Antibacterial activity of NESCAFÉ instant coffee beverages and pharyngitis-causing Streptococcus species

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Abstract

Coffee has been associated with many health benefits, one of them being its antibacterial action for which caffeine is widely believed to be responsible. This study investigated the antibacterial activities of decaffeinated and non-decaffeinated NESCAFÉ instant coffee against some pharyngitis-causing *Streptococcus* species – Group A Streptococcus, Group B Streptococcus, Group D Streptococcus and *Streptococcus pneumoniae* at eight different concentrations. NESCAFÉ brand of coffee, which is made and marketed by Nestlé, seems to be the most widely consumed instant coffee preparation the world over. Three sensitivity-testing methods were carried out: disc diffusion, well diffusion and Coffee Agar plate sensitivity testing. Zones of inhibition and growth on the plates were observed at 24 hours and 48 hours.

Results show no agreement between the disc and well diffusion methods with both showing inconsistent zones of inhibition on repeat experiments. However inhibition of growth on Coffee Agar plate was consistently shown with all *Streptococcus* species used. At the highest concentration of coffee solution in this study, which was 8g/100 ml, all the four *Streptococcus* species' growths were inhibited, with non-decaffeinated coffee acting much more strongly; this concentration is said not to be compatible with human health. Further studies are needed to understand the molecular mechanisms of the antibacterial action of these coffee beverages against the pharyngitis-causing *Streptococcus* species used in this study.

Keywords: *Antibacterial activity; Caffeine; Coffee; Molecular mechanisms; Streptococcus species*

Introduction

Coffee, the genus Coffea is a member of the family *Rubiaceae.* Its brews are widely consumed for its pleasant flavour and taste[1]. There are several varieties of coffee cultivated worldwide, but those that are commercially grown include *Coffea canephora* (*robusta*) and *Coffea Arabica*[2]. According to Brown (2007) coffee brew types (ground, instant, decaffeinated or espresso) vary depending upon the beans from which they are produced, how the beans are processed and the ingredients that are added. Usually *Coffea canephora (robusta)* are used for instant coffee[3].

Bioactive profile of coffee contains many of the most important constituents known to exist within functional foods - namely flavonoids (catechins, anthcyanins), ferrulic acids and caffeic acid – and biologically active components, such as nicotinic acid, trigonelline, quinolinic acid, tannic acid, pyrogallic acid and caffeine[4].

In coffee, the caffeine content varies widely depending on the type of coffee bean and the method of preparation used (http://www.ico.org/caffeine.asp). There are two basic techniques used to produce instant coffee powder: spray-drying and freeze-drying. The removal of caffeine from coffee would produce the decaffeinated forms of coffee, formerly by adding organic solvents (methylene chloride and ethyl acetate), and currently applied using the process of water-purification[3].

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Caffeine is widely consumed as a stimulant in the form of coffee, tea, soda beverages, chocolate, and many prescription and over-the-counter drugs[3, 5, 6]. For example, the average US daily consumption of caffeine is about 200 mg, with adults taking about 2.4 mg per kg per day and children aged between 5 and 18 years old taking 1.1 mg per kg per day[3].

Streptococcal species constitute one group of the causal organisms of bacterial sore throat, which is also known as pharyngitis[7], tonsillopharyngitis[8], or simply 'strep throat'[9]. The most clinically important ones are Group A Streptococcus (ß – haemolytic *Streptococcus pyogenes*) (GAS)[10] causes a majority of the pharyngitis cases[11, 9]. In addition to that, group B Streptococcus (GBS), group D Streptococcus (GDS) and *Streptococcus pneumoniae* are also implicated in the diagnosis of sore throat[12, 13, 14].

The antibacterial effect of coffee has been demonstrated in various studies carried out over the past 15 years[15], showing that growth of certain bacteria was either inhibited or stunted by different concentrations of coffee extract. For example, the growth of Gram negative bacteria *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* and Gram positive bacteria *Staphylococcus aureus*, *Bacillus cereus*, *Lactobacillus bulgaricus, Streptococcus lactis* and *S. faecalis* were inhibited[16], and in addition to that, decreases in bacterial growth were observed in *L. bulgaricus, E. coli, S. typhi* and *S. faecalis.*

It is widely believed that caffeine is the factor in coffee that is responsible for its antibacterial property[17]. A report showed that pure caffeine has a direct antibacterial effect[18]. Furthermore, caffeine works synergistically with some antibiotics, carbenicillin, ceftizoxime and gentamicin, which are effective on *P. aeruginosa* and *S. aureus* [19]. Coffee is also suggested to be anti-adhesive and thus provides an alternative method of caries prevention because it is able to reduce the adherence of *Streptococcus mutants* to dental surface[17]. It is possible that caffeine might also be playing a role in this. However, there are no reports in the literature on the effect of 'instant' coffee on GAS, GBS and *S. pneumoniae*: a few studies have been done on GDS.

Therefore, this study investigated the antibacterial activity of coffee on GAS, GBS, GDS and *S. pneumoniae* using two types of coffee beverages: instant non-decaffeinated and decaffeinated coffee under the proprietary name of NESCAFĖ, which seems to be the most widely consumed worldwide.

Materials and Methods

Bacterial strains

Bacterial strains were obtained as pure cultures on blood agar from the Microbiology Laboratory, Raja Isteri Pengiran Anak Saleha Hospital (RIPASH), Brunei Darussalam. The strains obtained were GAS, GBS, GDS and *S. pneumoniae*, all of which were previously isolated from throat swabs of patients with clinical symptoms of pharyngitis. Identification of the bacterial strains was done by their morphological and biochemical characterization. Bacterial strains were sub-cultured onto Blood Agar medium and Chocolate Agar medium by standard methods and incubated at 37°C for 24 hours.

Penicillin G as positive control

Stock solution of 10, 000 mg/L of penicillin G (SIGMA-ALDRICH) was made as described[20]. The content was vortexed well. 100 µl was decanted into each Eppendorf tube, for storage at -20°C. All the *Streptococcus* species used in this study were found to be sensitive to penicillin G[21]. The positive control is important because it ensures that the *Streptococcus* species had not mutated or changed their original sensitivity patterns during the study.

The pattern of zones of inhibition of growth in the *Streptococcus* strains were well correlated with penicillin G concentration. It was observed that the GAS and GBS were sensitive to 1 mg/L, while GDS and *S. pneumoniae* were sensitive to 10 mg/L. Thus to ensure that the zones of inhibition made by penicillin G did not interfere with the main observations in this study, the lowest concentration of penicillin G that showed zone of inhibition was chosen as positive control for the respective *Streptococcus* species, 1 mg/L for GAS and GBS, whilst 10 mg/L for GDS and *S. pneumoniae*.

Preparation of Coffee solution

Coffee grains (NESCAFÉ brand) were weighed, kept in labelled 150 ml beakers, and the beakers were covered with aluminium foil. The coffee was sterilised in a hot air oven at 160°C for one hour. Aliquots of 8 coffee solutions were made freshly for every experiment (10 g/L – 80 g/L). Each coffee solution was kept in the water bath (50°C) for about 15 minutes with shaking to ensure the uniformity of the coffee solutions.

Inoculum preparation by the direct colony suspension method

Streptococcus colonies were directly taken from a 24-hour subculture and aseptically inoculated into a 5 ml of sterile saline solution in a McCartney bottle. The bacterial suspension that matched or exceeded the density of a 0.5 McFarland standard (bioMerieux®) were estimated to contain 150,000,000 bacteria per ml and used fresh (within 15 minutes). *Streptococcus* species were uniformly spread by standard methods across a Blood Agar medium and a Chocolate Agar medium using a sterile swab for each species in any one experiment.

Disc diffusion test

Filter papers (Whatman® No. 1) were used and discs with 0.6 mm were punched out using a hole-puncher. Discs well wrapped in aluminium foil were sterilised in a hot air oven at 160°C for one hour. The sides of the agar plate were labelled around with the coffee concentrations. The discs were first planted on a Blood Agar medium and a Chocolate Agar medium. On to each of the discs 4 µl of coffee solutions and penicillin G solution were aseptically pipetted consecutively. The plate was allowed to stand for one hour for diffusion to take place and then incubated at 37°C in the incubator. Zones of growth inhibition surrounding the discs were measured at 24 hours and 48 hours under standard conditions.

Well diffusion test

Wells were aseptically punched out from the agar plate using pipette tips with a diameter of 6 mm. One of Blood Agar and Chocolate Agar medium was used for each *Streptococcus* species per experiment. 80 µl of coffee solutions and penicillin G were pipetted into the wells consecutively. The plate was allowed to stand for one hour for diffusion of coffee solutions and penicillin G to take place (the coffee solutions and penicillin G did not spill over onto the plate) and then incubated at 37°C and zones of growth inhibition surrounding the wells were measured at 24 hours and 48 hours.

Coffee agar medium preparation

Table 1 shows the recipe for Coffee Agar. The agar mixtures were autoclaved at 121°C for 20 minutes. The agar media were allowed to cool down in a water bath at 50°C. Defibrinated blood was then added gently but thoroughly mixed and then aseptically poured unto sterile Petri dishes in a laminar flow cabinet.

Culture of Streptococcus species on Coffee Agar

All the four *Streptococcus* species were inoculated from a 24 hours subculture onto Blood Agar medium. Bacterial suspensions were prepared following the direct colony method as described previously. The suspensions had uniform optical density measurements at 500 nm approximately, suggesting that the suspensions have equal amount of bacterial content[21]. 10 µl (estimated to contain 1.5 x 10^6 bacteria) of each suspension was streaked by standard method onto Coffee Agar medium with concentrations of 20 g/L, 40 g/L, 60 g/L, 80 g/L for both non-decaffeinated coffee and decaffeinated coffee. As positive controls, Blood agar and Chocolate Agar were used for each *Streptococcus* species. The cultures were incubated at 37°C and bacterial growth was observed after 24 hours and 48 hours.

The set of experiments were repeated twice with new batches of Blood Agar, Chocolate Agar and Coffee Agar.

Results

The sensitivity patterns of disc and well diffusion tests on GAS, GBS and *S. pneumoniae* were inconsistent. However, GDS showed an agreement between the two methods at 1g/100ml-3g/100ml decaffeinated and non-decaffeinated coffees.

Table 1: Recipe for making Coffee Agar.

	Non-decaffeinated coffee				Decaffeinated coffee			
Coffee concentrations (g/L)	20	40	60	80	20	40	60	80
Coffee mass (g)	5	10	15	20	5	10	15	20
Blood agar base (Difco) (g)	10	10	10	10	10	10	10	10
Distilled water (ml)	250	250	250	250	250	250	250	250
Defibrinated blood (ml)	12.5	12.5	12.5	12.5	12.5	12.5	12.5	12.5

Disc diffusion method

Zone of inhibition by coffee was much more apparent against GBS compared to the other *Streptococcus* species used in this study. Antibacterial property of coffee showed higher activity at non-decaffeinated form than decaffeinated form against GBS, GDS and *S. pneumoniae* but rarely visible against GAS. No pattern of inhibition around the discs was observed with increasing coffee concentration, thus making the method unreliable for use in this context (data not provided).

Well diffusion method

Decaffeinated coffee at 1g/100ml-3g/100ml was not able to inhibit the bacterial growth of GAS, and at 1-3g/100ml non-decaffeinated and decaffeinated coffee for both GBS and GDS (data not provided). Zones of inhibition were visible only with 4g/100ml and higher, and increased from 24 hours to 48 hours. For *S. pneumoniae*, only at 8g/100ml of both non-decaffeinated and decaffeinated coffee were zones of inhibition observed. The inhibitory effect of coffee that was not observed on GAS, GBS, GDS and *S. pneumoniae* at 24 hours later appeared at 48 hours.

Coffee Agar plate testing

The sensitivity patterns on Coffee Agar of GAS, GBS, GDS and *S. pneumoniae* were proportional to coffee concentrations (Table 2 and Table 3). Complete inhibition of all the four *Streptococcus* species at 24 hours are shown in 8g/100ml non-decaffeinated Coffee Agar. This similarity in the pattern was clearer at 48 hours.

Interestingly visual observation of the *Streptococcus* growth on Coffee Agar medium was able to show the decreasing degree of growth of the *Streptococcus* with increasing coffee content in the agar. Figure 1 and Figure 2 are the representatives of the growths on non-decaffeinated and decaffeinated coffee respectively. Similar results were obtained in the three sets of experiments carried out with different batches of Blood Agar, Chocolate Agar and Coffee Agar for each set.

Discussion

This study showed that with higher concentrations of both types of coffee preparation, the growth inhibition of the *Streptococcus* species increased. However, the minimum inhibitory concentration (MIC) of coffee solutions against all the *Streptococcus* species in this study was inconsistent. This is especially so with the disc diffusion and well diffusion methods. For example, using the disc diffusion method, the zone of inhibition on 6g/100ml non-decaffeinated coffee against GAS was 10 mm, whilst at its 8g/100ml it was 9 mm. This suggests that there is no direct correlation between minimum inhibitory concentrations and coffee concentrations. In addition, the disc and well diffusion methods showed no agreement. This is because *S. pneumoniae* showed zones of inhibitions on all the coffee concentrations by disc diffusion method, but none by well diffusion method.

It is true that the sensitivity of different bacteria to caffeine nonetheless can vary greatly depending on the concentration[18]. However the lack of agreement between the well and disc diffusion

Table 2: Growth of Streptococcus species on non-decaffeinated coffee agar.

		GAS		GBS		GDS		SP	
		24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
2g/100ml	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
4g/100ml	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
6g/100ml	1	+	+	+	+	+	+	+	+
	2	+	+	-	+	+	+	+	+
	3	-	+	-	+	-	+	-	+
8g/100ml	1	-	++	-	++	-	++	-	++
	2	-	-	-	-	-	+	-	-
	3	-	-	-	-	0	0	0	0

Key:
GAS, group A Streptococcus;
GBS, group B Streptococcus;
GDS, group D Streptococcus;
SP, *Streptococcus pneumoniae*;
+, bacterial growth was observed;
-, bacterial growth was not observed;
2g/100ml-8g/100ml, coffee concentration in the making of the coffee agar,
24 hours, observation at 24 hours;
48 hours, observation at 48 hours;
1-3, triplicate experiments;
0, no repetition of experiment was performed for the third time;
++, very small growths.

Table 3: Growth of *Streptococcus* species on decaffeinated coffee agar.

		GAS		GBS		GDS		SP	
		24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
2g/100ml	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
4g/100ml	1	+	+	+	+	+	+	+	+
	2	+	+	+	+	+	+	+	+
	3	+	+	+	+	+	+	+	+
6g/100ml	1	+	+	+	+	+	+	+	+
	2	+	+	-	+	+	+	-	+
	3	-	+	-	+	+	+	-	+
8g/100ml	1	+	+	-	+	+	+	+	+
	2	+	+	-	++	+	+	-	+
	3	-	+	-	++	-	++	-	+

Key:
GAS, group A Streptococcus;
GBS, group B Streptococcus;
GDS, group D Streptococcus;
SP, *Streptococcus pneumoniae*;
+, bacterial growth was observed;
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2g/100ml-8g/100ml, coffee concentration in the making of the coffee agar;
24 hours, observation at 24 hours;
48 hours, observation at 48 hours;
1-3, triplicate experiments;
0, no repetition of experiment was performed for the third time.
++, very small growth was observed.

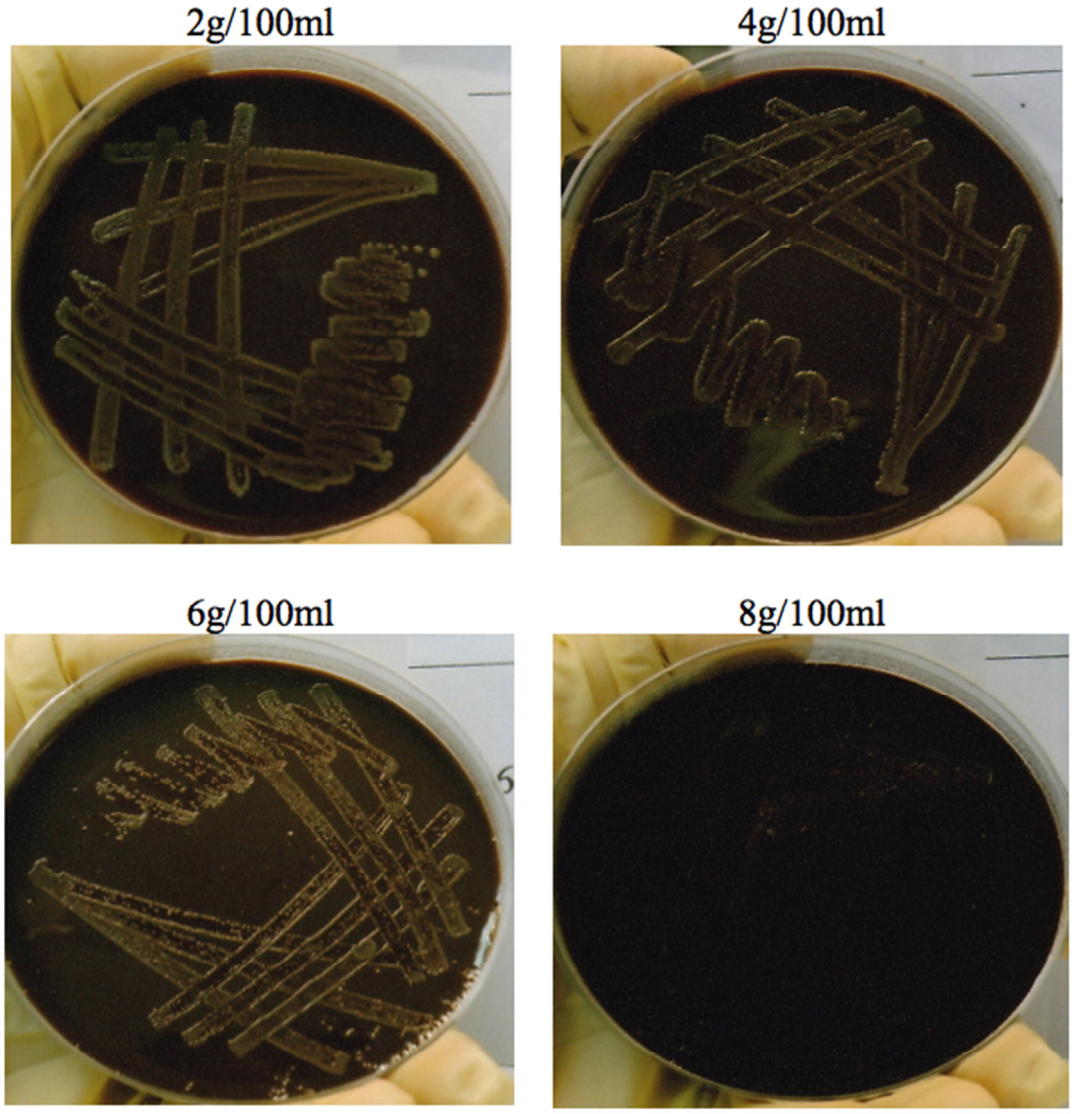

Figure 1. A representative diagram for growth of GAS on non-decaffeinated coffee agar media at 48 hours. Each plate represents coffee agar medium with the concentration as indicated on top of each plate diagram (2g/100ml, 4g/100ml, 6g/100ml and 8g/100ml). Complete inhibition of bacterial growth was observed at 8g/100ml non-decaffeinated coffee agar.

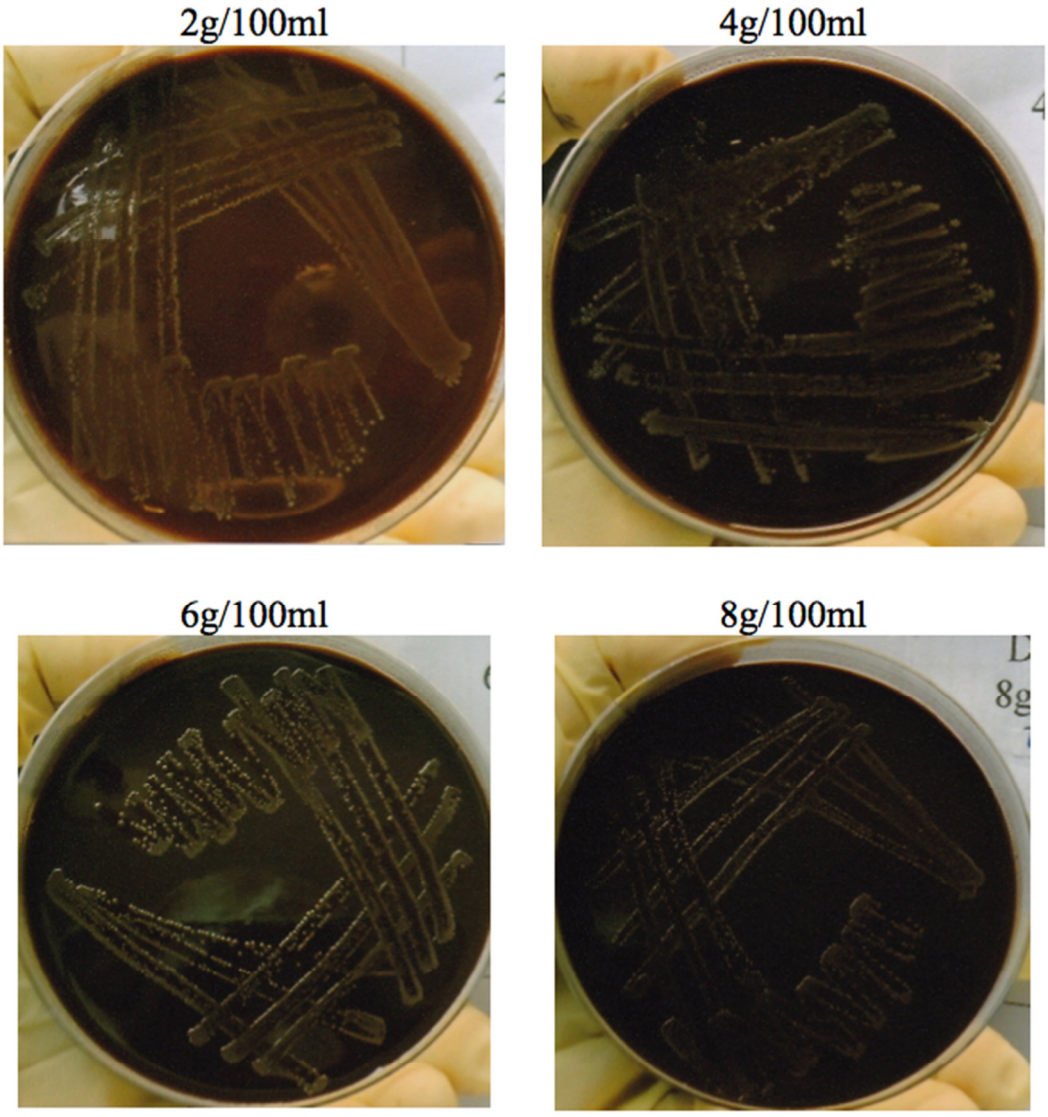

Figure 2. A representative diagram for growth of GAS on decaffeinated coffee agar media at 48 hours. Each plate represents coffee agar medium with the concentration as indicated on top of each plate diagram (2g/100ml, 4g/100ml, 6g/100ml and 8g/100ml). Comparatively minimal bacterial growth was observed at 8g/100ml decaffeinated coffee agar.

methods is most likely to be an artifact. Indeed, a report supported our findings when their investigation on the antibacterial effect of honey observed similar disagreements between the two methods which they suggested might be due to problems in achieving stable dispersion of the antimicrobial sample tested and/or non-uniform percolation of components in the antimicrobial sample[22]. On the contrary, another report showed that in their investigation of the antibacterial activity of green tea observed an agreement between the two methods[23]. Further studies are therefore needed towards understanding the reason(s) for the inconsistencies in these results. The fact that the positive control in our experiments showed zones of inhibition indicates that the condition of bacterial growth for the experiments was adequate enough to allow any antibacterial activity of coffee. Thus it is unlikely that the bacterial growth was defective.

Sensitivity testing using Coffee Agar plate method appears to be more suitable than both the well and disc diffusion methods. In our hands it revealed an antibacterial effect that correlates coffee concentrations. This is similar to a previous observation that used Coffee Agar in their study investigating the antifungal property of coffee[24]. Taking cognizance of the fact that the similarities in our observations occur despite the differences in the compositions of the growth media: the medium used by the previous report unlike ours did not contain blood[24], use of Coffee Agar certainly appears to be superior to the diffusion methods in investigations aimed at antimicrobial growth and sensitivity pattern testing of coffee.

Despite that, this study showed that the action of coffee against GDS seemed to be weaker compared to another report in which the bacterial growth inhibition was apparent after 5g/100ml, but this study showed that coffee was inhibitory against GDS at 8g/100ml[16]. However the author used a different method that is the plate count agar that indeed may be responsible for the differences in our observations. The plate count agar method does seem to be more sensitive than the method employed in our study.

We hypothesised that with prolonged contact with coffee, the bacterial growth would be retarded. Contrary to this however, from the three methods used in this study, *Streptococcus* species that were observed to have no growth at 24 hours were able to grow on media at 48 hours. Given that *Streptococcus* species possess *lux*S quorum sensing system[25, 26], it is most likely for the system to have triggered the synthesis of an enzyme that resulted in metabolizing the factor(s) that are responsible for the inhibitory property of coffee. Further studies are therefore needed in understanding the mechanism of antibacterial activities of coffee especially in relation to the control of the *lux*S gene.

This study made use of coffee manufactured under the proprietary name of NESCAFÉ, which anecdotally seems to be the most widely consumed coffee brand globally. The preparations come as non-decaffeinated which essentially means that the caffeine content in the coffee beans had not been removed as opposed to the decaffeinated form.

According to another report[27], 2g/100ml of non-decaffeinated coffee contains about 3% of caffeine. In this study, 8g/100ml, which is equivalent to 998mg of caffeine completely, inhibited the growth of *Streptococcus* species. Globally the average caffeine content that is consumed by an individual is about 200mg per day[3, 28]. A daily consumption of 500-600mg of caffeine over a period of time has been reported to increase the risk of developing "caffeinism" a syndrome characterised by a range of adverse reactions such as restlessness, anxiety, irritability, agitation, muscle tremor, insomnia, headache, diuresis, sensory disturbances (e.g. tinnitus), cardiovascular symptoms (e.g. tachycardia, arrhythmia) and gastrointestinal disturbances like nausea, vomiting, diarrhoea, etc)[5]. Thus, the consumption of the amount of coffee that completely inhibits the growth of Streptococcus in our experiment is inimical to health and is therefore unacceptable.

The word 'decaffeinated' suggests that the type of coffee preparation contains no caffeine. Indeed, for all Nestlĕ's varieties of decaffeinated Taster's Choice and NESCAFÉ, the caffeine content has been removed[3]. In this study, we have also observed detectable antibacterial activities in decaffeinated coffee. This suggests that caffeine might not be the only substance in coffee that has antibacterial activity or that 'decaffeinated' does not mean a complete absence of caffeine in NESCAFÉ decaffeinated coffee. The latter seems to be more plausible as report showed that 5 oz of NESCAFÉ decaffeinated coffee contained 3 mg which is 20 times lesser than caffeine in non-decaffeinated coffee: this is most likely to be responsible for its antibacterial activity[29].

While a report also observed antibacterial activity in decaffeinated coffee, its interpretation is that other substances other than caffeine could be responsible [16]. On their part, a report having made a similar observation concluded that caffeine is definitely antibacterial but its activity is dependent on other coffee components especially chlorogenic acids and trigonelline[30]. Furthermore, Dogasaki *et al*.[31] reported that protocatechuic acid, chlorogenic acid and caffeic acid are responsible for the antibacterial activities in coffee in a coffee beverage. Interestingly in an earlier work investigating the antibacterial property of roasted coffee against both *S. aureus* and

S. mutans bacteria, Daglia *et al*.[32] suggested that only one compound with strong antibacterial properties against both organisms was present.

The apparent disparities in these reports could be due a host of reasons which include differences in type[3], the degree of roast of the coffee[2] and the model organism(s) used given that the various workers used different bacterial model systems. Further studies are therefore needed in understanding the mechanisms of antibacterial properties of the coffee beverages used in this study.

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