Production of Slime Layer by *Staphylococcus epidermidis* Isolated From Corneal Infection

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Abstract:

A total of 37 *Staphylococcus epidermidis* isolates, isolated from corneal scraping of patients with bacterial keratitis and 20 isolates from healthy eyes (as control) (all isolates, isolated from, Ibn Al- Haietham eye hospital / Baghdad), were tested for slime production, 52.63% of all isolates were positive-slime production (23 isolates from patients and 7 isolates from controls). It was found that positive-slime producing *S. epidermidis* were exhibited a high resistance to antibiotics as compared to negative-slime producing isolates.

Key words: Slime Layer, *Staphylococcus epidermidis*, Keratitis.

Introduction:

The coagulase-negative staphylococci (CONS) are widely distributed over the surface of human body, where they constitute the majority of the common nasal bacterial micro flora. Among the CONS, *Staphylococcus epidermidis* is the most frequently isolated species and the most common species responsible for infection [1]. One important property of *S. epidermidis* which is responsible for its persistence and / or opportunistic invasion in the tissues is its ability to produce slime [2, 3]. Slime not only helps the organism in adhesion to host cells, but also protects it from phagocytosis and from the action of antibiotics [4].

Despite being important ocular pathogens, *S. epidermidis* have so far received little attention in ophthalmology. The purpose of this study was to identify, determine antibiotic susceptibility and slime production of *S. epidermidis* isolated from patients with bacterial Keratitis.

Materials and Methods:

Bacteria: isolates of *S. epidermidis* (CONS) from 57 patients, who attended the Ibn Al-Haietham Eye Hospital, Baghdad, during October 2001 to October 2002.

Subjects: of the 57 patients, 37 had come for treatment and investigation of keratitis and 20 (from healthy eyes) served as controls.

Methods:

1. Corneal scrapings: were performed in each case under the slit–lamp biomicroscope. The scrapings were taken from the base and the margins of the ulcer and were then smeared on glass slides for Gram - staining. The specimens also inoculated at 37 ºC on to blood and chocolate agar.

2. Conjunctival swabs: were obtained from 20 control subjects. In order to obtain an ideal swab for culture.

3. Isolation and identification of bacteria: culture material from corneal scrapings and swab were routinely plated on the following media: Blood agar and Chocolate agar with 10% CO_2 (at 37ºC for 24 hr.). In positive-culture cases: all bacteria (*Staphylococcus spp.*) were identified by API-system (API-staph) (Bio mereiux).

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4. Slime-production test: isolates were tested for slime production with the use of a technique described by Christensen *et al*., 1982 [5]. In brief, a loop of organisms from a pure growth on blood agar plate was inoculated onto 5ml of trypticase soy broth (oxoid), and incubated at 37ºC for 48 hr. the contents of the tubes were aspirated and the tubes were stained with 1% safranine (BDH) for 7min. A visible safranine stained film lining the wall of the tubes indicated a positive test.

5. Antibiotic susceptibility: The susceptibility of *S. epidermidis* isolates were performed by Kirby-Bauer disc diffusion assay [6]. We choose five effective antibiotics against most strains of corneal pathogens. The antibiotics and their concentrations/disc (µg) were: [Ciprofloxacin (5 µg), Gentamicin (10 µg), Cephalothin (30 µg), Rifampicin (5 µg) and Chloramphenicol (30 µg)] (oxoid).

6. Statistical analysis: Chi-Square test was used in the analysis of results [7].

Results and Discussion:

Slime test: A total of 57 CONS isolates were studied: 23 (62.16%) isolates from patients and 7(35%) from controls were positive-slime production. thus 30 (52.63%) of 57 isolates were positive-slime producers.

Antibiotics susceptibility: The results of antibiotic susceptibility testing of *S. epidermidis* isolates were isolated from patients with bacterial keratitis are given in Table (1)& Figure (1). The results showed a high resistance to rifampicin and chloramphenicol [16 (69.5%) and 14 (60.8%) respectively]. While cephalothin, ciprofloxacin and gentamicin had a low resistance [7 (30.43%), 8 (34.78%) and 10 (43.47%)] respectively in positive-slime producing isolates. While negative-slime producing isolates exhibited a low resistant against antibiotics, there was significant difference (X^2= 25.8, P < 0.05) between them. Table (2) &Figure (2) shows antibiotic resistance of control isolates. Results showed a low resistant against all antibiotics which are used particularly in negative-slime production isolates there was significant difference (X^2= 42.7, P < 0.05) between them.

Slime layer has been documented to be one of the virulence markers of *S. epidermidis* because of the close association of slime producing strains with infections related to indwelling medical devices including intraocular lenses [8]. Positive-slime producing isolates were isolated in high numbers from patients as compared to control [9].

We found a positive association between positive-slime production and resistance to antibiotics. This is supported by observation made presently in some reports [2, 9, 10], that slime not only helped the organism to colonize the host tissues, but it also protected it from the action of antibiotics.

Cephalothen and Ciprofloxacin a new broad spectrum antibiotics were found an effective agent in this study, they shows a low resistance for most of *S. epidermidis* isolates and bacteria had no chance to develop resistance to these antibiotics was expected to be slow because they requires chromosomal mutation, and resistance can not to be transferred by plasmid mediated mechanisms [11]. The results are similar to those reported by other authors [12], who found that slime layer and multi drug resistance were the important virulence factors of *S. epidermidis* in bacterial keratitis.

Studies on biofilms have shown that *S. epidermidis* is the most frequently isolated slime-producing CNS and is

also the most common cause of nosocomial infections in patients with catheters, medical implants or other invasive devices [13].

Table (1): Antibiotic resistance of keratitis isolates (No. of isolates=37).

Antibiotics	Positive Slime production (NO. of isolates=23)	%	Negative Slime production (NO. of isolates=14)	%
Chloramphenicol	14	60.8	2	15
Ciprofloxacin	8	34.78	2	15
Cephalothen	7	30.43	5	35
Rifampicin	16	69.5	4	29
Gentamycin	10	43.47	4	29

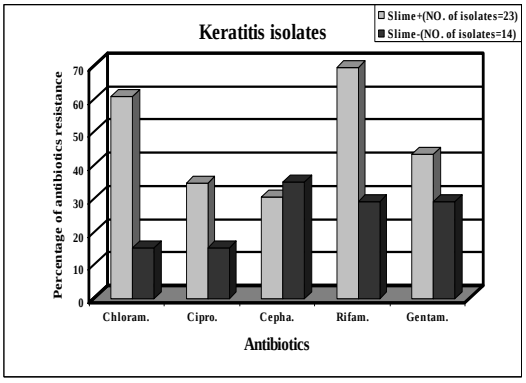

Fig. (1): Antibiotic resistance pattern of Keratitis isolates.

Table (2): Antibiotic resistance of control isolates (No. of isolates=20).

Antibiotics	Positive Slime production (NO. of isolates=7)	%	Negative Slime production (NO. of isolates=13)	%
Chloramphenicol	4	59	5	39
Ciprofloxacin	3	39	3	25
Cephalothen	3	44	5	39.5
Rifampicin	2	29.5	4	31
Gentamycin	2	29	3	23

In conclusion, our findings showed that the slime layer, was responsible for resistance to antibiotics

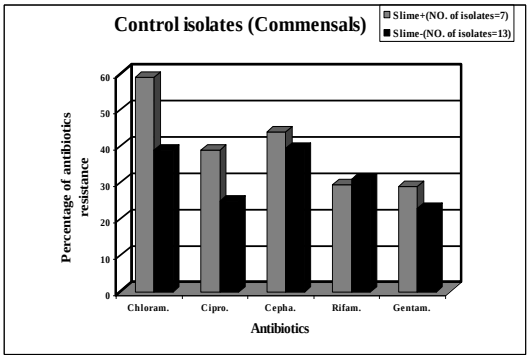

Fig. (2): Antibiotic resistance pattern of Control isolates.

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أنتاج الطبقة اللزجة من بكتريا المكورات العنقوديه للجلد المعزولة من أصابة القرنية

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الخلاصة:

من المجموع الكلي لـ 37 عزلة لبكتريا المكورات العنقوديه للجلد عزلت من قرنيات مرضى مصابين بالتهاب القرنية البكتيري و20عزلة عزلت من قرنيات عيون غير مصابة (السيطرة) (جميع العزلات, عزلت من مستشفى ابن الهيثم للعيون / بغداد), ظهر 52.63 % منها (23 عزلة من المصابين و7 من غير المصابين) منتجة للطبقة اللزجة وقد أظهرت النتائج إن جميع العزلات المنتجة لهذه الطبقة لها مقاومة عالية تجاه المضادات الحيوية مقارنة بالعزلات غير المنتجة.