



# Effect of Antimicrobial peptides and chemicals produced by animals on *Streptococcus pyogenes*

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## Abstract

There are about 700 million infections occur annually by *Streptococcus pyogenes* species around the world. Although the mortality rate of these infections does not exceed 0.1%. Among over 650 thousands cases which are invasive and severe, their mortality rate is 25%. Certainly early diagnosis and early treatment play an important role in severity prevention; failure of right diagnosis can lead to sepsis and death.

*Streptococcus pyogenes* species infect the throat, genitalia, rectum and even skin. Among healthy individual, there are 1% to 5% carrier of *Streptococcus pyogenes* infection in skin, rectum and vaginal without appearance of any signs or symptoms. Among children, about 17% are carriers of these bacteria. There are four methods of transmission of *Streptococcus pyogenes* species infection which are inhalation of droplets, contact with contaminated objects like surfaces or dust, skin contact and rarely contaminated food. These bacteria can cause deleterious diseases like pharyngitis rheumatic heart disease and scarlet fever which caused by streptococcal infections. Although pharyngitis is caused mainly by viral infection, about 15% to 30% of pharyngitis infections are streptococcal in origin. The rate of pharyngitis infection in adult is less than children infection due to children exposure in nurseries, schools, playgrounds as well as the low host immunity. Infection rate increases during winter time every year.

Metabolic type 1 clone is associated with *Streptococcus pyogenes* species infection. Mortality rate of *Streptococcus pyogenes* was high in developed country especially before the penicillin era, but the rate decreased after penicillin availability. So, the environmental factors play a vital role in streptococcal infection. Streptococcal infections are more frequently in men rather than women. With patient with risk factors like rheumatic heart disease, cancer, viral infections like covid-19 and flu and surgical incision, streptococcal infection occurs to 17% to 25% of those cases. Among children; chicken pox represents an important risk factor to increase possibility of streptococcal infection. However, 30% of people can be infected with *Streptococcus pyogenes* species without any previous risk factors. Rheumatic heart disease and scarlet fever occur after throat infection.

In this manuscript, some antimicrobial chemicals extracted from animals will be shown and their activity against *Streptococcus pyogenes* species using optical density analysis technique then their minimum inhibitory concentrations (MIC) will be determined as well as IC<sub>50</sub> to measure the potency to inhibit a biological function using programmes like Gene5, graph pad prism as well as testing antimicrobial activity of some chemicals which are found in animal secretions.

## Introduction

### *Streptococcus pyogenes* species serotyping

Serotyping of *Streptococcus pyogenes* species are based on its cell wall polysaccharide which represents the virulence factor on its surface. *Streptococcus pyogenes* species can be also serotyped according to its surface T-antigen. Bacteria's pili are 4 among 20 T-antigen which bacteria use them to adhere to the host cell. Finally, M protein was declared as a serotype which is encoded in 2016 with greater than 1200 alleles.

### Biofilm formation

Biofilm is a method which used by bacteria for communication as well as most bacterial

cells. In the biofilms gene expression is used for multiple purposes like defending against host immune system and short hydrophobic peptide regulation. The mode of transportation and processing factors are still unknown. Biofilm cytosol has a vital function which is binding to transcriptional regulators to increase short hydrophobic peptide production. Increasing short hydrophobic peptide increases biogenesis of biofilm [2]. Cysteine protease is considered as a virulence factor. In its absence, formation of biofilm is enhanced.

Animals are exposed to legion pathogens daily by intake, inhalation and call and their natural immunity plays a significant role in their resistance to infection. The role of

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antimicrobial peptides has been magnified apparently [3], and there's a promising proof has been discovered from decades reports that their antimicrobial action as necessary because the host protein, immune cells and phagocytes.

### Disease caused by *Streptococcus pyogenes*

Infections due to *Streptococcus pyogenes* are associated with its secretion of toxins. Infections of throat are associated with toxins which lead to scarlet fever. Other toxigenic *Streptococcus pyogenes* species can cause streptococcal shock syndrome which can lead to death [4].

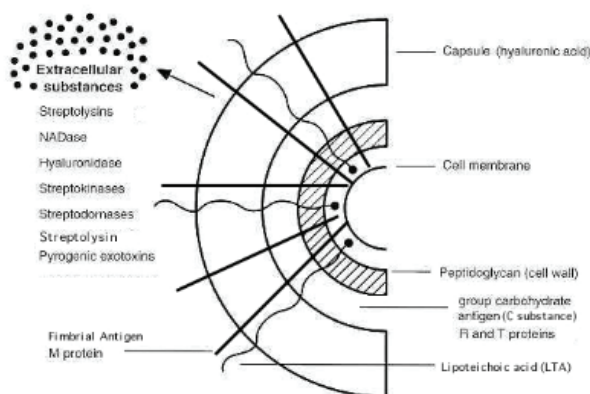
#### Examples of species secrete antimicrobial peptides:

Most species produce antimicrobial peptides as well as antimicrobial peptides made by bacterium, fungi, hydra, insects like bee produces mastoparan, melittin, poneratoxin, moricin, ceropin et al. [5], frogs turn out dermaseptin and magainin [6], craniate turn out defensins and cathelicidin [7] and even mammals produce conjointly defensins, cathelicidin and protegrin. ion amide is meant from C-terminal domain of human blood platelet atomic number 20 [8] and today, the foremost wide used antimicrobial amide is nisin that is thus far the sole Food and Drug Administration approved antimicrobial peptide and it's used as a synthetic preservative [9].

*Streptococcus pyogenes* is an extracellular microorganism that can live within the host according to his defence mechanism circumstances. To achieve this, *Streptococcus pyogenes* develop numerous strategies to attack the immune system e.g. virulence factors which prevent phagocytosis. Using murine model of skin infection, survival within phagocytic cell constitutes an additional strategy *Streptococcus pyogenes* use to destroy host defence and disseminate. The capacity of the intracellular bacteria can be determined using gentamicin treated neutrophils from infected mice and transferred into healthy mice. The ability of *Streptococcus pyogenes* may include addition mechanism to resist and exploit host inflammatory response.

### Antimicrobial Chemicals

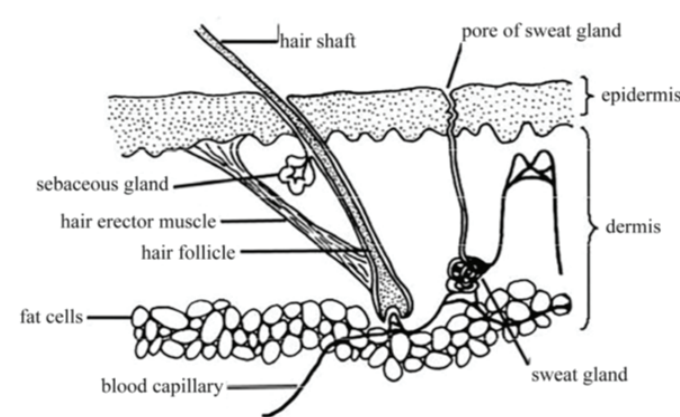
Animals of times use metabolites made by dependent bacterium for defence against microorganisms like bacteria, parasites and alternative pathogens. Secretion from the sweat and oily glands from some animals like giraffes and deer and secretion from preen glands of birds are used for this purpose though these chemicals are employed by the bird apparently.



**Figure 1.** cell surface structure of *Streptococcus pyogenes* and its secretion cause virulence

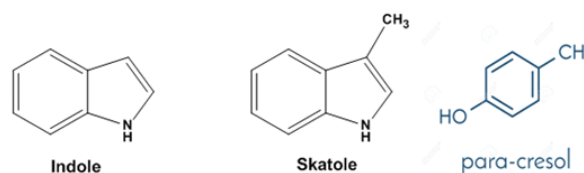
### Types of anti-biofilm agents which produced naturally

Pathogenic microorganisms are concerned in scientific research. Biofilm-linked infections are resistant to be treated due to antimicrobial resistance. Due to multidrug resistance of the conventional antibiotics as well as their toxicity in some doses led researchers to find natural anti-biofilm agents. Natural extract and natural product-based anti-biofilm agents are more efficient and safer than the conventional antibiotics with also fewer side effects. Types of anti-biofilm agent are various e.g. antimicrobial bio-surfactants, phytochemicals, antimicrobial peptides and chemicals and microbial enzymes, mechanism of action of the agents mentioned above is mostly interference with pathways of biofilm-forming and metabolism pathway as disruption of polymeric substances, adhesion mechanism and their minimal inhibitory concentrations. Natural anti-biofilm reagents are working against many types of microorganisms; this can help scientists to use them as combinations to kill several microorganisms safely. Alternative example, the glands and hair of giraffes contains several varied bactericide chemicals like indole, alkyl group indole or skatole and phenol. There mixture and combination turn out synergistic result quite victimisation all one by one which might be used for defence against being as phenol is employed wide for preservation of internal secretion.



**Figure 2.** The anatomy of giraffes' skin showing deep and superficial dermis with sweat, sebaceous glands and hair.

The Giraffe produces these chemicals which might be detected by totally different ways, methylene chloride or DMSO of hair samples of male and female Giraffes were analysed by gas chromatography/ mass spectroscopy that produces two chemicals which are indole and 3-methyl indole which are powerfully accountable to the robust scent of the giraffe, alternative chemical extracts are p-cresol, heptanal, octanal, nonanal, benzaldehyde, octane, hexadecenoic acid and tetradecanoic acid.



**Figure 3.** The chemical structure of main chemicals of giraffes' hair indole, skatole and p-cresol.

## Application in bio-nanotechnology

*Streptococcus pyogenes* species have characteristic properties, which are used to produce superglue [10,11] and also used for improving antibody therapy [12].

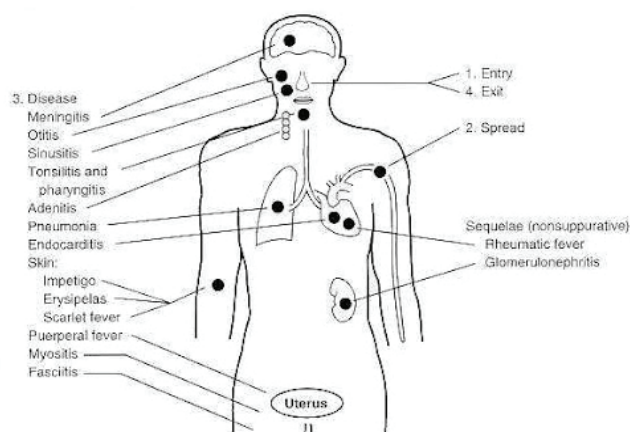
### *Streptococcus pyogenes* and CRISPR technique

Genome editing using CRISPR system from this bacterium [13] which is used for DNA destruction of the viral invasion to prevent the infection was adopted to alter DNA and then RNA.

### Pathogenic mechanism of acute rheumatic fever

The pathogenesis of acute rheumatic fever is a result from the virulence factor of *Streptococcus pyogenes* and host. T-cells play an important role in integration between infected tissue and epitopes of type 5 M-protein [14]. B-lymphocytes play a major role in activating antibodies target M-protein in cardiac tissue as myosin [15]. Interestingly anti-myosin antibodies attack also cardiac endothelium [16]. So, because antibody against M-protein develops in the patient infected with group (A) streptococcal pharyngitis, antibody can fix the complement, which in turn damage and activate cytokines and chemokine which attract and activate the t-lymphocytes. So, mimicry reaction between specific epitopes of M-protein and cardiac tissue leads to damage of endothelium of cardiac valves by B lymphocytes and T-lymphocytes.

Streptococcus is a gram-positive bacterium which may cause pharyngitis, impetigo and erysipelas & cellulitis. It is very sensitive to penicillin and for bio nanotechnology it produces superglue and enhances effectiveness of antibody therapy. Its DNA and RNA are altered by CRISPR. It is grown on brain heart infusion medium [17].



**Figure 4.** Human anatomy and the most important streptococcal diseases which infect human being. Entry and exit through nasal droplets, spread and diseases e.g. meningitis, otitis, sinusitis, tonsillitis, pharyngitis, adenitis, pneumonia, endocarditis, impetigo, erysipelas, scarlet fever, puerperal fever, myositis, fasciitis, rheumatic fever and glomerulonephritis.

### Therapeutic applications of animal secretions

Animal peptides and chemicals are broad spectrum antimicrobial agents that have disinfectant and static activities against gram positive, gram negative, protozoa and fungi.

Erysipelas, for example, is an acute inflammation of skin

characterized by lymphatic vessels involvement. It is found mostly in infants and adults aged more than 30 years. Always erysipelas was found mostly on face. However, it could be found also in 85% of infections in legs and feet [18]. It is characterized by sore throat and can be found also on the same position of surgical incisions, especially when the surgery includes trunk or extremities. The lesions are characterized by toxicity and fever. The rash itself is scarlet colour or has the same colour of salmon. Blood cultures are positive in 5% of patients. Facial erysipelas is self-limited within 10 days. The cornerstone of treatment involves mainly penicillin. Superficial infections may be treated orally within 10 days. While more aggressive infections may need parenteral treatment with antibiotics like nafcillin, clindamycin or third generation cephalosporin as ceftriaxone.

Surgical treatment is required as well as antimicrobial therapy. Due to the inoculum effect, penicillin may be less effective in addition to its serious side effects as hypersensitivity reactions which lead to anaphylactic shock and death [19] so the most appropriate antibiotics are natural antibiotics.

In some animals e.g., arthropod, the haemocyte that could be a current cell accountable to blood coagulation in class blood platelet, aggregates at the position of the wound cathartic antimicrobial agents and physiological systems that they're expressed in giant half represent the division of host animal weapons system which referred to as natural immunity.

The study of those antimicrobial animal secretions was concerned from totally different animal species as well as frogs, mammals and even sharks. each basic studies of those categories of antimicrobials and their biological systems in addition as apply them for human therapeutic application are concerned as sprays, patches, socks for leg ulceration to diabetic patients. Animal derived antimicrobial peptides and chemicals may be used for plants to repel and kill pests and insects.

## Materials and methods

### Chemicals

#### Overview of MIC testing

To summarise minimum inhibitory concentration test, a culture of pure microorganisms had been grown in the appropriate broth. The culture had been standardized using recognized standard microbiological techniques to obtain concentration equals nearly 1 million cells/ml. the more standardized microbiological culture the more reproducible obtained test results.

The antimicrobial substance frequently had been diluted, usually 1:1 using the appropriate diluent. After dilution of the antimicrobial, volume of the standard volume equals the volume of diluted antimicrobial had been added for each dilution vessel until reaching the microbial concentration nearly to 500000 cells/ml.

Incubate the serial diluted antimicrobial substance at the proper temperature according to the type of the microorganism for the right duration which is between 18 to 24 hours, the longer incubation duration, the more reproducible results obtained.

Observe the serial diluted vessels after incubation to screen microbial growth which can be detected by turbidity or pellet formation in the bottom of vessel, the last diluted vessel which that had not demonstrated growth, turbidity or pellet formation determines the minimum inhibitory concentration of the antimicrobial agent.



#### Advantages of MIC:

- Easy to prepare and straightforward which permits reproducibility
- Can be done on a very small scale without needing to use high amounts of the antimicrobial agent which is very important for antimicrobial agents used experimentally e.g. antimicrobial peptides which are synthesized biologically

#### Disadvantages of MIC:

- The variations of parameters of MIC test can affect the apparent results of MIC. For example, the long incubation will show higher MIC and lower inoculum concentration can result apparent lower MIC
- In bacteriostatic antimicrobial agent, some microorganisms will stop growing but not killed, there can be still the same number of cells waiting until the antimicrobial agent is neutralized

### Microbiology culture preparation

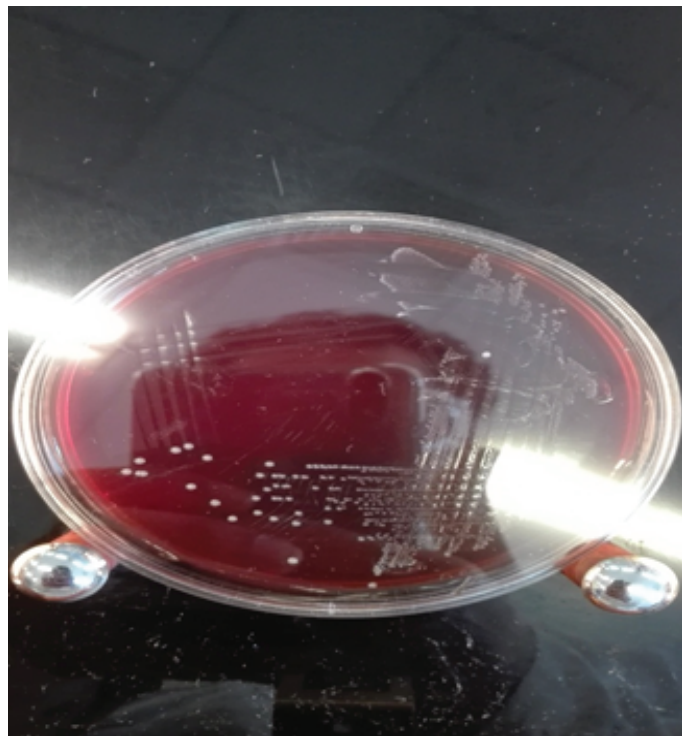
It depends mainly culturing each microorganism in its proper culture media and incubation in the proper temperature. You need brain heart infusion culture medium

Prepare amount per 500 ml of water and to prepare Incubate this bottle of brain heart infusion overnight in the autoclave in 121 Celsius degrees.

*Streptococcus pyogenes* is grown in brain heart infusion broth.

Prepare 10 tubes for each media then fill each with 10 ml of the culture and bring Petri dishes containing each individual microorganism.

Prepare culture media for each microorganism by the following:



**Figure 5.** Incubating *Streptococcus pyogenes* culture in 37.5 degrees.

Label each bottle with the name of the culture media and the intended microorganism (*Streptococcus pyogenes*)

Take a wire loop and place the wire loop in the flame of the Bunsen burner until glowing red along its length for sterilization then leave the loop to cool down

Petri dish had been picked up and open the lid had been opened to make a streak then the lid of the tube had been opened of the appropriate culture media and loop had been dipped into the bottle.

These steps had been repeated for each microorganism then incubated overnight each one in the right incubation temperature.

The culture had been diluted by adjusting the ratios between bacterial culture and broth.

**Table 1.** Dilution culture of *Streptococci* shows the ratios and amounts of culture media and broth in ml.

Name of M. O	Ratio	Culture (ml)	Broth (ml)
Streptococci	1:57	0.5	28

### Optical density analysis

In a clean tube the solvent only is put just to tare the device then from 10 ml of the tube aseptically 1/20 culture had been prepared to solvent by insertion 50µl of culture against 950 µl T.E buffer (tris and EDTA) and they had been measured at wave length 600 nm then cultures had been diluted to 0.5 in absorbance 0.063 e.g. if 6.3 O. D 500 µl 49.5 ml=1/100. The resulted value had been multiplied in 20 to obtain the final optical density.

If optical density is so high, 1/40 ratio could have been prepared which had been represented by 25 µl of culture mixed with 975 µl of T.E.

### Chemicals preparation

Using DMSO as a solvent extract indole, methyl indole (skatole) and p-cresol

Four of 24 well plates had been prepared for each individual microorganism and had been divided as follows:

**Table 2.** Scheme shows the content of the 24 well plates .

	1	2	3	4	5	6
A	1	2	3	4	5	6
B	1	2	3	4	5	6
C	7	8	9 (+)	10 (-)	Empty	Empty
D	7	8	9 (+)	10 (-)	Empty	Empty

according to their contents and concentrations of broth, cells and chemical 1 refers to 20 mM, 2 refers to 10 mM, 3 refers to 5mM, 4 refers to 1mM, 5 refers to 500 µM, 6 refers to 250 µM, 7 refers to 25 µM, 8 refers to 12.5 µM, 9 represents positive control contains cells without chemicals and 10 represents negative control doesn't contain any cells or chemicals but contains just media

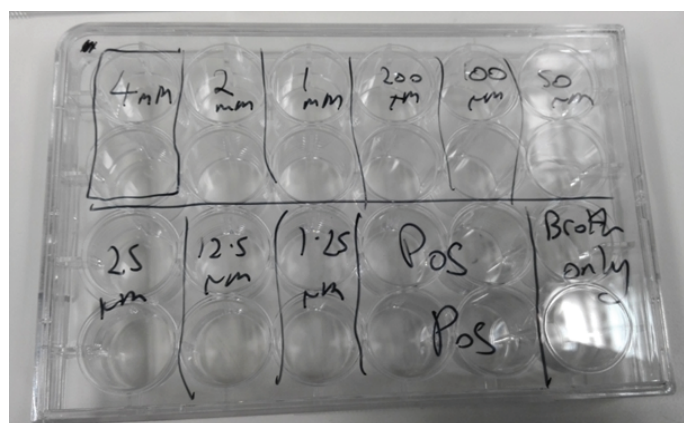
**Table 3.** The ratios and concentrations of media and cells required in each well according to fig. 3 before adding chemical.

Code	Well number	Ratios	Concentrations
1	A1 and B1	1	20 mM
2	A2 and B2	$\frac{1}{2}$	10 mM
3	A3 and B3	$\frac{1}{2}$	5 mM
4	A4 and B4	$\frac{1}{5}$	1 mM
5	A5 and B5	$\frac{1}{2}$	500 $\mu$ M
6	A6 and B6	$\frac{1}{2}$	250 $\mu$ M
7	C1 and D1	$\frac{1}{10}$	25 $\mu$ M
8	C2 and D2	$\frac{1}{2}$	12.5 $\mu$ M
9	C3 and D3	Positive control	just Cells
10	C4 and D4	Negative control	No cells
Empty	C5 and D5	Empty	Empty
Empty	C6 and D6	Empty	Empty

In the First plate put 20  $\mu$ M of indole to 500  $\mu$ M of the cells and media in all wells except the last eight wells, put the cells only without chemicals in two wells as a positive control and in another two wells put the media only as a negative control.

In the Second plate 20  $\mu$ M of skatole was placed to 500  $\mu$ M of the cells and media in all wells except the last eight wells, put the cells only without chemicals in two wells as a positive control and in another two wells put the media only as a negative control.

In the Third plate put 20  $\mu$ M of p-cresol to 500  $\mu$ M of the cells and media in all wells except the last eight wells, put the cells only without chemicals in two wells as a positive control and in another two wells put the media only as a negative control.

**Figure 6.** Scheme shows the content of the 24 well plates according to their contents and concentrations of broth, cells and chemicals.

In the Fourth plate put 20  $\mu$ M of blend of indole, cresol and skatole to 500  $\mu$ M of the cells and media in all wells except the last eight wells, put the cells only without chemicals in two wells as a positive control and in another two wells put the media only as a negative

Repeat this process for each plate with changing microorganisms used by making four plates for each microorganism each individual one contains indole, methyl indole (skatole), p-cresol and blend then label each plate according to the type of cells, media, chemical used control according table. 3 then incubate them overnight.

**Table 4.** The ratios and concentrations of chemicals with media and cells required in each well according to fig. 3 after adding chemical.

Code	Well number	Dilutions	Concentrations
1	A1 and B1	1	4000 $\mu$ M
2	A2 and B2	$\frac{1}{2}$	2000 $\mu$ M
3	A3 and B3	$\frac{1}{2}$	1000 $\mu$ M
4	A4 and B4	$\frac{1}{5}$	200 $\mu$ M
5	A5 and B5	$\frac{1}{2}$	100 $\mu$ M
6	A6 and B6	$\frac{1}{2}$	50 $\mu$ M
7	C1 and D1	$\frac{1}{2}$	25 $\mu$ M
8	C2 and D2	$\frac{1}{2}$	12.5 $\mu$ M
9	C3 and D3	$\frac{1}{10}$	1.25 $\mu$ M
10	C4 and D4	Positive control	Chemicals
Empty	C5 and D5	Positive control	Chemicals
Empty	C6 and D6	Negative control	Broth only

### Optical density analysis with chemicals

Using Gen5 software enter each plate individually to get the results showing the optical density through fluorescence assays of each well which evaluate the effect of each chemical and the response of the microorganism to it, input the results into excel sheets showing the concentrations of wells, names of microorganisms and chemicals used. Optical density is more preferred than ultraviolet spectrometry because the ultraviolet wave itself had an antibacterial activity which may interfere with the results of chemical effect on microorganisms. Calculate the percentage of potency by the equation:

$$\% \text{ of potency} = 100 - (\text{O.D of chemical} / \text{O.D of Blend}) * 100$$

### Statistical analysis for microorganisms

Using GraphPad prism input the data obtained from the excel sheet of gene 5 server then make non-linear regression to obtain graph showing the growth inhibition of each microorganism as well as calculation IC50, this curve can be fitted by dose response inhibition and then drawing log inhibitor by  $\mu$ M versus response to obtain variable slopes consist of four parameters and when you had more optical density you will need to reduce concentration and hence the lower IC is indication to the higher potency which means that the minimum dose of chemical is effective.

## Results

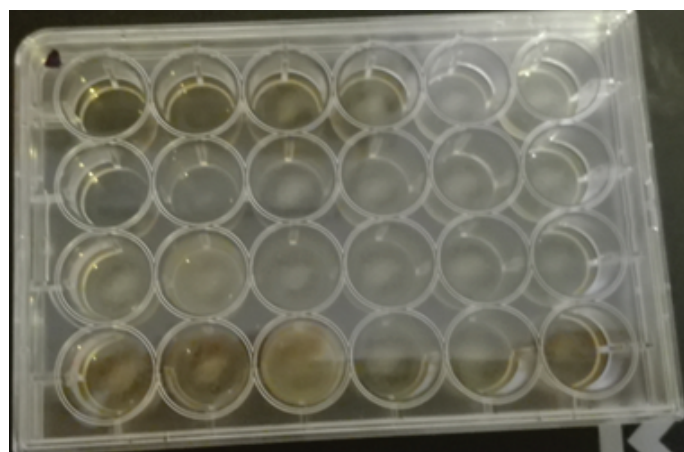
### Optical density measurement

**Table 5.** Optical density results for *Streptococcus pyogenes* according to readings multiplied by 20.

	Reading	Optical density
Blank	Zero	Zero
Streptococci	0.13	2.6

### Chemical preparation and Optical density analysis with chemicals

It depends on the types and species of microorganisms as well as types and concentrations of chemicals, as shown in the following figures there are some microorganisms affected with chemicals in given concentration and others has not affected enough with the chemicals and need higher concentration. For



**Figure 7.** The effect of blend on *Streptococcus* showing growth inhibition in concentrations from 4 mM until 2mM.

**Table 6.** Values of optical density values between concentrations from 4 mM until 1.25µM as well as positive and negative controls related to the effect of blend on *Streptococci*.

Concentration					Mean
4mM	0.102	0.105			0.1035
2 mM	0.185	0.321			0.253
1mM	0.284	0.375			0.3295
200 µM	0.526	0.529			0.5275
100 µM	0.518	0.485			0.5015
50 µM	0.523	0.464			0.4935
25µM	0.561	0.559			0.56
12.5µM	0.884	0.57			0.727
1.25µM	0.513	1.019			0.766
Positive controls	0.498	0.458	0.459	0.392	0.45175
Negative controls	0.299	0.234			0.2665

optical densities means of results related to each concentration is calculated including positive and negative controls.

### *Streptococcus pyogenes*

#### Blend

It is showing that it killed cells in concentrations from 4 mM until the concentration of 2mM and after that it wasn't effective against cells.

#### Cresol

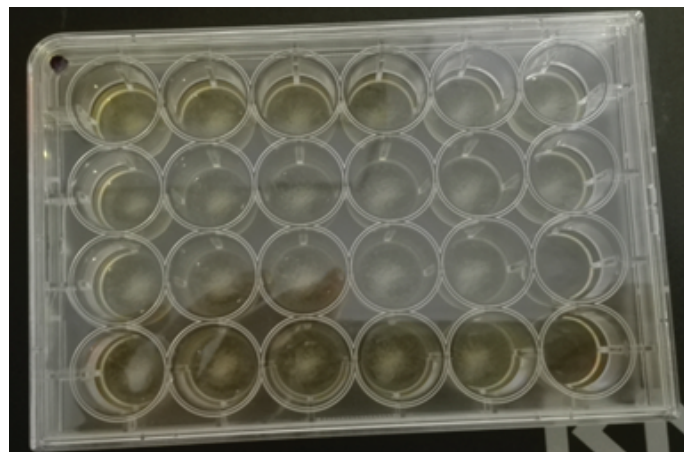
It is showing that it did not kill cells in any concentrations from 4 mM until the concentration of 1.25µM.

#### Indole

It is showing that it killed cells in only concentration of 4 mM.

#### Skatole

It is showing that it killed cells in concentrations from 4 mM until the concentration of 2mM and after that it wasn't effective against cells.



**Figure 8.** The effect of cresol on *Streptococcus* showing no growth inhibition in any concentrations.

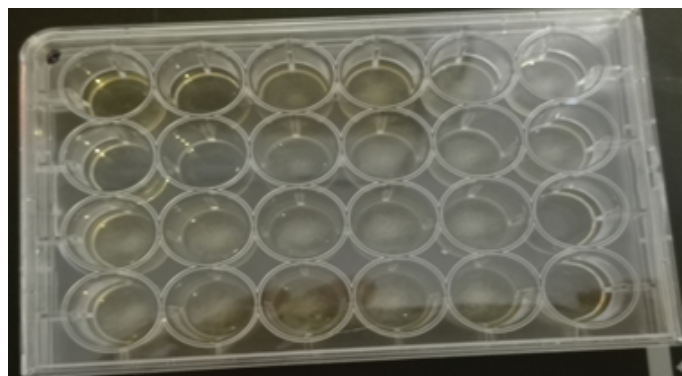
**Table 7.** Values of optical density values between concentrations from 4 mM until 1.25µM as well as positive and negative controls related to the effect of cresol on *Streptococci*

Concentration					Mean
4mM	0.481	0.472			0.4765
2 mM	0.545	0.575			0.56
1mM	0.514	0.517			0.5155
200 µM	0.446	0.437			0.4415
100 µM	0.419	0.383			0.401
50 µM	0.442	0.374			0.408
25µM	0.485	0.477			0.481
12.5µM	0.48	0.477			0.4785
1.25µM	0.467	0.458			0.4625
Positive controls	0.448	0.425	0.415	0.373	0.41525
Negative controls	0.096	0.098			0.097



**Table 7.** Values of optical density values between concentrations from 4 mM until 1.25 $\mu$ M as well as positive and negative controls related to the effect of indole on Streptococci.

Concentration					Mean
4mM	0.104	0.112			0.108
2 mM	0.342	0.407			0.3745
1mM	0.381	0.452			0.4165
200 $\mu$ M	0.496	0.481			0.4885
100 $\mu$ M	0.476	0.445			0.4605
50 $\mu$ M	0.481	0.431			0.456
25 $\mu$ M	0.521	0.506			0.5135
12.5 $\mu$ M	0.506	0.509			0.5075
1.25 $\mu$ M	0.494	1.165			0.8295
Positive controls	0.46	0.427	0.439	0.372	0.4245
Negative controls	0.091	0.095			0.093



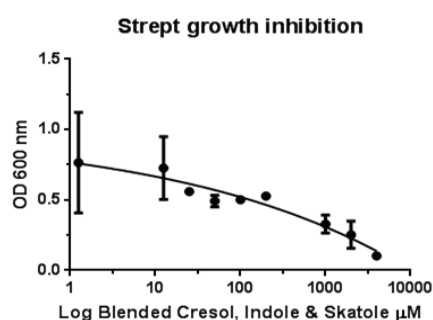
**Figure 10.** The effect of skatole on Streptococcus showing growth inhibition in concentrations from 4 mM until 2mM.

### Statistical analysis

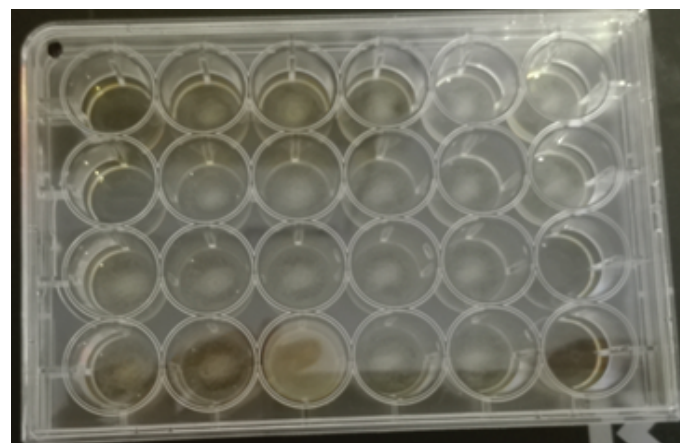
Making graphs illustrating microorganism growth inhibition by drawing a graph between OD 600 by nm and logarithm of chemicals by  $\mu$ M given to obtain the inhibitory concentration IC<sub>50</sub> and R square which will lead to measure the potency according to the values of the previous tables.

### Streptococci

#### Blend



**Figure 11.** GraphPad shows optical density by Nano meter against log of blend related to Streptococci.



**Figure 9.** The effect of indole on Streptococcus showing growth inhibition in concentration of 4 mM.

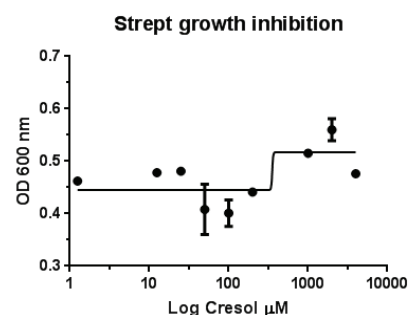
**Table 9.** Values of optical density values between concentrations from 4 mM until 1.25 $\mu$ M as well as positive and negative controls related to the effect of skatole on Streptococci.

Concentration					Mean
4mM	0.107	0.11			0.1085
2 mM	0.108	0.112			0.11
1mM	0.363	0.329			0.346
200 $\mu$ M	0.548	0.523			0.5355
100 $\mu$ M	0.491	0.468			0.4795
50 $\mu$ M	0.502	0.46			0.481
25 $\mu$ M	0.536	0.568			0.552
12.5 $\mu$ M	0.553	0.535			0.544
1.25 $\mu$ M	0.537	0.479			0.508
Positive controls	0.496	0.468	0.439	0.406	0.45225
Negative controls	0.09	0.091			0.0905

The graph shows OD 600 against log concentration of blend, as seen log IC<sub>50</sub> equals approximately 16.93 and R square is 0.7475 $\mu$ M.

#### Cresol

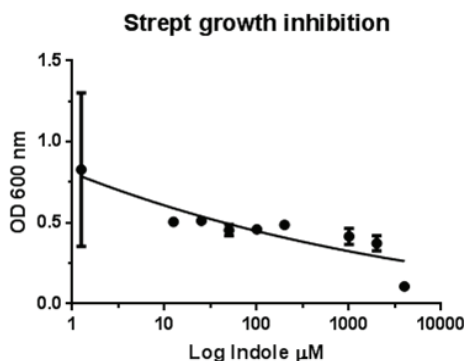
The graph below shows OD 600 against log concentration of cresol, as seen IC<sub>50</sub> equals approximately 353.8  $\mu$ M and R square is 0.4775 $\mu$ M



**Figure 12.** GraphPad shows optical density by Nano meter against log of cresol related to Streptococci.

### Indole

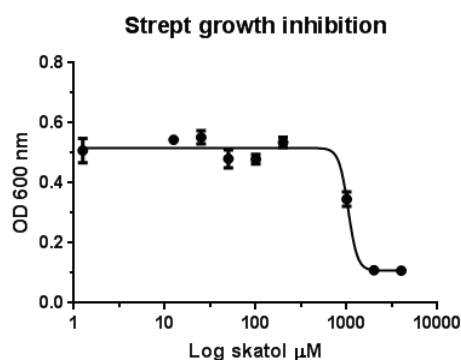
The graph below shows OD 600 against log concentration of indole, as seen IC<sub>50</sub> equals approximately 0  $\mu$ M and R square is 0.5599 $\mu$ M



**Figure 13.** GraphPad shows optical density by Nano meter against log of indole related to *Streptococci*.

### Skatole

The graph below shows OD 600 against log concentration of skatole, as seen the IC<sub>50</sub> average is between 750.6  $\mu$ M to 1439  $\mu$ M where IC<sub>50</sub> equals approximately 1039 $\mu$ M and R square is 0.972 $\mu$ M



**Figure 14.** GraphPad shows optical density by Nano meter against log of skatole related to *Streptococci*.

## Discussion

The previous figures were showing that blend of indole, skatole and cresol has synergistic effect more than using each chemical alone and, at the same time, it plays a major role in diluting the toxic effect of p-cresol. In case of observation statistical analysis resulted from graph pad prism, although the IC<sub>50</sub> is ambiguous in some graphs. However, this can help in determining the dose which is effective and economic in case this combination applied for medicinal uses.

### Streptococci

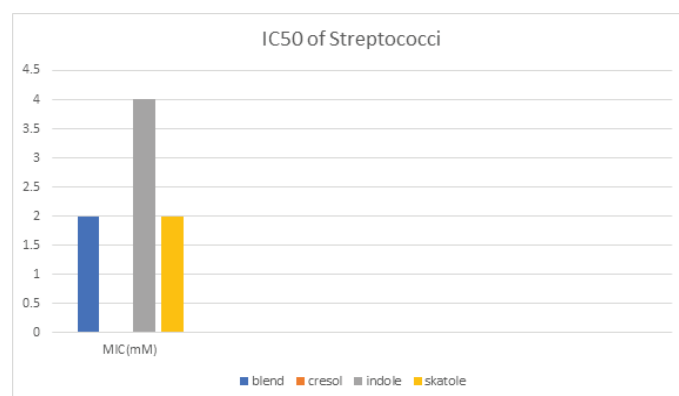
The chemical preparation showed cell death at least concentration with blend 2mM, no cells were killed with cresol,

4 mM with indole and 2 mM with skatole when using graph pad it showed IC<sub>50</sub> with cresol is 353.8  $\mu$ M and IC<sub>50</sub> with skatole is 1039  $\mu$ M which indicates its therapeutic action as well as blend

P-cresol individually is not effective against *Streptococcus* which cannot be used in this mixture and this is advantageous to avoid its toxic effect on kidney. Though P-cresol particularly is employed in internal secretion as a preservative, the mechanisms underlying internal secretion resistance accompanies oftentimes with chronic uropathy (CKD) aren't understood, however the retention of renally excreted compounds plays a significant role during this method. one in every of these compounds is p-cresyl salt (PCS) that is protein-bound uremic poisonous substance that results from amino alcanoic acid metabolism by internal bacterium *Norma flora* thus it's supposed that P-cresyl sulphate is concerned in to chronic uropathy associated internal secretion resistance. Administration of p-cresyl salt to mice with traditional urinary organ operate for four weeks triggered internal secretion resistance, loss of fat mass and posture of distribution of fat in muscle and liver that is comparable to CKD options. Mice treated with PCS obtained altered internal secretion signalling in muscle. Subtotal cutting out crystal rectifier to internal secretion resistance and dyslipidaemia, and when giving prebiotic arabin-x-ylo-oligosaccharide that reduced blood serum PCS by decreasing internal organ production of p-cresol, prevented these metabolic disturbances. And after they were taken along, the data counsel that PCS contributes to internal secretion resistance which is targeting PCS is also therapeutic against CKD [20].

It's notable that uremic syndrome could be a consequence of the retention of solutes that are cleared by healthy kidneys. P-cresol may be thought of as an image of protein-bound uremic poisonous substance. It's analogous with medication, that the not-protein certain fraction of p-cresol exerts toxicity. This side had not been evaluated nor have the factors poignant the free fraction of p-cresol.

Generally, hypoalbuminemia and total p-cresol increase the free fraction of p-cresol, patients hospitalized with infections are detected with high level of free p-cresol. In vitro, free p-cresol high level contains a negative result on WBC production, thus dangerous nutrition might exacerbate the toxicity of p-cresol [21] however if p-cresol are used locally, this might decrease its toxicity and facet effects. So, the blend is more synergistic in fungal infection and skatole is the most potent component in this combination.



**Figure 15.** Chart showing IC<sub>50</sub> for *Streptococci* in presence if indole, methyl indole, skatole and blend



## Conclusion

Cloning and expression with yeasts can be done further with other protein peptides as lactoferrin and lactotransferrin which is found in the milk of many species of mammals and has antimicrobial activity. It can be extracted from milk of whales, camels and dolphins depending their hydrophobicity and  $\beta$ -sheets which facilitates its penetration to the cell wall of microbes.

Lactoferrin is a bioactive 80-kDa iron binding glycoprotein which has antiviral, antibacterial, antifungal and anticarcinogenic activity which its efficiency can be measured by measuring  $\alpha$  helices and  $\beta$  sheets, water hydrophobicity, negatively charged residues and estimated half-life using bioinformatics programmes like NCBI, ensemble, ExPasy-protparam, multiple sequence alignment, Uniprot and clone manager.

For chemicals, the blend of indole, skatole and cresol can be used for topical uses to protect the body against dermatological disturbances. This blend can be used topically as patches, ointments or creams especially for some dermatologic disorders.

Further studies are needed to understand the overall properties of this combination and its effect against more microorganisms and its inhibitory effect to liver microsomal enzymes.

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