Growth Rate and Antibiotic Sensitivity Effect of Some Natural and Petroleum Based Materials on Staphylococcus aureus

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ABSTRACT
Numerous bacteria expose to different materials every day. Bacterial genome mainly composed of a single double-stranded circular DNA molecule, which can easily undergo changes or mutations upon the exposure to many substances. The aim of this study was to evaluate the mutagenic effect in term of growth rate and antibiotic sensitivity of some natural and petroleum based materials on Staphylococcus aureus. Exposure to each of Bunsen and acetone lower the growth rate of bacterial cells compared to diesel and engine oil that dramatically stimulate their growth. Tobacco based products and the low concentrations of tea and coffee accelerate the growth. The high concentrations of caffeine inhibit the bacterial growth. Wild type bacteria was sensitive to most of used antibiotic and gained resistance to many of them after the exposure to the petroleum products. Similarly, tobacco and tea, which accelerate the growth of cells, make them also completely resistant to the antibiotics that inhibit the synthesis of cell walls. Based on the obtained results, it can be concluded that even natural products can induce bacterial gene mutations such as antibiotic resistance.

Keywords: Mutagenic, Exposure, Bacteria, Antibiotic Resistance Growth Rate

I. INTRODUCTION
Since antibiotic discovery, bacteria was able to develop resistance after few years from its first use. This development, together with the existing paucity in the antibiotic pipeline, renders every antibiotic into a non-renewable resource that should be carefully rationed [1, 2]. Recently, the consumption of power drinks, cigarette, soft drinks and even hot drinks such as coffee and tea become necessary in almost everybody’s life [3]. Everyday billions of microorganisms expose to caffeine containing drinks such as coffee and tea, and nicotine-containing materials such as cigarettes and olfactory, either inside or outside our bodies. Petroleum based product, such as diesel bunsen and engine oils also have been of a great concern in term of their potential carcinogenicity or mutagenic effect. Geno-toxicity or gene alteration is the change that may occur in the genetic material due to the exposure to certain material [4]. Genotoxicity testing of chemical, pharmaceuticals and even industrial food products prior to commercialization is requested by regulatory agencies. The bacterial mutagenicity test or what’s known as “Ames test” was considered having the highest accuracy of carcinogenic prediction [5]. Ames test or Salmonella test is a short term test have been used to measure the mutagenicity using bacteria. It is originally designed to check whether a specific material able to induce genetic damage (gene mutation) or no [6, 7]. This test is one of the famous and unexpansive tests that have been used as an initial screen to determine the mutagenic potential of new chemicals and drugs. However, some evidences suggest that it always results in false-positive responses when the bacterial mutagenicity test is used to predict carcinogenicity [8].

The present study conducted to evaluate the effect of some stimulants containing materials caffeine (coffee and tea) and nicotine (cigarette and olfactory) and some petroleum based product (bunsen, diesel and engine oil) on Staphylococcus aureus following new approach to estimate the effect of these materials on the growth rate and potential antibiotic resistant mutagenesis.

II. METHODOLOGY
2.1. Mutagenic Materials
Bunsen, diesel, and engine oil have been selected as chemical mutagens, and all were obtained from chemistry laboratory of faculty of science, Al-asmarya Islamic university. Nescafe and green tea have been used as caffeine containing materials, which were obtained from local market. Cigarette and olfactory were selected as nicotine containing materials and were obtained from local reliable supplier.

2.2. Test Microorganism
Staphylococcus aureus, which was kindly obtained and identified in microbiology lab of faculty of science, Al-asmarya Islamic university. The culture was grown on nutrient agar and stored at 4°C as single colonies.
2.3. Preparation of Mutagenic Conditions
Different concentrations from each material were prepared and equal amount from each of them separately were mixed with nutrient broth. Constant number of bacteria added to each mixture and left for 48 h. Bacterial suspension were prepared and measure its count prior the addition and constant volume were added. Mustard have been added as an emulsifier to all the mixtures. Distilled water were used as control.

2.4. Effect of Growth Rate
Detection the effect of the material on growth rate were done by comparing the number of bacteria after the exposure to each material with the control. The results were written as increase, decrease or no effect.

2.5. Antibiotic Sensitivity Assay
Evaluation of antibiotic sensitivity was done using disk diffusion assay as described in [9] and following all the quality control procedures. Various antibiotic were used including; amoxicillin, ampicillin

vancomycin gatifloxacin ciprofloxacin clindamycin trimethoprime sulfamethoxazole. All the antibiotics obtained from Thermo Scientific, USA, and selected based on the four different mechanism of action.

III. RESULTS
Bacterial growth rate in this study compared to wild type (control) which is the cells that didn’t exposed to any material. Caffeine containing materials tend to accelerate the bacterial growth in there low concentration, and decrease it in the high concentration as presented in table 1. Only bunsen from the petroleum based materials inhibited the growth, unlike the engine oil and diesel, which dramatically enhanced the bacterial growth. Nicotine which is found in cigarette and olfactory also enhance the growth rate compared to the wild type.

Table 1: Effect of materials on the growth rate of Staphylococcus aureus

| Material   | Concentration %25 | Concentration %50 | Concentration %75 |
|------------|------------------|--------------------|-------------------|
| Control    | =                | =                  | =                 |
| Engine oil | ++               | ++                 | ++                |
| Diesel     | ++               | ++                 | ++                |
| Bunsen     | --               | --                 | --                |
| Olfactory  | +                | +                  | ++                |
| Cigarette  | ++               | ++                 | ++                |
| Tea        | +                | +                  | ++                |
| Nescafe    | ++               | -                  | --                |

*(+++) steadily increase, (+) increase, (=) no change (--) steadily decrease, (-) decrease.

Induction of antibiotic resistant mutation was evaluated using 8 antibiotics with different 4 mechanism of actions. Table 2 present the results of antibiotic resistance evaluation. Wild type bacteria was highly sensitive to all tested antibiotics except trimethoprim which was weakly sensitive to it.

Table 2: Antibiotic sensitivity evaluation of Staphylococcus aureus (mm)

| Material | Amo | Amp | Van | Gat | Cip | Cli | Gen | Tri |
|----------|-----|-----|-----|-----|-----|-----|-----|-----|
| Control  | 20  | 18  | 26  | 38  | 33  | 38  | 32  | 10  |
| Engine oil | 22 | 5   | 30  | 30  | 10  | 10  | 30  | 35  |
| Diesel   | 14  | 23  | 20  | 35  | 34  | 43  | 33  | 18  |
| Bunsen   | 15  | 17  | 28  | 35  | 38  | 45  | 33  | 16  |
| Olfactory | 5  | 5   | 30  | 26  | 28  | 45  | 30  | 14  |
| Cigarette | 5  | 5   | 23  | 30  | 33  | 28  | 38  | 18  |
| Tea      | 5   | 22  | 19  | 35  | 33  | 26  | 36  | 14  |
| Nescafe  | 28  | 25  | 38  | 36  | 35  | 43  | 43  | 18  |
Figure 1(a): Antibiotic comparison between the wild type and mutated bacteria

Figure 1(b): Antibiotic comparison between the wild type and mutated bacteria
IV. DISCUSSION

Engine oil, after the exposure to engine oil the growth of S. aureus noticeably enhanced compared to the wild type, similar observation was noticed by KM Krasnogomsk et al. [10] they reported that freshwater bacteria display enhanced growth after exposure to used motor oil. Changing the medium or the environmental conditions may enforce the bacteria to undergo some gene alteration in attempt to adapt with the situation. This can be also observed as bacterial sensitivity to antibiotics also changed. The bacteria become highly resistance to ampicillin, ciprofloxacin and clindamycin. Surprisingly, its sensitivity dramatically increased to trimethoprim antibiotic, with a zone of inhibition reached 35mm after it was only 10 in the wild type.

Diesel and bunsen are two different types of fuel that made from crude oil at petroleum refineries. In this study the effect of these two substances on the bacterial growth was completely different; diesel enhanced the growth, while bunsen reduced it. Many studies reported the ability of bacteria to grow in diesel containing media but not diesel [11, 12]. In term of antibiotic sensitivity, the results in table 2 didn’t show much difference among bunsen and diesel nor to the wild type, only slight changes have been observed. The effect on the growth rate may explained by acceleration the movement and separation of cells, and for the bunsen, the cells growth maybe inhibited due to the presence of bunsen as it have been confirmed in many studies [13-15].

Nicotine is the primary addictive and dependence producing substance in smokeless tobacco products, and nicotine dependence drives the development and maintenance of addiction to tobacco products [16]. In this study tobacco containing material dramatically enhanced the growth of bacteria, especially cigarette extract. Similar results was obtained by M Liu et al.[17] they test 20 type of bacteria, and found that the growth of 14 of them significantly enhanced after the exposure to tobacco extract. DAA Putra et al. [18] also reported that tobacco leaves extract prevent S. aureus biofilm formation and accelerate its growth. In term of antibiotic sensitivity, after the exposure to tobacco products, the bacteria become completely resistant to the antibiotic that affect cell wall. amoxicillin, ampicillin are two β-lactam antibiotics that inhibit the synthesis of bacteria cell wall [19]. Exposing bacterial cells to tobacco extracts allow them to develop such a mutation that prevent the two antibiotics from affecting them, unlike the other types of antibiotics.

Finally, caffeine is a chemical compound, which acts as stimulant. In this study, the effect of low concentration of coffee and all the concentrations of tea enhance the growth of bacteria. Suggesting that this due to the low caffeine concentration, as the high concentration of coffee reduce the growth in a noticeable manner. A. Saxena et al. [20] studied the effect of caffeine on bacteria and reported that it steadily enhanced its growth. For antibiotic sensitivity analysis, the results was similar to tobacco based materials. Suggesting that the stimulants affect the bacteria in the same manner. Figure 1 from a to g present antibiotic comparison between the wild type and the exposed bacteria to each substance separately.

V. CONCLUSION

From this study, it can be concluded that there is a significant effect of tested materials on the bacteria in term of growth rate and antibiotic sensitivity. Gaining antibiotic resistance mutation after the exposure to such materials can be of great interest. More studies should be done to understand the mechanism of this induction.

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