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Seromycin, an Effective Treatment for Tuberculosis in Tamsk, Russia

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ABSTRACT

This study was conducted to determine the minimum inhibitory concentration of cycloserine on 6 isolates of tuberculosis from Tomsk, Russia. Using the broth microdilution (BM) method, the isolates were found to be sensitive to 32 μg/ml of seromycin after 3 weeks of treatment. These results conformed to the control strain, irrespective of the amount of inoculum used. There was an indirect relationship between the amount of culture treated and the MIC of seromycin. All this show that seromycin was an effective treatment for tuberculosis in Tomsk, Russia.

KEYWORDS: *Seromycin, Tuberculosis, Sensitivity, Colonies, Russia*

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INTRODUCTION

Seromycin is a tuberculosis drug that's used to treat tuberculosis-infected patients (Singh, 2011). However, it is associated with side effects, and therefore its usage has become limited (Singh, 2011). Some of the complications patient's experiences are confusion, convulsions, depression, dysarthria, headache, paresis, psychosis, somnolence, tremor, vertigo, and breast feeding (Singh, 2013). In spite of these side effects, seromycin is used in the treatment of renal and hepatic impairment (Singh, 2013). The virulence in *Mycobacterium tuberculosis* is due to the structure of the bacterium. The alpha branched hydrophobic lipid layer serves as a barrier for chemical compounds to enter and kill it (Singh, 2011). As a result, drugs are being synthesised to prevent these bacterium surviving as facultative intracellular parasites in macrophages (Singh, 2011; Singh 2013). Due to the slow generation time of tuberculosis, *Mycobacterium tuberculosis* multiplies rapidly in the lungs. The generation time is between 15 and 20 hours (Todar, 2011). This study was conducted on 6 tuberculosis isolates from Tomsk, Russia. The drug, seromycin was administered at different concentrations to determine the MIC after 21 days of experimentation. The MIC was defined as the lowest concentration of seromycin that was used to inhibit the growth of tuberculosis after 3 weeks.

MATERIALS AND METHODS

a. Bacterial strains

Six tuberculosis isolates from the province of Tamsk, Russia, were frozen in cryovials that contained 0.5 ml freezing solution. The freezing solution was made up of 2g protease peptone and 16 ml glycerol. The experimental names of the isolates have been changed for confidentiality purposes. The experimental numbers start with the letter 'R' as represented in the Appendix, and tables accompanying the results.

b. Seromycin

Seromycin was purchased directly from Sigma-Aldrich Quimica, S.A. (Steinheim Germany). Aqueous solutions that were buffered to pH 10 were prepared on the day of performing MIC experiments. The method used was recommended by the manufacturer, Sigma-Aldrich Quimica (2008). The protocol involved making a 100 × concentrated 64 µg/ml cycloserine stock solutions by dissolving the required amount of seromycin in sodium carbonate (Na_2CO_3). The resultant seromycin solution was subsequently filter-sterilised using a 0.22 µm sterile bell filter (Sarstedst, Numbrecht, Germany).

c. MIC determination

The 6 Tamsk Isolates were recovered or grown in Middlebrook 7H9 broth. In order to obtain the exponential growth phase, cultures were thereafter transferred to Middlebrook 7H11 at 37°C. When the cultures reached the third week of growth, they were suspended in individual tubes that contained 4.5 ml phosphate buffered saline (PBS), 0.05 % Tween 80, and 4 – 6 glass beads. The tubes were thereafter vortexed for 5 minutes and allowed to settle for approximately 45 minutes. Following this, the upper supernatant was aspirated and adjusted to a McFarland standard of 1 (10^7 colony forming units/ml) (National Committee for Clinical Laboratory Standard, 2002). Sterile triple distilled water was used to standardise the bacterial culture. Colony counts, or inoculums (herein also referred to as inocula), were performed on the respective isolates to determine the cfu/ml.

The Broth Microdilution (BM) procedure was performed in 24-well tissue culture plates. The 7H9 broth (supplemented with 10% oleic acid-albumin-dextrose-catalase (OADC)) was adjusted to pH 7.2. Thereafter, the three 64 µg/ml-labelled wells on each of the plates, were treated with 1800 µl of broth and 20 µl seromycin. All the plate wells that didn't contain seromycin served as drug-free controls.

In contrast, wells that contained 64 µg/ml of seromycin in triplicate were two-fold diluted to 1 µg/ml. $H_{37}R_v$ was used as the control strain whenever a set of isolates or an isolate were being tested. For each well in the plate, 100 µl of 10^5 cfu/ml of culture was inoculated. The plates were thereafter incubated for 7, 14, 21 and 28 days in order for trends in the sensitivity pattern to be obtained, and the MIC was thereafter determined after 21 days. The MIC of seromycin, in this study, is defined as the lowest concentration at which seromycin completely inhibited the growth of Tamsk tuberculosis isolates at a pH of 7.2 (Singh, 2012). This was the standardised protocol used for the interpretation of the results that are to follow.

d. Calculation of colony counts

Colony counts were calculated for the 10^{-3} plate cultures. This was performed to determine the number of colonies present at this concentration of culture. Since 20 µl of culture was plated, the proportion of culture in 1000 µl was established. Thereafter the equation below was used to determine the cfu/ml:

Equation:

$$\text{cfu/ml} = \frac{\text{(no. of colonies x dilution factor)}}{\text{Volume of culture plate}}$$

$$= \text{No of colonies} \times \text{dilution factor} \times \frac{1000\ \mu l}{20\ \mu l}$$

RESULTS AND DISCUSSION

Mycobacterium tuberculosis is a disease of public health concern (Sharma and Mohan, 2007). It occurs when infected patients are in close proximity to individuals who are uninfected (Singh, 2013). *M. tuberculosis* is a disease that affects the lungs, and thus it is a respiratory illness (Zhang and Amzel, 2002; Todar, 2011). However, in recent times, tuberculosis has become manageable due to the vast majority of anti-tuberculosis agents that are available on the market. Rifampicin, ampicillin, and seromycin are examples of drugs that are available to treat tuberculosis infections (WHO, 2012). These infections are categorised as primary, secondary, and tertiary depending on the severity of tuberculosis infection (Singh, 2011). In this study, 6 clinical isolates were studied when they were treated with seromycin (Singh, 2013). These isolates were each tested in triplicate, and the results were

then duplicated experimentally. The 6 isolates were all susceptible to seromycin. This means that seromycin treatment was a good treatment agent against the tested bacterial innocula. In terms of seromycin susceptibility, it was found that isolates R 101, R 102 and R 103 (Table 1) exhibited a single shift in MIC reading from to 16 and 32 μg/ml. The other 3 isolates, viz. R 99, R 100 and R 104 (also presented in Table 1), exhibited a different shift in MIC patterns in comparison to the mentioned 3 isolates. R 98 and R 100 had a single shift in MIC from days 7 to 21, while isolate R 104 was equally susceptible at day 7 and 14 to 8 μg/ml of seromycin, while at 3 weeks R 104 was inhibited at 32 μg/ml of seromycin.

Table 1: Behaviour of tuberculosis to seromycin treatment

Isolate number	Minimum Inhibitory Concentration (MIC)				Inoculum CFU /mL
	Day 7	Day 14	Day 21	Day 28	
R99	16	16	32	32	6×10^6
R100	8	8	32	32	7×10^6
R101	8	16	32	32	7×10^6
R102	8	16	32	32	7×10^6
R103	8	16	32	32	7×10^6
R104	8	8	32	32	6×10^6

Seromycin is a second-line tuberculosis drug that is associated with side effects (Anon, 1965; Singh, 2011; Singh, 2013). In this study, the isolates had very close inoculum counts. The innocula ranged between 6 and 7 x 10^6 CFU/ml. In all isolates, the mycobacteria were not susceptible to higher concentrations of seromycin, above 32 μg/ml, after 4 weeks of incubation. This means that these mycobacteria acquired resistance to seromycin after 28 days. The control strains tested in comparison to these experimental isolates conformed to the MIC of the tested strain. With an exception of C (presented in Table 2), all the control isolates were inhibited at 32 μg/ml. This means that the test experiment was standardised, and that the concentration of seromycin used was in keeping with the media conditions. pH 7.2 was found to be ideal for tuberculosis isolate responses to seromycin (Singh, 2012). The MIC of isolate C (Table2) did not correlate with that of R 101, since 8 μg/ml seromycin was required to inhibit the multiplication of $H_{37}R_v$ after 2 weeks. Only isolate R 102 and R 103 (Table 1) had the same MIC susceptibility profile to seromycin as D (Table 2) and E (Appendix table) respectively. The remainder had a mixture of responses to seromycin. Isolates G, H, I and J (Table 3) showed favourable responses to seromycin. All of these $H_{37}R_v$ isolates had the same sensitivity pattern, with exception of H, which became resistant to seromycin about a week earlier in the experiment. H required 32 μg/ml after 14 and 21 days for growth inhibition.

Table 2: Behaviour of the control $H_{37}R_v$ to seromycin treatment

Inoculum CFU / mL	Control Strain Set	Day 7	Day 14	Day 21	Day 28
4×10^6	A	8	16	32	32
4×10^6	B	8	16	32	32
5×10^6	C	8	8	16	32
5×10^6	D	8	16	32	32

Inoculums are used to optimise and standardise the protocols used in MIC tests in different bacterial cultures. In this study, the $H_{37}R_v$ strains tested had lower inoculums compared to the Russian strains tested. The lowest inoculum counts were 3 x 10^6, and this could have contributed to an earlier acquired resistance to seromycin. According to Petrini and Hoffner (1999) and Singh (2012), resistance acquisition in tuberculosis is not attributed to plasmid insertion of resistance genes in tuberculosis; instead resistance is induced due to administered tuberculosis agents (Petrini and Hoffner, 1999; Singh, 2011).

Table 3: $H_{37}R_v$ isolates showing good MICs in response to seromycin treatment

Inoculum CFU/mL	Key	Day 7	Day 14	Day 21	Day 28
5 x 10^6	G	8	16	32	32
3 x 10^6	H	8	32	32	> 32
6 x 10^6	I	8	16	32	32
7 x 10^6	J	8	16	32	32

In total, there were six isolates that had colony counts of 7 x 10^6, with the rest lying between 6 and 4 x 10^6. Since the antibiogram of the patients tested in Russia were not provided, the fact that the colony counts varied, justified the results found. Furthermore, Singh (2012, 2013) suggested that it was also possible for patients to have been on combination therapies, or utilising other forms of treatment options. From this study, it can be taken that irrespective of whether colony forming units have an influence on the concentration of seromycin used by the bacteria (Singh, 2013), the uniformity among the tested isolates, and $H_{37}R_v$, after 3 weeks, show that the seromycin is an effective treatment for tuberculosis infection.

Table 4: MIC results of seromycin in Tamsk tuberculosis isolates in comparison to the control strain $H_{37}R_v$ (Appendix Table)

Number	Experiment No.	Day 7	Day 14	Day 21	Day 28	Inoculum (CFU/mL)
1.	A	8	16	32	32	4 x 10^6
2.	R. 99	16	16	32	32	6 x 10^6
3.	R. 99	16	16	32	32	6 x 10^6
4.	R. 99	16	16	32	32	6 x 10^6
5.	B	8	16	32	32	4 x 10^6
6.	R. 100	8	8	32	32	7 x 10^6
7.	R. 100	8	8	32	32	7 x 10^6
8.	R. 100	8	8	32	32	7 x 10^6
9.	C	8	8	16	32	5 x 10^6
10.	R. 101	8	16	32	32	7 x 10^6
11.	R. 101	8	16	32	32	7 x 10^6
12.	R. 101	8	16	32	32	7 x 10^6
13.	D	8	16	32	32	7 x 10^6
14.	R. 102	8	16	32	32	7 x 10^6
15.	R. 102	8	16	32	32	7 x 10^6
16.	R. 102	8	16	32	32	7 x 10^6
17.	E	8	16	32	32	4 x 10^6
18.	R. 103	8	16	32	32	7 x 10^6
19.	R. 103	8	16	32	32	7 x 10^6
20.	R. 103	8	16	32	32	7 x 10^6
21.	F	8	16	32	32	7 x 10^6
22.	R. 104	8	8	32	32	6 x 10^6
23.	R. 104	8	8	32	32	6 x 10^6
24.	R. 104	8	8	32	32	6 x 10^6

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