



The Microbial World: Control of Microbial Growth

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Abstract

This report details the results of four experiments that investigated the effect of physical and chemical techniques on the control of microbial growth, which have been carried out through three laboratory sessions in BIO60002 at Islamia University of Bahawalpur Pakistan. In the first set of experiments (Part 1), moist-heat sterilisation by autoclave, the antimicrobial activity of six disinfectants and antiseptics against *Bacillus subtilis*, antibiotic sensitivity profile of *Staphylococcus epidermidis* and *Escherichia coli* and the Minimum Inhibitory Concentration (MIC) of phenol on *E. coli* were evaluated. In the second set (Part 2), the bactericidal effects of moist heat (60°C) and Ultraviolet (UV) irradiation was evaluated against *B. subtilis* and then class-wise results were compared with four species. *B. subtilis* endospores were eliminated in both packing conditions in the autoclave. The efficacy of the disinfectants also differed, with White King and Tea Tree Oil having the widest zone of inhibition. The MIC of phenol was found as 1:200. The results of the UV treatment were very effective, destroying all colonies of *B. subtilis* within 40 seconds, while the thermoresistance of endospores is well known, and they did not always reduce colony counts at 60°C.

Keywords: Chemical Techniques; Microbial Activities; Disinfectants

Introduction

Control of microbial growth is an essential part of clinical, food safety and industrial microbiology. These may be biological or nonbiological agents used to inhibit or destroy microorganisms and it is important to know the relative effectiveness of these agents to use them correctly. Disinfection and antisepsis are the reduction of microbial numbers on inanimate and living surfaces, respectively, while sterilisation is the complete destruction of all microbes including endospores [1].

Moist heat sterilisation is done by autoclaving at 121°C and 15 psi for at least 15-20 minutes, which is enough to denature essential proteins and kill endospores [2]. If packing of the autoclave is not done properly, there may be air pockets that will not allow steam to penetrate and thus the efficiency of sterilisation will be affected. The spores produced by *B. subtilis* are among the most heat-resistant biological structures known and are used as a test organism for validating sterilisations [3].

Chemical Disinfectants and Chemical Antiseptics have different modes of action and spectrum of action. The modes of action of chlorine-based compounds like White King rely on oxidative damage to cellular macromolecules, while chlorhexidine causes damage to the membrane and Betadine liberates free iodine as the active antimicrobial agent [4]. Bacteria vary in their susceptibility to these agents, being more susceptible to certain agents than others, due to differences in cell-wall architecture between Gram-positive and Gram-negative bacteria.

Antibiotic sensitivity test is carried out by Kirby-bauer disc diffusion method which classifies the isolates as Sensitive (S) or Resistant (R) depending on the diameter of zone of growth inhibition. The size of the inhibition zone indicates susceptibility as well as diffusion properties of the antibiotics [5].

The MIC is the lowest concentration of the antimicrobial agent that shows a definite inhibitory effect on the growth of bacteria and plays a crucial role in determining clinical doses [6]. The process of UV Irradiation of DNA at around 260 nm produces pyrimidine Dimers, primarily thymine Dimers, which disrupt the ability of DNA to replicate and cause cell death or mutation [1].

The objective of this investigation was to assess the relative effectiveness of autoclave sterilisations, selected disinfectants, antibiotics (6 species), phenol as a disinfectant, UV and dry heat treatment for controlling the growth of representative bacteria species.

Materials and Methods

Experimental methods used were as per the BIO20002/BIO60002 Laboratory Manual [7] of



OPEN ACCESS

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Received Date: 07 Aug 2026

Accepted Date: 16 Sep 2026

Published Date: 18 Sep 2026

Citation:

Asghar A, Ali H. The Microbial World:
Control of Microbial Growth. WebLog J
Microbiol. wjm.2026.i1801. <https://doi.org/10.5281/zenodo.23103418>

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Swinburne University of Technology, Hawthorn campus, in Semester 1, 2026 at the microbiology laboratories of Swinburne University of Technology, Hawthorn campus.

In autoclave sterilisation (Part 1 Experiment 1): Two McCartney bottles were prepared with spores of *B. subtilis* – one was tightly packed with cotton wool in a can (Bottle A) and the other was packed without cotton wool (Bottle B). The two bottles were autoclaved at 121°C for 15 minutes. After cooling, a loopful from each bottle was streaked onto Nutrient Agar (NA) and incubated at 37°C for 48 hours, and then checked for growth or no growth [8-12].

Efficacy of disinfectant was evaluated by disc diffusion (Experiment 2). *B. subtilis* was swab inoculated on NA plates to prepare *B. subtilis* lawn. Six disinfectants were used: chlorhexidine 0.5% (Chlorhexidine), white king (chlorine bleach), Savlon 1:20, Dettol 1:20, Tea Tree Oil and Betadine. These were poured onto the surface with the inoculated surface as sterile filter paper discs. Plates were incubated at 37°C for 48 hours and the diameter of the zone of inhibition was recorded semi-quantitatively (S+ to S++++ for sensitive and R for resistant). Additionally, the results of the *E. coli* and *Staphylococcus aureus* tests were gathered.

The same disc diffusion principle was utilised in antibiotic sensitivity testing, Experiment 3. Lawns of *S. epidermidis* and *E. coli* were set up on NA and seeded with six Oxoid antibiotic discs (Penicillin G (P), Ampicillin (Amp), Tetracycline (TE), Chloramphenicol (C), Streptomycin (S), Sulphafurazole (SF)). Plates were incubated for 24 hours at 37°C. The results were reported as sensitive (S, zone diameter ≥12 mm) or resistant (R, zone diameter <12 mm).

A serial 2-fold dilution of phenol (stock: 1:50) was performed for the MIC determination (Experiment 4) in nutrient broth in 5 tubes (final concentrations: 1:100, 1:200, 1:400, 1:800, 1:1600); the 6th tube contained nutrient broth only as a positive growth control. The *E. coli* broth culture (0.1 mL) were added to each of the tubes, and then incubated at 37°C for 24 hours. The turbidity was estimated by visual inspection and growth was indicated as either present (+) or absent (–).

Bacteria *B. subtilis* was subjected to heat treatment and UV irradiation for Part 2. An overnight culture was diluted 10× into nutrient broth. Heat treatment: 3 mL of the diluted suspension was placed in a 60°C water bath and 0.1 mL aliquots were plated onto NA at 1, 2, 3, 4 and 5 minutes. Aliquots of the same dilution were added to NA plates and allowed to dry, then exposed to UV light for 5, 10, 20, 40, and 80 seconds. Each plate was incubated at 37°C for 24 hours before counting the colonies. The results of class-wide experiments were summed for each isolate group: *B. subtilis*, *E. coli*, *S. epidermidis* and *Serratia marcescens*.

Results

The autoclave sterilisation results of *B. subtilis* endospores at two packing conditions are shown in Table 1.

After autoclaving, there was no growth in either of the bottles, so both conditions of packing were considered to be sterilising.

The antimicrobial efficacy of six disinfectants and antiseptics against *B. subtilis* is summarised in Table 2.

Growth inhibition of *B. subtilis* occurred with all six agents to a greater or lesser extent. The largest zones of inhibition (S++++) were obtained by White King and Tea Tree Oil while Savlon and Dettol

Table 1: Growth of *Bacillus subtilis* after 15 minutes of autoclave treatment at 121°C under 2 packing conditions.

Treatment Condition	Bottle	Growth Observed
Improperly packed (cotton-wool insulated)	Bottle A	No growth
Properly packed (no insulation)	Bottle B	No growth

Table 2: *Bacillus subtilis* (Gram positive) sensitivity to six disinfectants as tested by disc diffusion method. S = Sensitive, R = Resistant, number of + = relative zone size.

Disinfectant	<i>B. subtilis</i> (Gram-positive)
White King (chlorine bleach)	S++++
Betadine (povidone-iodine)	S+++
Savlon 1:20	S++
Tea Tree Oil	S++++
Dettol 1:20	S++
Chlorhexidine 0.5%	S+++

Table 3: Disc diffusion sensitivity of *Staphylococcus epidermidis* and *Escherichia coli* to antibiotics. S = Susceptible; R = Resistant.

Antibiotic	<i>S. epidermidis</i> (Gram-positive)	<i>E. coli</i> (Gram-negative)
Penicillin G (P)	S	R
Ampicillin (Amp)	S	S
Tetracycline (TE)	S	S
Chloramphenicol (C)	S	S
Streptomycin (S)	S	S
Sulphafurazole (SF)	R	S

Table 4: After 24 hours incubation at 37°C, growth of *Escherichia coli* in 2-fold serial dilutions of phenol (stock: 1:50). – = no growth, + = growth.

Tube	Phenol Concentration	Growth (<i>E. coli</i>)
1	1:100	–
2	1:200	–
3	1:400	+
4	1:800	+
5	1:1600	+
PC (Control)	0 (broth only)	+

Table 5: The colony count of *Bacillus subtilis* after heat treatment at 60°C for different periods of time (10-6 dilution).

Heating Duration (60°C)	Colonies Formed (<i>B. subtilis</i>)
Control (0 min)	~11 (class avg)
1 min	5
2 min	7
3 min	9
4 min	13
5 min	7

were the least effective (S++).

The antibiotic sensitivity profiles of *S. epidermidis* and *E. coli* are shown in Table 3.

S. epidermidis was resistant to only one of the six antibiotics tested - Sulphafurazole - while others were sensitive. *E. coli* on the other hand was resistant to Penicillin G but sensitive to all other agents tested.

Table 6: Colony counts for *Bacillus subtilis* (10-6 dilution) following UV irradiation at 260 nm for varying durations.

UV Exposure Duration	Colonies Formed (<i>B. subtilis</i>)
Control (0 s)	~11 (class avg)
5 s	5
10 s	5
20 s	2
40 s	0
80 s	0

The MIC of phenol against *E. coli* is given in Table 4.

Tubes 1 and 2 (phenol concentrations 1:100 and 1:200, respectively) showed no growth while growth was observed in all the remaining tubes. Therefore, in these experimental conditions, the MIC of phenol was 1: 200. Positive control did not reveal any change in bacterial viability during the experiment.

The individual results of the heat treatment of *B. subtilis* at 60°C are shown in Table 5 and the UV irradiation results are shown in Table 6.

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