Sex Pheromone-Responsive Conjugative Antibiotic Resistance in *Enterococcus faecalis*: How to Control it?

Dear editor

Enterococcus faecalis strains are opportunistic pathogens for human. They are one of the most common bacteria involved in nosocomial (hospital-acquired) infections and are notorious for being resistant to multiple antibiotics and harboring plasmids that confer pheromone response. The sex pheromone system in *E. faecalis* plays important role in transfer of antibiotic resistance genes. The sex pheromones produced by *E. faecalis* are small peptides consisting of 7–8 amino acids with low molecular weights (<1000), secreted extracellular under chromosomal control. Up to now, five types of sex pheromones were discovered. Each type of these pheromones is specialized in transmitting a specific trait or traits between bacterial strains. More than 20 plasmids that respond to these sex pheromones were discovered in the genus *E. faecalis*.[1]

The aim of this title is to study of antibiotic resistance gene transfer among isolates of *E. faecalis* which has the ability to produce sex-pheromones in order to control the pheromone–responsive transmissible plasmids to other *E. faecalis* strains.

The local *E. faecalis* bacteria were isolated from hospitalized patients at Hilla City, Iraq. The Ej-medium was used for isolation of *E. faecalis* in pure culture.[2] The local isolates were characterized by resistant to Tetracycline and sensitive to Rifampicin. The standard strains; *E. faecalis* FA2-2 were used for production of sex pheromone. While *E. faecalis* OG1RF was used in mating experiment as recipient cells which characterized by resistant to Rifampicin and Fusidic acid, and sensitive to Tetracycline. The sex pheromone is secreted from the bacteria into the medium, Todd–Hewitt broth. The sex pheromone was purified by using sephadex column (diameter: 3.4 cm and height: 12.7 cm) and sephadex gel DEAE-Sephadex A25. The Lowry method was used for the purpose of estimating the amount of purified sex pheromone. The clumping assay was used for detecting the activity of purified sex pheromone.[3]

The local isolates were selected that characterized by resistant to Tetracycline and sensitive to Rifampicin. They were used as donor cells in mating experiment. The standard strains, *E. faecalis* OG1RF were used as recipient cells and they are resistant to Rifampicin and Fusidic acid, and it sensitive to Tetracycline. The conjugation experiment between *E. faecalis* isolates were divided into 2 categories: cCF10-induced bacterial conjugation, and non-sex pheromone-induced bacterial conjugation.[4] To control on action of the sex pheromone cCF10, the bacterial conjugation experiment was repeated with the use of chloramphenicol according to what was stated in Dunny *et al.*[5] The antibiotic solution of chloramphenicol was added at a final concentration of 50 (μg/mL) to the suspension of donor cells, then treated with the sex pheromone, at different times, in four separate experiments. In the first experiment, the antibiotic was added to the donor cells immediately after treating them with the sex pheromone, and in the second experiment the antibiotic was added 15 min after treating the donor cells with the sex pheromone, and in the third experiment the antibiotic was added after half an hour of treatment, and in the fourth experiment the antibiotic was added an hour after the treatment. a control groups for each step without adding the antibiotic were done.

The results of bacterial conjugation in the current study showed in Table 1, revealed the sex pheromone (cCF10) play an important role in an increasing conjugation frequency and transferring resistance trait to tetracycline among *E. faecalis* isolates. The frequency of conjugation of sex pheromone-inducing experiment was increased in high level, 1.6×10^{-1}, when compared with the conjugation frequency of the same isolates without the addition of the sex pheromone, 1.3×10^{-5} cell/mL.

The current study showed in Table 2 the addition of chloramphenicol at a final concentration (50 μg/mL) to the donor cells directly with the treatment of the sex pheromone to it led to preventing any increase in the conjugation frequency, but the antibiotic did not affect the conjugation frequency when it was added after 15, 30, and 60 min of treating the sex pheromone to the donor cells.

Table 1: Frequency of bacterial mating among *E. faecalis*

Type of conjugation experiment	Frequency (conjugated cell/mL)
Sex pheromone-inducible conjugation	1.6×10^{-1}
Sex pheromone-uninducible conjugation	1.3×10^{-5}

Table 2: The effect of chloramphenicol antibiotic agent on bacterial conjugation induced by the sex pheromone cCF10

No. of conjugation experiment	Time of chloramphenicol addition to donor cell treated with sex pheromone	Effect of chloramphenicol on frequency
1	O	+
2	15	-
3	30	-
4	60	-
5	Control	-

In the current study, the local isolate was chosen according to its resistance to tetracycline because the pheromone cCF10 is specific signal for induction of a single plasmid that harboring gene of tetracycline resistance. The results in Table 1 indicate the sex pheromone works to induce bacterial conjugation between the donor and recipient cells, and thus greatly increases the process of transmission and spread of genes between bacterial cells. It may lead to four or more times more transfer of plasmids compared to normal uninduced conjugation with the pheromone.[3,6]

The increase in conjugation frequencies of the isolates treated with the cCF10 sex pheromone indicates that the added cCF10 sex pheromone stimulated the donor cells to produce aggregation proteins on their surface, which bind with the binding proteins on the surface of the recipient cells. It allows the transfer of plasmids significantly between conjugated cells.[4,7]

In controlling the effect sex pheromone test, the local isolate was chosen, because in addition to its resistance to tetracycline, it is also sensitive to chloramphenicol. The last antibiotic was selected for the purpose of inhibiting the synthesis of aggregation proteins. They are surface proteins that play an important role in conjugation by formation of conjugal tube, and then gene transfer.

The results in Table 2 explain the fact that the addition of the antibiotic and the sex pheromone together "to the donor cells at the same time, the antibiotic has led to stopping the activity of the sex pheromone in stimulating the genes located on the plasmid responding to it from the synthesis of aggregation substances on the surface of the donor cells, and therefore there was no increase in the frequency of transmission of plasmids between mated cells, this indicates that the antibiotic affected the conjugation frequency, while when the antibiotic was added after (15, 30, and 60 min) of adding the sex pheromone to the donor cells, the sex pheromone may have been able to stimulate synthesis of aggregation substances during these periods, so the antibiotic did not affect the conjugation frequency.[6,8]

The chloramphenicol antibiotic did not kill the bacterial cells when exposed to it during those periods, because the periods of exposure to the antibiotic were not sufficient to kill the bacterial cells (Dunny, personal communication).

The results of the current study are consistent with the findings of Dunny *et al*.[5] that the addition of some antibiotics to bacterial cells immediately after the addition of the sex pheromone. It leads to stopping the activity of the sex pheromone, which prevents cells from clumping, and this indicates the inhibition of the synthesis of clustering proteins on the surface of the donor cells through the interference of antibiotics with the RNA, but the addition of these antibiotics to bacterial cells at 15 min after adding the pheromone did not prevent cell aggregation, which indicates the bacteria's ability to synthesize aggregation proteins as a result of the effect of the sex pheromone.

From this study, we concluded the effect of cCF10 sex pheromone on gene transfer that responsible for tetracycline resistance can be controlled by using protein synthesis-inhibition antibiotic agents such as chloramphenicol.

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Conflicts of interest
There are no conflicts of interest.

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