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COMPARISON OF ANTIBIOTIC SUSCEPTIBILITIES OF FOUR SPECIES OF COAGULASE-NEGATIVE STAPHYLOCOCCI ISOLATED FROM NORMAL SKIN AND ACNE LESIONS: A STATISTICAL APPROACH

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دراسة أحصائية للمقاومة للمضادات الحيوية لأربعة أنواع من المكورات العنقودية السالبة لتجلط البلازما مستخلصه من لطخات حب الشباب ومن الجلد السليم

عزلت عشرة سلالات من كل نوع من المكورات العنقودية السالبة لتجلط البلازما وهي *S. cohnii, S. capitis, S. hominis, S. epidermidis* من لطخات حب الشباب ومن الجلد السليم (ومجموعها ٨٠ سلالة) وقد أختبرت حساسيتها لعشرة أنواع من المضادات الحيوية. ظهرت فروقات معنوية بين الأنواع ووجد أن الأنواع المعزولة من لطخة حب الشباب كانت أكثر مقاومة من تلك المعزولة من الجلد السليم. وقد كان للمضادين الحيويين الكنتامايسين والجنتامايسين تأثيراً فعالاً مقارنة بالإمبسلين والبنسلين والستريتومايسين والتتراسايكلين .

Key words: Coagulase-Negative Staphylococci, Skin and Acne lesions

ABSTRACT

Ten strains of each of four species of coagulase negative staphylococci: *S. epidermidis, S. hominis, S. capitis,* and *S. cohnii* isolated from both acne lesion and normal skin (total 80 strains) were tested for their antibiotic susceptibilities. Statistical analysis has shown significant differences among strains. Strains of acne lesions were more resistant. The antibiotics clindamycin and gentamycin were found to have superior effect over ampicillin, penicillin, streptomycin and tetracyclin.

INTRODUCTION

Wide variety of coagulase negative staphylococci are normal skin resident and could cause infections when foreign bodies are implanted (1) or when massive medical insult to host defences is incured (2). *Staphylococcus epidermidis* accounted for approximately 50% of staphylococcal colonist in the normal skin, the rest were other coagulase negative species (3). Their numbers increase significantly with the development of acne lesion which is a pathological condition of sebaceous follicle (4).

When a coagulase negative isolate is acting as a pathogen, its sensitivity is tested to the antibiotics commonly used for *S. aureus* (5). Because acne is chronic, its treatment usually must be given continually. This may lead eventually to the emergence of antibiotic resistant organisms (6).

It is important therefore, to determine whether antibiotic therapy for acne increases the number of multiple-resistant staphylococci at the skin surface from where they can be easily disseminated.

This study was undertaken to test antibiotic susceptibility of four species of coagulase negative staphylococci isolated from normal skin and acne lesions to have a better understanding of their pathogenic potentialities.

MATERIALS AND METHODS

Subjects

One hundred and fifty male and female subjects aged 14-24 years (patients of the private clinic of Dr. Al-Rubaiee and from the Basrah General Hospital, in addition to secondary school and university students) were subjected to this experiment. One hundred and five persons with mild to moderate acne and the rest were normal. They received no oral or topical therapy for at least one month prior to this investigation.

Bacteriological methods

Bacteria were sampled from the normal face using cotton swabs (7) dipped in 2 ml Brain Heart Infusion Broth (BHIB.). A circular area (16 mm^2 approximately from the unprepared skin of the face (cheek and forehead) were stroked and the swab was returned to the tube and sealed. Comedons were expressed by comedon extractor from acne lesions after wiping the skin with 70% ethyl alcohol, and collected on swabs dipped in BHIB. (8). Care being taken to avoid contamination of the sample with blood.

Colony forming units of members of staphylococci were determined by cultivating swabs on Brain Heart Infusion Agar (BHIA.) and incubated aerobically at 37°C for 24-48 h. The identification tests were as follows: Gram stain, production of acid from glucose, Catalase, Oxidase, Coagulase, gelatinase, hemolysin and lecithinase (9, 10).

Antibiotic sensitivity

The method of Bauer *et al.* (11) was adopted for testing antibiotic susceptibilities of coagulase negative staphylococci toward ten antibiotics by disk diffusion method using Muller Hinton Agar (MHA.). The following concentrations (μ per disk) of antibiotics (Oxoid) were used:(1) Ampicillin (10), (2)Chloramphenicol (30), (3)Clindamycin (10), (4)Gentamycin (10), (5)Erythromycin (15), (6)Kanamycin (30), (7)Neomycin (30), (8)Penicillin (10), (9)Streptomycin (10) and (10)Tetracycline (30).

Plates were incubated at 37°C for 24 h. The inhibition zones were measured and the average of duplicate plates was used to estimate the susceptibility according to Difco (12). The strains were coded as resistant or susceptible with intermediate strains being included in the resistant class. The zone diameters of inhibition of five isolates of each species was considered for comparison.

RESULTS

Coagulase negative staphylococci recovered from both (1)acne lesion and (2)normal skin were (1)Staphylococcus epidermidis, (2)*S. capitis*, (3)*S. cohnii* and (4)*S. hominis*. Percentage frequencies of each species in either sites were determined (13). Ten strains of each species from each site were selected randomly to test their antibiotic susceptibilities.

The experimental scheme has been designed as a -3-way factorial completely randomized design (CRD) (14), where the model for this CRD is:

$$Y_{ijkl}=\mu+\alpha_i+\beta_j+\gamma_k+(\alpha\beta)_{ij}+(\beta\gamma)_{jk}+(\alpha\beta\gamma)_{ijk}+e_{ijkl}$$

(i = 1, 2; j = 1, 2, 3, 4; k = 1, 2, ..., 10; l = 1, ..., 5)

where Y_{ijkl} denotes the length of inhibition zone diameter (mm) of killed bacteria of the *l*th replicate of the *k*th antibiotic of *j*th bacteria from *i*th person. At 1% significance level, we have found that there is a significant difference between the mean of each main factor and the interaction X antibiotic. A revised least significant difference (RLSD)

Table 1
Bacteria Means Comparisons

Bacteria	Y.j..	**
S. epidermidis = β_1	18.47	ab
S. capitis = β_2	19.86	a
S. cohnii = β_3	19.58	a
S. hominis = β_4	15.86	b

$\alpha = 0.01$, RLSD = 1.533

Means without the same letter in each column are significantly different at the 1% level.

Table 2
Antibiotic Means Comparisons

Antibiotic	$Y_{..k.}$	**
Ampicilline = γ_1	15.850	ef
Chloramphenicol = γ_2	19.850	bc
Clindamycin = γ_3	23.275	a
Gentamycin = γ_4	21.000	ab
Erythromycin = γ_5	20.175	bc
Kanamycin = γ_6	18.675	bcd
Neomycin = γ_7	18.550	cd
Penicillin = γ_8	16.100	ef
Streptomycin = γ_9	17.200	de
Tetracycline = γ_{10}	13.750	f

$\alpha = 0.01$, RLSD = 2.40

Means without the same letter in each column are significantly different at the 1% level.

Table 3
Interaction Means Comparisons

Interaction	$Y_{jk.}$	**	Interaction	$Y_{jk.}$	**
$(\beta\gamma)11$	12.7	mn	31	19.2	bcdefghij
$(\beta\gamma)12$	21.5	bcde	32	20.5	bcdefg
13	23.1	abc	33	21.3	bcdef
14	22.6	abcd	34	20.2	bcdefg
15	20.2	bcdefg	35	22.9	abc
16	19.9	bcdefg	36	19.5	bcdefgh
17	17.9	cdefghijklmn	37	18.5	cdefghijkl
18	14.1	hijklmn	38	23.1	abc
19	17.0	defghijklmn	39	17.1	defghijklmn
110	15.7	fghijklmn	310	13.3	klmn
21	18.5	cdefghijkl	41	13.0	lmn
22	18.8	cdefghijk	42	18.6	cdefghijkl
23	27.8	a	43	20.9	bcdef
24	24.9	ab	44	13.1	klmn
25	18.3	cdefghijklm	45	19.3	bcdefghi
26	18.8	cdefghijk	46	16.5	efghijklmn
27	23.0	abc	47	14.8	ghijklmn
28	13.7	ijklmn	48	13.5	jklmn
29	21.0	bcdef	49	13.7	ijklmn
210	13.8	hijklmn	410	12.2	n

$\alpha = 0.01$, RLSD = 5.97

Means without the same letter in each column are significantly different at the 1% level.

procedure for multiple comparisons has been used to indicate the differences and also the superiority of each mean over other's (15).

Tables 1, 2 and 3 illustrate the final results of the multiple comparisons which can be described as follows: Two means in the ** column are declared significantly different only if their respective letters are completely different and certain mean is said to be superior to another if its corresponding letters precede the other corresponding letter (following the alphabetical order).

Results of the statistical analysis have shown significant differences between acne lesion and normal skin in their means, where the acne lesions have been affected by amount of (19.14) compared to the normal skin (17.74). The results of the statistical analysis have also shown significant differences among the species of coagulase negative staphylococci (Table 1), where *S. capitis* and *S. cohnii* have superior susceptibilities over *S. hominis*, and there was no significant differences between *S. epidermidis* and *S. hominis*.

Concerning the effect of antibiotics, our analyses showed (Table 2) superiority of the antibiotics clindamycin and gentamycin which were significantly different from others, whereas ampicillin, penicillin and tetracycline were the least effective. Finally, considering the interaction effect between the species and antibiotics, the analyses indicated (Table 3) the best interaction combination was *S. capitis* X clindamycin compared to other combinations, whereas the least interaction combination was *S. hominis* X tetracycline.

From Table (3) two findings can also be deduced.

(i) Comparing the effect of antibiotics on each species

Table 4
Comparisons of the Effects of Antibiotics on Each Species

Bacteria	*S. epidermidis*		*S. capitis*		*S. cohnii*		*S. hominis*	
Antibiotic	$Y_{.1k.}$	**	$Y_{.2k.}$	**	$Y_{.3k.}$	**	$Y_{.4k.}$	**
Ampicillin	12.7	mn	18.5	cdefghijkl	19.2	bcdefghij	13.0	lmn
Chloramphenicol	21.5	bcde	18.8	cdefghijk	20.5	bcdefg	18.6	cdefghijkl
Clindamycin	23.1	abc	27.8	a	21.3	bcdef	20.9	bcdef
Gentamycin	22.6	abcd	24.9	ab	20.2	bcdefg	13.1	klmn
Erythromycin	20.2	bcdefg	18.3	cdefghijklm	22.9	abc	19.3	bcdefghi
Kanamycin	19.9	bcdefg	18.8	cdefghijk	19.5	bcdefgh	16.5	efghijklmn
Neomycin	17.9	cdefghijklmn	23.0	abc	18.5	cdefghijkl	14.8	ghijklmn
Penicillin	14.1	hijklmn	13.7	ijklmn	23.1	abc	13.5	jklmn
Streptomycin	17.0	defghijklmn	21.0	bcdef	17.1	defghijklmn	13.7	ijklmn
Tetracycline	15.7	fghijklmn	13.8	hijklmn	13.3	klmn	12.2	n

Means without the same letter in each column are significantly different at the 1% level.

Table 5
The Resistance of Bacteria as Compared to Every Antibiotic

Antibiotic	Ampicillin		Chloramphenicol		Clindamycin		Gentamycin		Erythromycin		Kanamycin		Neomycin					
Bacteria	$Y_{.j1.}$	**	$Y_{.j2.}$	**	$Y_{.j3.}$	**	$Y_{.j4.}$	**	$Y_{.j5.}$	**	$Y_{.j6.}$	**	$Y_{.j7.}$	**	$Y_{.j8.}$	**	$Y_{.j9.}$	**	$Y_{.j10.}$	**
S. epidermidis	12.7	mn	21.5	bcde	23.1	abc	22.6	abcd	20.2	bcdefg	19.9	bcdefg	17.9	cdefgh ijklmn	14.1	higklmn	17.0	defghi jklmn	15.7	fghijk lmn
S. capities	18.5	cdefg hijkl	18.8	cdefg hijk	27.8	a	24.9	ab	18.3	cdefgh ijklm	18.8	cdefgh	23.0	abc	13.7	ijklmn hijk	21.0	bcdef	13.8	hijklmn
S. cohinii	19.2	bcdef gkij	20.5	bcdefg	21.3	bcdef	20.2	bcdefg	22.9	abc	19.5	bcdefgh	18.5	cdefg hijkl	23.1	cdefg	17.1	defghi jklmn	13.3	klmn
S. hominos	13.0	lmn	18.6	cdefg hijkl	20.9	bcdef	13.1	klmn	19.3	bcdefghi	16.5	efghij klmn	14.8	ghijklmn	13.5	jklmn	13.7	ijklmn	12.2	n

Means without the same letter in each column are significantly different at the 1% level.

separately as shown in Table (4), it was found that clindamycin is superior for *S. epidermidis* over ampicillin, penicillin, streptomycin and tetracycline, whereas the least effective antibiotics for this species were ampicillin and penicillin. We can discuss, likewise, for the other three species.

(ii) Considering the resistance of bacteria to every antibiotic separately (Table 5), it appears that *S. epidermidis* is more resistant than *S. capitis* and *S. cohnii* to ampicillin.

DISCUSSION

In inflammatory acne, tetracycline and erythromycin are the drugs of choice as they are effective in reducing bacterial population and relatively free of side effects. A 50% or more decrease in number of acne lesions occurs after 8-12 weeks of treatment; therefore, multiple resistant coagulase negative staphylococci rose steadily as treatment progress (6, 16). This was clearly documented in the present study where significant differences were found between means of acne lesions and normal skin species. Statistical analysis confirmed that clindamycin was superior over most antibiotics; it is, therefore, an effective alternative to be used when moderate to severe acne fails to respond adequately to tetracycline, erythromycin and cortimoxazole (17).

Naido (18) reported that plasmids are able to mobilise resistance factors (covering resistance to tetracycline, erythromycin and chloramphenicol) that exist as distinct plasmid in both donor and recipient strains of staphylococci. In addition, Naido and Noble (19) concluded that epidemiologically independent gentamycin-resistant coagulase negative staphylococci such as *S. epidermidis, S. cohnii* could transfer conjugative plasmids to *S. aureus, S. capitis, S. epidermidis* and *S. hominis* fairly readily. Furthermore, both *S. epidermidis* and *S. aureus* were reported to share a number of bacteriophages (20) and that transduction may allow a ready mean for multiple resistance to be acquired to fully virulent *S. aureus* strains. These are crucially important prospects which inevitably lead to the emergence of multiple-resistance strains as indicated by Naido and Noble (19) that plasmid transfer occurs at a faster rate on human skin than in cultures.

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