

GENE TRANSFER AMONG DIFFERENT SPECIES OF *STAPHYLOCOCCUS* ISOLATED FROM HUMAN

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ABSTRACT: Twenty one *Staphylococcus* isolates were isolated from Zagazig University Hospital from patients belong different locations in Sharkia. All these isolates were isolated from different parts of human body and identified genetically and microbiologically. The isolates were obtained and characterized in the Dept. of Medical Microbiology Zagazig University. Resistance to antibiotics (streptomycin, chloramphenicol, penicillin and rifampicin) was examined for all of isolates. The highest conjugation frequencies was 2.14×10^{-8} between *S. aureus* 5 and *Staphylococcus* spp. 12. This study shows that transferring of genens among different species of *Staphylococcus* bacteria isolated from different parts of human body can occur. Conjugation frequencies have been enhanced when plates were incubated at 25°C for 48 hour. Addition of calcim chloride increased the frequency about 12.70 fold increase than control. Numbers of donor and recipient with a ratio of 2 : 1 ml/ml resulted in maximum number of transconjugants.

Key words: *Staphylococcus*, sensitivity, conjugation, factors.

INTRODUCTION

Staphylococci are spherical Gram-positive bacteria, which are immobile and form grape-like clusters (Ryan and Ray, 2004 and Todar, 2005).

The genus *Staphylococcus* contains both pathogenic and non-pathogenic organisms. They do not

produce endospores but are highly resistant to drying, especially when associated with organic matter such as blood, pus, and other tissue fluids. Most staphylococci are found routinely on the surface of the skin. Breaks in skin and mucous membranes allow entrance of these organisms into the body where they may cause disease. The

three major species include *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. The latter two are rarely implicated in disease, but have been isolated in cases of endocarditis and urinary tract infections under certain circumstances. *S. aureus* is considered the pathogenic strain, causing abscesses, boils, carbuncles, acne and impetigo. Less commonly, pneumonia, osteomyelitis, endocarditis, cystitis, pyelonephritis, and food poisoning have been attributed to this organism (Lowy, 1998 and Archer and Climo, 2001). These three strains of staphylococci can be distinguished from each other by a number of biochemical tests (Foster, 1996).

Conjugation involves cell-to-cell contact and the movement of host DNA by conjugative plasmids or transposons and can result in the largest replacements of hundreds of kilobases in vitro (Lloyd and Buckman, 1995 and Milkman *et al* 1999). With both transduction and conjugation, the donor DNA is frequently abridged by endonuclease cutting and exonuclease shortening before incorporation into the recipient chromosome (Milkman *et al* 1999).

The aim of this work is to assess the potential gene transfer among human clinical isolates of staphylococci bacteria. Only conjugation as a process of transferring antibiotic resistance and pathogenic factor has been detected. Factors that optimizing this process have also investigated.

MATERIALS AND METHODS

This study was carried out at Microbial Genetic Laboratory, Fac. Agric. Zagazig Univ.

Isolation of *Staphylococcus* Bacteria

Data in Table 1 shows the host, source and locations of *Staphylococcus* isolated from Zagazig University Hospital as human clinical from different locations in Sharkia.

Bacterial Isolates

There were twenty one isolates of other staphylococci, including 13 isolates identified as *Staphylococcus aureus*, one isolate as *Staphylococcus saprophyticus*, one isolate of *Staphylococcus epidermidis*, one isolate as *Staphylococcus cohnii* and five of *Staphylococcus* species. The isolates were identified by conventional

Table 1. List of *Staphylococcus* isolates and their sources

S/N	Isolate code	Scientific name	Host	Age	Source
1	Sau1	<i>Staphylococcus aureus</i>	Child	Newborn	Blood*
2	Sau2	<i>Staphylococcus aureus</i>	Child	Newborn	Blood*
3	Sau3	<i>Staphylococcus aureus</i>	Child	Newborn	Blood*
4	Sau4	<i>Staphylococcus aureus</i>	Child	Newborn	Blood*
5	Sau5	<i>Staphylococcus aureus</i>	Child	Newborn	Blood*
6	Sau6	<i>Staphylococcus aureus</i>	Child	Newborn	Blood*
7	Sau7	<i>Staphylococcus aureus</i>	Child	Newborn	Blood*
8	Sau8	<i>Staphylococcus aureus</i>	Child	Newborn	Blood*
9	Ss9	<i>Staphylococcus sp</i>	Female	42 y	Nipple discharge*
10	Sau10	<i>Staphylococcus aureus</i>	Male	67 y	Aspirate from post operate wound****
11	Sau11	<i>Staphylococcus aureus</i>	Female	30 y	Vaginal swab*
12	Ss12	<i>Staphylococcus sp</i>	Female	19 y	Urine*
13	Ssapro13	<i>Staphylococcus saprophyticus</i>	Female	43 y	Urine*
14	Sau14	<i>Staphylococcus aureus</i>	Male	40 y	Diabetic foot***
15	Sau15	<i>Staphylococcus aureus</i>	Female	70 y	Knee Joint*****
16	Sep16	<i>Staphylococcus epidermidis</i>	Female	27 y	Recurrent boils*
17	Sau17	<i>Staphylococcus aureus</i>	Female	45 y	Bloody Aspirate**
18	Ss18	<i>Staphylococcus sp</i>	Female	39 y	Rt.conjunctivitis*
19	Ss19	<i>Staphylococcus sp</i>	Female	39 y	Lt.conjunctivitis*
20	Ss20	<i>Staphylococcus sp</i>	Male	54 y	Rt.conjunctivitis**
21	SC021	<i>Staphylococcus cohnii</i>	Female	32 y	Urine*

* Zagazig University Hospital - **Menia el kameh - ***Abo kaper -

****Belbies - *****Abo hamad

biochemical tests based on manual for clinical microbiology (Bannerman, 2003).

Growth Media

Staphylococcus isolates were inoculated on mannitol salt agar (MSA) media at 37°C, nutrient agar media (NA) and nutrient broth media (NB).

Gram Staining

Loopfull from isolated colonies was examined with Gram stain Microscopically for characteristic cellular morphology and the purity of culture were tested.

Rabbit Plasma

A sample of rabbit plasma were taken on EDTA for coagulase test prepared according to manufacturer's instructions: 0.1g of EDTA/ ml.

Coagulase Test

The Coagulase Test is an important indicator for the pathogenicity of *Staphylococcus* strains. 15 colonies grown on selective media as transconjugants have been selected randomly to assess their abilities to coagulation. The same number have also been used in the donor and recipient isolates.

Rabbit plasma was used for applying the test. 1/6 dilution of

plasma in saline (0.85% NaCl) was prepared and distributed in small test tubes, then colonies of the staphylococci were placed on this tubes and incubated at 37°C, for up to 4 hours and examined at 1, 2 and 4 hours for clot formation. The negative tubes were left at room temperature over night and re-examined (Colle *et al* 1996).

Catalase Test

The catalase test was used to detect the presence of the enzyme catalase in bacteria, which catalyses the breakdown of hydrogen peroxide (H₂O₂) with the release of free oxygen, applying by smearing bacteria on microscopic slide and adding few drops of hydrogen peroxide. Bubbling indicated a positive catalase test. (Foster, 1991 and Todar, 2005).

Mannitol Fermentation Test

15 colonies grown on selective media as transconjugants have been selected randomly to assess their abilities in fermentation. The same number have also been used in the donor and recipient isolates. Typical pathogenic staphylococci ferment mannitol and form yellow colonies with yellow zones, while typical non-pathogenic staphylococci do not ferment mannitol and form red colonies (Blair *et al* 1967.

Chapman, 1945 and Finegold and Baron, 1989).

Antibiotics

Streptomycin with concentrations (6, 8, and 12 mg/ml), chloramphenicol with concentrations (0.5, 1, and 3 mg/ml), penicillin with concentrations (0.5, 1, and 3 mg/ml) and rifampicin with concentrations (0.5, 1, and 3 mg/ml) were used in this investigation. Antibiotics were added directly into molten NA media before pouring.

Biological Buffer Solution

Phosphate buffer with different values of pH were, (5, 6, and 7), KH_2PO_4 and $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$ were used.

Acetate buffer with value of pH were, (4), sodium acetate 0.1N (8.204 g/l) and acetic acid 0.1N (6.005 ml/l).

Carbonate buffer with different values of pH were, (9.2, 10), sodium carbonate 0.1N (10.6 g/l), sodium bicarbonate 0.1N (8.401 g/l).

Sensitivity of Staph Isolates

All isolates were checked with different concentrations of each antibiotic by added one ml of each isolate on surface of complete media.

The Optimization Factors Influencing Conjugation

Effect of temperature

One ml from each donor and recipient was placed on complete media and incubated at different degrees (5°, 25°, 30°, 42°, 45° and 37 °C as control). After incubation time, the growth harvested in 10ml phosphate buffer and number of the donor, recipient, and transconjugants have been calculated.

Effect of salts

The influence of salts on conjugation between *Staphylococcus* isolates has been experimented. The salts of KCl and NaCl (monovalent), CaCl_2 and MnCl_2 (divalent) have been used in this study. For each salt individual stock solution have been prepared and then, autoclaved. Different concentrations of each salt have been added to NA media. One ml from each donor and recipient was placed on plates that contain different concentrations of the used salts and incubated for 4 days.

Effect of mating time

One ml from each donor and recipient was placed on complete media and incubated at 37°C for different mating times (12h, 24h,

48h, 3 and 5 days) and number of the donor, recipient, and transconjugants have been calculated.

Effect of density

Different ratios of the mixture (donor: recipient) were added. These ratios were (1:1, 2:1, 3:1, 1:2 and 1:3 ml/ml). This experiment was applied on complete media and incubated at 37°C. For every ratio, number of transconjugants and number of donor and recipient cells have been counted.

Effect of biological pH

The components of NA have been dissolved in different biological buffer solutions instead of sterile distilled water. So, different pH values have been obtained. These pH values were (4, 5, 6, 7, 9.2 and 10) and numbers of donor, recipient and transconjugants have been calculated.

Conjugation Assay

All donor and recipient isolates were inoculated and incubated at 37°C for 24 hours on shaker incubator. Equal volumes (1 ml) of donor and recipient were added on surface of complete media plates and incubated at 37°C for 24 hours. The growth washed

by 10 ml phosphate buffer and removed by spreader to sterile flasks. Then, serial dilutions were used (0.1ml spread on selective media (Str. 6mg/ml, Chl. 0.5mg/ml)) but only neat concentration has been shown transconjugants. For every donor, number of transconjugants and number of donor and recipient cells have been counted.

RESULTS AND DISCUSSION

Phenotypic Characteristics of Staphylococci Isolates

All isolates were gram-positive and cocci and were identified as *Staphylococcus* bacteria. All strains gave positive reaction with catalase test (Table 2). *Staphylococcus aureus* strains 1-8, 10, 11, 14, 15 and 17 were pathogens to human. They gave positive results with coagulase testes and were able to ferment mannitol. Strains *Staphylococcus spp* 9, 12, 18, 19 and 20 did not form a clot in coagulase test and did not ferment mannitol. *S. saprophyticus* 13 and *S. cohnii* 21 did not form a clot in coagulase test but still able to ferment the mannitol. *S. epidermidis* 16 gave negative reaction with both of tests.

Table 2. Pathogenic characterization of *Staphylococcus* species bacteria isolated from teaching hospital of Zagazig University

Strains	Catalase test	Coagulase test	Mannitol Fermentation
<i>S. aureus</i> 1	+	+	+
<i>S. aureus</i> 2	+	+	+
<i>S. aureus</i> 3	+	+	+
<i>S. aureus</i> 4	+	+	+
<i>S. aureus</i> 5	+	+	+
<i>S. aureus</i> 6	+	+	+
<i>S. aureus</i> 7	+	+	+
<i>S. aureus</i> 8	+	+	+
<i>Staphylococcus</i> spp. 9	+	-	-
<i>S. aureus</i> 10	+	+	+
<i>S. aureus</i> 11	+	+	+
<i>Staphylococcus</i> spp. 12	+	-	-
<i>S. saprophyticus</i> 13	+	-	+
<i>S. aureus</i> 14	+	+	+
<i>S. aureus</i> 15	+	+	+
<i>S. epidermids</i> 16	+	-	-
<i>S. aureus</i> 17	+	+	+
<i>Staphylococcus</i> spp. 18	+	-	-
<i>Staphylococcus</i> spp. 19	+	-	-
<i>Staphylococcus</i> spp. 20	+	-	-
<i>S. cohnii</i> 21	+	-	+

The classical phenotypic identification of staphylococci by Kloos and Schleifer, (1975), Kloss and Bannerman, (1994) remains the gold standard for reference laboratories. In addition, *S. aureus* strains were isolated from a teaching hospital and identified by gram staining, colonial morphology, tests for clumping factor and tests of coagulase (Seguin *et al* 1999). Furthermore, the production of staphylocoagulase (coagulase) is still the most identifying characteristic of *S. aureus*.

However, new techniques depending on molecular analysis have been recently used to improve time require for the diagnosis of bacteria in clinical microbiology laboratories (Martineau *et al* 2000).

Antibiotic Sensitivity Patterns

Data in Table 3 illustrates the antibiotic sensitivity patterns among *Staphylococcus* species isolated from human. One of the patterns designated group 1 was sensitive to the four antibiotics represented by five isolates (No., 7, 9, 10, 18 and 20). The isolates with the second pattern designated group 2 which were resistant to streptomycin and chloramphenicol only represented by four isolates (No., 4, 8, 15 and 17). The third

pattern and group 3 represented by the rest of isolates were sensitive to penicillin, rifampicin and either streptomycin or chloramphenicol.

These results could be useful for the clinical point view especially in the treatment of staphylococci causing diseases.

Many studies reported that antibiotic resistance determinants were borne as plasmids in *S. aureus* (Minshew and Rosenblum, 1973, Stiffler *et al* 1974, Kloss *et al* 1980, Cohen *et al* 1982, Goering and Ruff, 1983, Gotz *et al* 1983 and Keller *et al* 1983).

Naidoo, (1984) reported that many hospital strains of *S. aureus* were multiply antibiotic resistant and the resistances being plasmid encoded. In addition, methicillin resistance (Beta lactamase-resistant penicillin) in *S. aureus* and other antibiotic resistance genes were located on a locus that had certain features of a pathogenicity island called PAI (Schmidt and Hensel, 2004).

Conjugation Experiments between Human *Staphylococcus* Species

Results in Table 4 represent the conjugation frequencies per recipient in some conjugation experiments. This means that *S. aureus* 2 and *S. aureus* 5 isolated

Table 3. Patterns of antibiotic sensitivity in human staphylococci bacteria

<i>Isolate No.</i>	<i>Str.</i>	<i>Pen.</i>	<i>Rif.</i>	<i>Chl.</i>
Sau1	-	-	-	+
Sau2	-	-	-	+
Sau3	-	-	-	+
Sau4	+	-	-	+
Sau5	-	-	-	+
Sau6	+	-	-	-
Sau7	-	-	-	-
Sau8	+	-	-	+
Ss9	-	-	-	-
Sau10	-	-	-	-
Sau11	+	-	-	-
Ss12	+	-	-	-
Ssapro13	-	-	-	+
Sau14	-	-	-	+
Sau15	+	-	-	+
Sep16	-	-	-	+
Sau17	+	-	-	+
Ss18	-	-	-	-
Ss19	-	-	-	+
Ss20	-	-	-	-
Sc021	-	-	-	+

Str = streptomycin, Pen = penicillin, Rif = rifampicin,

Chl = chloramphenicol.

++ = resistance

- = sensitive

Table 4. Conjugation frequencies among *Staphylococcus* species isolated from human

Donor	Cfu/ml 10^{10}	recipient	Cfu/ml 10^{10}	No. transconjugants 10^2	Marker	Conjugation frequency per recipient 10^{-8}
<i>S. aureus</i> 2	36	<i>Staphylococcus spp.</i> 12	14	10	chl+	0.714
<i>S. aureus</i> 5	20	<i>Staphylococcus spp.</i> 12	14	30	chl+	2.14
<i>S. aureus</i> 11	18	<i>S. epidermids</i> 16	40	6	Str+	0.15
<i>S. aureus</i> 11	18	<i>S. saprophyticus</i> 13	12	18	Str+	1.5
<i>S. aureus</i> 14	15	<i>Staphylococcus spp.</i> 12	14	20	chl+	1.42

from the blood of newborn child can transfer chloramphenicol resistant gene into *Staphylococcus spp.* 12 which isolated from the urine of a girl of 19 years old.

Moreover, *S. aureus* 11 that isolated from vagina of a women of 30 years old can transfer streptomycin resistant gene into *S. saprophyticus* 13 and *S. epidermidis* 16 isolated from recurrent boils of a lady of 27 years old. In addition *S. aureus* 14 can transfer chloramphenicol resistant gene isolated from the diabetic foot from a man of 40 years old into *Staphylococcus spp.* 12 which isolated from the urine of a girl of 19 years old.

This clearly shows that the potential of gene transfer among *Staphylococcus species* clinical isolates from different parts of human body is a range wide and transfer can occur between different sex and ages.

Factors Controlling Conjugation

In all these experiments, *S. aureus* isolate number 5 was used as donor with *Staphylococcus spp.* isolate number 12 as recipient.

Effect of salts

Effect of mono cations

The effect of mono cations (Na^+ and K^+) on conjugation with

the same anion Cl^- has been tested in this study (Tables 5 and 6).

No enhancement in number of transconjugants and subsequently conjugation frequency per recipient has been observed in both mono cations. It seem that Na^+ or K^+ have no stimulation effect on conjugation between *Staphylococcus species*. No transconjugants have been detect at 1800 mM of NaCl and 400-500 mM of KCl.

Effect of di-cations

Two di-cations, Mn^{++} and Ca^{++} sharing the same anion Cl^- have been tested. Fold increase than control (at 5mM) of MnCl_2 has a little response in enhancing conjugation frequency. No transconjugants have been detected in concentration 50-200 mM of MnCl_2 (Table 7).

All the used concentrations of CaCl_2 have a good stimulation influence on conjugation process. The maximum number of transconjugants was observed using 400 mM of CaCl_2 with 12.7 fold increase than those observed in control (Table 8).

The addition of different concentrations of calcium chloride (50- up to- 1200 mM) to the agar plates enhanced the mechanism of

Table 5. Effect of NaCl on conjugation between *Staphylococcus* bacteria

Concentration mM	No. Transconjugants 10^2	Conjugation frequency per recipient 10^{-8}	Fold increase than control
Control (0.0)	34	2.42	-
100	13	0.92	0.0
200	21	1.5	0.0
300	25	1.78	0.0
400	27	1.92	0.0
500	28	2.0	0.0
600	30	2.14	0.0
700	23	1.64	0.0
800	20	1.42	0.0
900	12	0.85	0.0
1000	8	0.57	0.0
1200	6	0.42	0.0
1500	6	0.42	0.0
1800	0.00	0.00	0.0

Recipient = 14×10^{10} cfu/ml

Table 6. Effect of KCl on conjugation between *Staphylococcus* bacteria

Concentration mM	No. Transconjugants 10^2	Conjugation frequency per recipient 10^{-8}	Fold increase than control
Control (0.0)	30.0	2.14	-
50	13.0	0.92	0.0
100	15.0	1.07	0.0
200	27.6	1.97	0.0
300	13.3	0.95	0.0
400	0.00	0.00	-
500	0.00	0.00	-

Recipient = 14×10^{10} cfu/ml

Table 7. Effect of MnCl₂ on conjugation between *Staphylococcus* bacteria

Concentration mM	No. Transconjugants 10 ²	Conjugation frequency per recipient 10 ⁻⁸	Fold increase than control
Control (0.0)	28.0	1.64	-
2	25.0	1.47	0.0
5	30.0	1.76	1.07
10	21.0	1.23	0.0
50	0.00	0.00	-
100	0.00	0.00	-
200	0.00	0.00	-

Recipient = 17×10^{10} cfu/ml**Table 8. Effect of CaCl₂ on conjugation between *Staphylococcus* bacteria**

Concentration mM	No. Transconjugants 10	Conjugation frequency per recipient 10 ⁻⁸	Fold increase than control
Control (0.0)	37.0	2.46	-
50	50.0	3.33	1.35
100	64.0	4.26	1.72
200	120.0	8.0	3.24
300	207.0	13.8	5.59
400	470.0	31.3	12.70
500	394.0	26.26	10.64
800	200.0	13.3	5.41
1200	80.0	5.3	2.16
1500	0.00	0.00	-

Recipient = 15×10^{10} cfu/ml.

gene transfer by conjugation between staphylococci bacteria. The fold increase than these observed in control ranged from

1.35 up to 12.7. These data do agree with others. Naidoo and Noble. (1981) observed that transfer of gentamicin resistance

between *S. hominis* and *S. aureus* strains enhanced when CaCl_2 was added. The fold increase was about 8-10 fold. However, calcium chloride has no effect on antimicrobial resistance transfer from *S. epidermidis* to *S. aureus* (Forbes and Schaberg, 1983).

Effect of mating time

The highest number of transconjugants and subsequently conjugation frequency was observed after 48 hour (Conjugation frequency per recipient was 0.38×10^{-8}), (Table 9). Transconjugants have been detected after 12 h, 24h, 3d and 5 days but with less efficiency (Conjugation frequency per recipient was 0.21×10^{-8} , 0.21×10^{-8} , 0.17×10^{-8} and 0.15×10^{-8} at these mating times, respectively). The results of this study showed that mating time between donor and recipient of 48 hour gave the highest gene transfer by conjugation. However, Mitra *et al* (1995) found that transfer of drug resistance between *S. aureus* occurred maximally between 6 and 18 hour post incubation but in nutrient broth rather than agar plates.

Effect of temperature

The highest number of transconjugants and subsequently conjugation frequency was observed at 25°C (Conjugation frequency per recipient was 0.49×10^{-8}),

Table 10. Transconjugants have been detected at 30 , 37 and 42°C but with less efficiency (Conjugation frequency per recipient was 0.38×10^{-8} , 0.17×10^{-8} and 0.19×10^{-8} at these temperatures, respectively). Results showed that environmental conditions may affect the rate of transfer.

Effect of pH values

Six pH values were studied, 4.0, 5.0, 6.0, 7.0, 9.2 and 10.0. Number of viable cells have been observed in extreme acid (pH 4.0). At (pH 5.0, 6.0 and 7.0), (Table 11) conjugation frequency was (0.59×10^{-9} , 0.89×10^{-9} and 0.16×10^{-9}) at these values, respectively. At pH 9.2, the highest number of transconjugants was observed, and conjugation frequency was 7.46×10^{-9} and dropped up to 0.08×10^{-9} at pH 10.0.

Effect of donor and recipient numbers

In all the previous experiments, 1 ml of donor and recipient was mixed together on the agar plate with nearly equal number. In this experiment, the ratio 1:1 per volume has been changed into 2:1 and 3:1 donor to recipient and 1:2, 1:3 donor to recipient. Data are shown in (Table 12).

Table 9. Effect of mating time on conjugation between *Staphylococcus* bacteria

Mating time	No. Transconjugants 10^2	Conjugation frequency per recipient 10^{-8}
12 hour	3.0	0.21
24 hour	3.0	0.21
48 hour	5.4	0.38
3 days	2.4	0.17
5 days	2.2	0.15

Recipient = 14×10^{10} cfu/ml.

Table 10. Effect of temperature mating on conjugation between *Staphylococcus* bacteria

Temperature $^{\circ}\text{C}$	No. Transconjugants 10^2	Conjugation frequency per recipient 10^{-8}
5°	0.00	0.00
25°	6.9	0.49
30°	5.4	0.38
37°	2.5	0.17
42°	2.7	0.19
45°	0.00	0.00

Recipient = 14×10^{10} cfu/ml.

Table 11. Effect of pH values on conjugation between *Staphylococcus* bacteria

pH Values	No. Transconjugants 10^3	Conjugation frequency per recipient 10^{-9}
4.0	0.00	0.00
5.0	4.0	0.59
6.0	6.0	0.89
7.0	1.1	0.16
9.2	50.0	7.46
10.0	0.6	0.08

Recipient = 6.7×10^{12} cfu/ml.

Table 12. Effect of donor and recipient numbers on conjugation

Ratio (donor : recipient) ml/ml	No. Transconjugants 10^2	Conjugation frequency per recipient 10^{-8}
1 : 1	2.0	0.14
2 : 1	4.0	0.28
3 : 1	2.0	0.14
1 : 2	0.6	0.04
1 : 3	0.00	0.00

Recipient = 14×10^{10} cfu/ml, Donor = 13.8×10^{10} cfu/ml.

The highest number of transconjugants was observed when ratio between donor and recipient was 2:1 ml/ ml. Number of transconjugants was 4×10^2 with conjugation frequency of 0.28×10^{-7} per recipient. These results do not agree with others. Since Evans and Dyke, (1988) found that optimum transfer by conjugation between *S. aureus* strains was detected when number of donor and recipient were equal.

Conjugation as a mechanism of gene transfer has been reported in many studies. Naidoo and Noble (1978) observed the transmission of gentamicin resistance between two strains of *S. aureus* on human skin. Moreover, Naidoo and Noble (1981) detected the transfer between two different species. *S. hominis* and *S. aureus* isolated from a hospital patients. Also, plasmid was transferred between *S. aureus* and *S. epidermids* (Jaffe *et al* 1982). In addition, Forbes and Schaberg, (1983) transferred antimicrobial resistance from *S. epidermids* to *S. aureus*. Evans and Dyke (1988) confirmed the transfer of plasmid pJE1 between *S. aureus* bacteria. Furthermore, a chromosome copy of transposon Tn551 was transferred between *S. aureus* (Stout and Iandolo, 1990). Moreover, penicillin resistance

plasmid was transferred by *S. aureus* strains (Mitra *et al* 1995).

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النقل الجيني بين أنواع مختلفة من الـ *Staphylococcus* المعزولة من الإنسان

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أمكن عزل أحادي وعشرون عزلة من الـ *Staphylococcus* من مستشفى جامعة الزقازيق لمرضى من أماكن مختلفة داخل محافظة الشرقية. كل هذه العزلات تم عزلها من أجزاء مختلفة من الجسم البشري، تم التعرف عليها وراثيا وميكروبيولوجيا. كما تم الحصول على العزلات و تمييزها في قسم الميكروبيولوجي الطبي بجامعة الزقازيق. كما تم اختبار مقاومة كل العزلات لعدد من المضادات الحيوية. (الأسترينوميسين، الكلورامفينيكول، البنسيلين و الرفامبيسين)، أعلى نسبة نقل كانت 1.4×10^{-1} بين العزلة رقم 5 و العزلة رقم 12.

أوضحت هذه الدراسة إمكانية حدوث انتقال الجينات بين أنواع مختلفة من بكتريا الـ *Staphylococcus* المعزولة من أجزاء مختلفة من الجسم البشري، ظهرت زيادة في نسبة النقل عند تحضين الأطباق على 25 درجة مئوية لمدة 48 ساعة. بالإضافة إلي زيادة نسبة النقل مع كلوريد الكالسيوم ، حيث كان الفرق الزائد عن الكونتروال حوالي 12.7، نتج أقصى عدد للخلايا المحولة وراثيا عندما كانت نسبة المعطي الي المستقبل 1:2 مل/مل.