	7.50±0.03	7.32±0.01	0.00	0.26	35.22
2		6.78±0.14	0.05	0.23	41.23		7.12±0.01	0.00	0.45	58.84
4		6.70±0.13	0.02	0.31	52.18		6.97±0.0.03	0.00	0.64	70.55
EMB										
2	6.86±0.35	6.36±0.30	0.01	0.49	71.25	7.79±0.01	7.14±0.01	0.00	0.65	77.82
4		6.19±0.33	0.00	0.66	79.10		6.83±0.002	0.00	0.97	89.28
8		6.08±0.40	0.01	0.77	80.83		6.48±0.02	0.00	1.32	95.18
CAP										
1.25	6.69±0.26	6.66±0.23	0.41	0.04	14.22	7.71±0.04	7.75±0.03	0.47	-0.04	-9.60
2.5		6.65±0.22	0.41	0.05	16.39		7.56±0.00	0.02	0.15	29.97
5		6.62±0.24	0.16	0.07	19.05		7.38±0.00	0.00	0.33	53.67
AM										
0.50	5.95±0.50	5.94±0.51	0.07	0.01	1.57	7.78±0.03	7.55±0.01	0.00	0.24	42.46
1.00		5.93±0.51	0.19	0.02	0.85		7.42±0.01	0.00	0.36	56.78
2.00		5.84±0.54	0.08	0.12	11.28		7.22±0.02	0.00	0.56	72.82
KAN										
2.5	7.10±0.14	7.06±0.12	0.25	0.04	12.48		7.24±0.05	0.01	0.12	51.44
5.00		7.02±0.12	0.11	0.08	19.89	7.55±0.06	7.06±0.04	0.00	0.44	68.06
10.00		6.93±0.15	0.03	0.17	31.58		6.82±0.05	0.00	0.68	81.30
LEV										
0.25	7.04±0.18	7.10±0.19	0.01	-0.06	-15.06	7.81±0.01	7.77±0.01	0.06	0.04	8.98
0.50		7.10±0.21	0.17	-0.06	-21.64		7.75±0.01	0.01	0.06	13.95
1.00		7.04±0.21	0.94	0.00	-6.10		7.62±0.01	0.00	0.19	35.75
OFL										
0.5	6.45±0.50	6.52±0.48	0.10	-0.07	-12.84	7.73±0.03	7.69±0.01	0.15	0.05	10.92
1		6.46±0.49	0.86	0.00	2.76		7.51±0.01	0.00	0.22	40.61
2		6.43±0.51	0.10	0.02	3.20		7.24±0.01	0.00	0.49	67.97

* Mean ± standard error in three different experiments. ** *p* value of ≤ 0.05 was considered statistically significant using unpaired, two tailed student's *t* test.

Table (3) susceptibility of *M. bovis* (ATCC® 35734™) (BCG Pasteur) to antituberculosis drugs (MOI, 3-10 CFUs:1 macrophage)

M. bovis (ATCC® 35734™) (BCG Pasteur)										
	Two days' treatment					Five days' treatment				
Drug conc. mg/L	Control (untreated) Log CFUs/ml*	Drug (treated) Log CFUs/ml*	*P* value**	Log reduction	% of inhibition	Control (untreated) Log CFUs/ml*	Drug (treated) Log CFUs/ml*	*P* value**	Log reduction	% of inhibition
INH										
1	4.56±0.11	4.04±0.03	0.01	0.52	71.45		6.19±0.01	0.00	0.53	70.65
2		3.81±0.03	0.00	0.75	83.19	6.72±0.04	6.15±0.01	0.00	0.57	73.17
4		3.90±0.05	0.01	0.66	78.99		6.22±0.02	0.00	0.51	68.97
RIF										
0.125	6.62±0.004	6.12±0.03	0.00	0.50	68.49	5.92±0.02	4.95±0.02	0.00	0.97	89.18
0.25		5.91±0.04	0.00	0.71	80.54		4.71±0.03	0.00	1.21	93.79
0.50		5.45±0.03	0.00	1.17	93.25		4.49±0.01	0.04	1.43	96.30
SM										
1	5.93±0.02	5.90±0.01	0.24	0.03	7.02	6.21±0.04	6.15±0.02	0.23	0.06	12.93
2		5.87±0.01	0.07	0.06	12.48		6.05±0.02	0.02	0.16	30.61
4		5.74±0.02	0.00	0.19	34.89		5.95±0.0.03	0.01	0.26	45.58
EMB										
2	4.56±0.11	3.90±0.08	0.01	0.65	78.41	6.72±0.04	6.37±0.04	0.00	0.35	55.03
4		4.10±0.07	0.03	0.45	65.94		6.21±0.002	0.00	0.51	69.08
8		4.01±0.02	0.01	0.54	73.04		6.16±0.02	0.00	0.56	72.43
CAP										
1.25	5.04±0.03	4.96±0.06	0.25	0.09	17.04	4.84±0.01	4.86±0.03	0.72	-0.01	-2.86
2.5		4.99±0.05	0.25	0.06	11.39		4.69±0.05	0.03	0.15	28.57
5		4.92***		0.13	25.69		4.74±0.03	0.04	0.10	20.95
AM										
0.50	5.04±0.03	5.05±0.00	0.92	0.00	-0.25	6.25±0.04	6.21±0.01	0.38	0.04	9.03
1.00		4.99±0.01	0.19	0.05	11.72		6.19±0.01	0.19	0.06	13.40
2.00		4.87±0.02	0.01	0.18	33.67		6.07±0.01	0.01	0.18	33.96
KAN										
2.5	5.04±0.03	4.87±0.05	0.03	0.17	32.67	6.25±0.04	6.16±0.01	0.08	0.09	19.00
5.00		4.96±0.01	0.06	0.09	18.70		6.13±0.01	0.03	0.12	24.61
10.00		4.88***		0.17	32.67		5.99±0.02	0.00	0.25	44.55
LEV										
0.25	5.97±0.02	5.98±0.06	0.81	-0.02	-5.11	6.21±0.04	6.16±0.02	0.26	0.06	12.24
0.50		5.99±0.03	0.58	-0.02	-5.11		6.3±0.08	0.41	0.08	14.97
1.00		6.03±0.04	0.21	-0.06	-16.52		6.06±0.03	0.03	0.15	28.57
OFL										
0.5	5.04±0.03	5.01±0.00	0.29	0.04	8.73	6.25±0.04	6.12±0.02	0.03	0.21	26.48
1		4.94±0.03	0.08	0.10	21.20		6.12±0.01	0.03	0.21	26.17
2		5.03±0.02	0.65	0.02	4.24		6.04±0.01	0.00	0.21	38.94

* Mean ± standard error in three different experiments. ** p value of ≤ 0.05 was considered statistically significant using unpaired, two tailed student's t test. *** The Mean of three different wells in one experiment.

4. DISCUSSION:

Evaluation of drug activity against intracellular growing mycobacterium is not only important to determine ability of the drug to accumulate inside the macrophages but also to ensure that the drug is effective intracellularly. As the growth of the mycobacteria in the host has been observed to be quite different from its growth *in vitro.* So that upregulation of mycobacterial genes may be differed between both systems. The drug target (e.g. an enzyme in a key pathway) that may be upregulated *in vitro* may not be present at the same level or may not be expressed at all when the mycobacteria grow intracellularly. Which may give different drug profile against extracellular and intracellular mycobacterium (Barrow, 2001). The current study showed the activity of nine of most

common antituberculosis drugs against intracellular growing BCG Connaught and BCG Pasteur strains.

INH was found to be the second drug after RIF to be active against both BCG strains. However, after 5 days' treatment, its inhibitory effect against intracellular BCG Connaught (up to 97.6%) was more than those against intracellular BCG Pasteur strain (up to 73.2). which may indicate that BCG Pasteur strain had less susceptibility to INH than BCG Connaught, intracellularly, even in its obtainable serum level in human (4 µg/ml). Comparing to the MTB susceptibility to INH, BCG Connaught seemed to be quite similar to MTB and *M. bovis,* while BCG Pasteur was less susceptible. The previously published results showed that INH was highly effective against intracellular MTB as it inhibited more than 99% at 0.20 µg/ml after 7 days' treatment (Wright et al., 1996 and Jhamb et al., 2014)**.** In addition, INH obtainable serum level in human (4 µg/ml) caused 97.6% & 99.2% inhibition of MTB and M. bovis inside J774 cells after 5 days' treatment, respectively (Rastogi et al., 1987) or 99.65% after 7 days (Rastogi et al., 1996). Also it was reported that INH inhibited the mycobacterial growth at 0.05 µg/ml (Brennan et al., 2008).

RIF showed the most potent effective drugs against the intracellular BCG strains in the current study. Its activity against both BCG strains was quite the same. Comparing to its activity against MTB, it seemed to be higher against BCG strains. in this respect Rastogi et al. (1987) stated that 0.1 µg/ml RIF inhibited 55% of MTB growing in J774 cells after 5 days' treatment. And when the cells treated with RIF serum level (15 µg/ml) there was 99.06% inhibition. In the current study, 0.125 inhibited 89.2-90.6 of both BCG strains tested and 0.5 µg/ml inhibited 99.2% of BCG Connaught and 96.3 % of BCG Pasteur after 5 days.

There was another study that treated MTB growing in J774 and human MM6 cells with 0.016 µg/ml (3 fold higher than MIC). and it has been found that it inhibited 95% of MTB growing in J774 cells and 99.5% of that growing in MM6 cells after 7 days of treatment (Wright et al., 1996)**.** Additionally, it was found that RIF serum level inhibited about 99.99% of MTB growing in human macrophages after 7 days' treatment (Rastogi et al., 1996)**.**

As presented in table 2 & 3, EMB was had significant growth inhibition on both BCG strains after 2 and 5 days of drug addition. This inhibition was more obvious against BCG Connaught after 5 days' treatment that showed exposure dependent inhibition (time x concentration). While BCG Pasteur growth inhibition was quite the same between the two-time point of cells lysis.

Previously, 7 days' treatment with 4 µg/ml EMB to J774 cells and human MM6 cells infected with MTB caused 96.98 and 99.2 % growth inhibition respectively (Wright et al., 1996)**.** Comparing to our results 4 µg/ml EMB activity against BCG Connaught (89.28 % inhibition after 5 days) were more close to MTB than that of BCG Pasteur (69.1 %).

Another study tested the EMB serum level (6 µg/ml) against MBT in human macrophages. The growth inhibition was around 86.7 % after 3 days and around 98.8 % after 7 days (Rastogi et al., 1996). In the recent study, 8 µg/ml caused 80.8-95.18 inhibition to BCG Connaught after 2-5 days of drug addition, respectively. While BCG Pasteur growth was inhibited by 73.04-72.4 after 2-5 days' treatment.

The highest tested concentration of SM (4 µg/ml), as can be seen in table 2 & 3, inhibited 52.2% of BCG Connaught growth after 2 days of treatment and increased up to 70.6% after 5 days. While BCG Pasteur was inhibited by 34.9% and 45.6 % after 2 and 5 days, respectively. This indicating that SM was more active against BCG Connaught than BCG Pasteur.

It has been found that SM at 1 µg/ml had no inhibition on MBT gowning inside J774 cells after 2 or 5 days' treatment. While 10 µg/ml inhibited only 50 % of mycobacterial load after 5 days of treatment (Rastogi et al., 1987)**.** Comparing to the current obtained results BCG Connaught was more susceptible to SM than BCG Pasteur and MTB.

In the current study AMK and KAN were also found to be more active against BCG Connaught than BCG Pasteur after 5 days' treatment. KAN at 10 µg/ml inhibited BCG Connaught and BCG Pasteur by 31.6-81.3% and 32.7-44.6% after 2 and 5 days of treatment, respectively. Previously, it has been found that the same concentration inhibited MTB growing in human THP1 cells by 44-58% after 3-7 days of treatment, respectively (Giovagnoli et al., 2013). While their serum level (KAN 30 µg/ml and AMI 20 µg/ml) caused 90 % and close to 99% killing of *M. tuberculosis*-growing in macrophages (Brennan et al., 2008).

The inhibitory effect of CAP against both BCG strains was found to be accepted statistically ($P \leq 0.05$) only at 2.5 and 5 µg/ml after 5 days of drug addition. 2.5 µg/ml cause nearly the same percent of growth inhibition of both strains (28-

30%). While 5 µg/ml inhibited 53.7 % of BCG Connaught and 20.95 % of BCG Pasteur.

The CAP activity against MBT had been evaluated in human macrophages. At 10 µg/ml, there was 63-88% inhibition after 3-7 days of treatment, respectively (Giovagnoli et al., 2013). And at 30 µg/ml, there was around 75-95% inhibition after 3-7 days of treatment, respectively (Rastogi et al., 1996)**.**

OFL showed no activity against both BCG strains after 2 days' treatment. While after 5 days' treatment the maximum inhibition was 67.97% of BCG Connaught and 38.9 for BCG Pasteur strain at concentration 2 µg/ml. Comparing to intracellular MTB susceptibility to OFL, it had been reported that OFL at 2 µg/ml inhibited MTB growing in human THP1 cells by 40% and up to 100% after 3 and 7 days after treatment, respectively (Giovagnoli et al., 2013). Another study reported that OFL serum level (5 µg/ml) cause more than 99% inhibition of MBT gowning inside J774 cells or human macrophages after 5-7 days of treatment. And more than 90% inhibition when compared to initial bacterial count (just before drug addition) giving indication that OFL has bactericidal activity against MTB after 5 days (Rastogi and Blom-Potar, 1990 and Rastogi et al., 1996).

Depending on exposure dependent inhibition (time x concentration) of OFL against tested BCG strains may indicate that the susceptibility of BCG Connaught is closer to that of MTB than BCG Pasteur. And the intracellular susceptibility of both BCG strains to OFL was different.

LEV also showed no activity against both BCG strains after 2 days' treatment. Even after 5 days' treatment the maximum inhibition at 1 µg/ml was 35.7% and 28.57 % for BCG Connaught and BCG Pasteur, respectively. The intracellular susceptibility of both BCG strains to LEV was quite similar to each other. But absolutely different from MTB as it was reported that LEV was active against *M. tuberculosis*-infected macrophages at 0.5 µg/ml (Brennan et al., 2008). previous study showed that LEV was had bactericidal effect against *M. tuberculosis*-infected J7441 cells at concentration of 1.547 and 0.878 to 1.708 µg/ml after 4 and 7 days' treatment, respectively (Andreu et al., 2012)**.**

In conclusion: BCG Connaught showed more susceptibility to the activity of isoniazid, ethambutol, kanamycin, ofloxacin, streptomycin, amikacin and capreomycin than BCG Pasteur growing in J774A.1 cells. This primary screening of intracellular antituberculosis drug profile showed difference between two of BCG daughter strains and previously publish results against MTB. The serum level investigation of all antituberculosis drugs and their combination against all BCG daughter strains is recommended. To determine the most effective drug regime to be used for treatment of BCG complications. Which will help in avoiding of emergence of drug resistance BCG.

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