INFLUENCES OF BACTERIA ON ECZEMA: BACTERIAL AND IMMUNE ASPECTS

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ABSTRACT

This study aimed to investigate the most common aerobic bacteria associated with Eczema, preparation of bacterial crude antigen from the predominant bacterial isolate as well as determination of total IgE and IgE specific for bacterial antigen using ELISA test.

Fifty (50) out of (80) eczematic patients were showing positive skin swab culture with dominance of S. aureus (58%) from both adults and children , Corynebacterium , diphtheroid species took the second rank of isolation (22%) followed by S.epidermedis (16%) and Micrococcus species (10%).

Patients with positive swab cultures from both sexes and two age groups showed more IgE specific for S. aureus crude antigen (65.5%) than that of *S. aurous* standard strain. More esosinophil absolute counts were found among patients than control group. We can conclude that bacterial infections associated with eczema makes it more severe via induction of certain mediators (IgE) in this study and probably through other mediators. S. took the first rank of isolation.

Introduction

Eczema is an acute or chronic non contagious skin disease of global distribution and affects all age groups and both sexes (1, 2).

Regarding the causative triggering factors, eczema may be due to endogenous or exogenous factors or both (1, 2, 3).

Many pioneers emphasized that infectious agents, (Bacteria, Viruses, Fungi and parasites) were playing a role in the etiopathogenesis of allergic and non allergic disorders like dermatitis, asthma and rhinitis (4,5,6). It is necessary to distinguish between infected eczematoid dermatitis as an example of auto sensitization and infected eczema in which eczema is already existed and complicated by secondary bacterial or viral invasion of the broken skin (7,8).

Staphylococcus aureus was found at high concentration in over 90% of eczematous lesions and consequently these has been considerable interest in the contribution of *S. aureus* colonization and infection to the severity of eczema (9,10,11). The mechanism by which microorganisms can cause eczema is not understood, In spite of many mentioned studies in this field emphasized the link between bacteria and allergy, still there is a great need of studies to show more of microbial role in eczema etiology. So this study was devoted.

Materials and Methods:

Patients and Control Groups:

Eighty (80) eczematic patients who attended the Clinic Dermatology and Venereology Department in Ramadi General Hospital during the period extending from April 2005 to March 2006. Patients were from both sexes and their ages were from 3-70 years.

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Twenty six (26) healthy individuals from both sexes were selected resembling the same age groups of patients as negative control as they did not show a history of eczema and atopy after investigation. Both test and control groups were submitted to a clinical examination, questionnaire tests as well as laboratory investigations for skin swabs and blood specimens.

Blood Specimens:

Aseptically 5 ml Of venous blood were collected from each individual of test and control groups. Two mls of each sample were transferred rapidly to clean, dry and sterile EDTA tube and shacked gently, the specimens were used for differential leucocytic count using Leishman stained smears following (12). Residual part of each blood sample was transferred to serum tube (free of anticoagulant), and let to coagulate for serum collection using centrifuge at 3000 rpm for 5 minutes. Collected sera were kept in labeled cryotubes (Nalgen, USA) at -20 °C to be used for total IgE and specific IgE estimation.

Skin swabs collection:

Sterile swabs were wetted with sterile normal saline and the dry lesions of patients were smeared aseptically and transferred to the laboratory for bacteriological studies. Moist skin lesions were smeared directly (22). These swabs were examined as following:

1. Direct smears

A drop of sterile normal saline was placed on a clean slide and a suitable piece of bacterial colony was mixed on a slide to make a smear, dried with air, fixed with flame and then stained with Gram stain (14).

2. Cultivation

Skin swabs were inoculated on Blood agar, MacConkey agar and Chocolate agar. The streaked plates Blood agar and MacConkey were incubated aerobically while Chocolate agar was cultivated under 5-10 % CO₂ using Gas pack system at 37 °C for 24 - 48 hours .Bacterial isolates were identified and confirmed using the suitable media and tests suitable for each following (14). Isolates of *Staphylococcus aureus* were kept frozen on Brain Heart Infusion broth with 10% Glycerol to be used later for bacterial antigen preparation to be used later for ELISA test for specific IgE (15).

Preparation of bacterial antigen:

Three sterile blood agar plates were seeded with sufficient amounts of well identified preserved bacterial isolates of *Staphylococcus aureus* after its refreshment. Plates were incubated at 37 °C for overnight; growth was harvested with sterile L form glass rode after flooding agar surface with 0.3% formalized saline.

Growth suspension was collected and poured in sterile centrifuge tube aseptically, and then each 5 ml of this suspension was centrifuged at 2500 rpm in cooled centrifuge for 10 minutes. The supernatant was decanted and pellets were resuspended in 5 ml of sterile resuspending buffer with gentle vortex shaking. The suspension was centrifuged for 5 minutes at 2500 rpm in cooled centrifuge, supernatant was discarded and pellets resuspended in 2 ml of sterile Tris/EDTA/Nacl (TEN) buffer.

Suspension was sonicated aseptically at ice melting temperature for 30 minutes at 2400 cycle/second.

[Precautions were considered to prevent contamination]. Sterility test was done to sonicate and protein concentration was determined using Biuret test (16). Sonicate suspension was kept frozen at -20 °C till be used (15).

Preparation of crude bacterial antigen discs:

Two mls of frozen crude *Staphylococcus aureus* antigen (contained 4 mg/ml protein) was thawed and

one ml of this suspension was diluted two folds with sterile phosphate buffer saline PH7.2. Each one ml of diluted bacterial crude antigen suspension was employed to impregnate 100 discs prepared as described bellow:

Watman blotting papers No. 1 were used for paper discs preparation using paper puncher to get 0.5 cm in diameter discs. These discs were sterilized by UV light illuminator for overnight. A sample from these discs was submitted to sterility test.

Antigen impregnated discs were kept semidried in clean and sterile cases as described by Biomaghreb kits. These discs were used as test antigens for the specific IgE determination by ELISA test (5, 17).

Serological Tests:

Total IgE Determination Biomaghreb Kit for Total IgE (Biomaghreb, Tunisia) was employed for the total IgE determination using ELISA test as mentioned by (17).

IgE Specific for *Staphylococcus aureus* Prepared Antigen:

ELISA test was used in the same manner of that used for environmental, (HD) and House Dust Mites (HDM) except that the antigen discs were employed were impregnated with

Crude antigens prepared from *Staphylococcus aureus* isolated from patients (5,17).

Statistical Analysis Of Results:

Data were analyzed using Statistical analysis was performed by using the statistical program SPSS Ver. 10 (18).

Results

Distribution of patients according to age and sex:

Females within age group 1 (3- 17) years showed higher rate of eczema than males, while adult males

showed higher rate of eczema than females (P < 0.05) (Table-1).

Skin Swabs Investigations:

Skin swabs cultures:

Out of (80) studied eczematic patient skin swabs (50) patients (62.5%) showed positive cultures while (30) patients (37.5%) showed negative cultures (Table-2). Adult females showed higher ratio of positive cultural results (68.42%) than males (54.54%) (P<0.05), while no significant difference was found between sexes within age group (3-17) years (Table-2).

(38) out of (65) bacterial isolates were from adults while (27) were from samples of age group (3-17) years.

Age group (18 - 70) years of both sexes showed the highest number of bacterial isolates 38 (58.4%) (Table-3).

Within patient groups *Staphylococcus aureus* showed the highest number (29 out of 65), Diphtheroid species came next (11 out of 65). While *Staphylococcus epidermidis*, Micrococcus spp. and *Streptococcus pyogenes* showed lower rate of isolation 8:65, 5:65 and 4:65 respectively. Then isolates of Gram negative bacteria Proteus spp. came next 3:65 and Escherichia coli 2:65.

Streptococcus fecalis, Bacillus spp. and Pseudomonas aeruginosa showed the lowest rate of isolation 1: 65 for each of them as shown in (Table-4). One isolate of Staphylococcus aureus was isolated from normal carrier (control) young male within the age group (3-17) years (Table-4).

Mixed bacteria *Staphylococcus aureus* and Diphtheroid spp. showed the highest number (3 out of 17), *Streptococcus pyogenes* and Diphtheroid spp., *Staphylococcus epidermidis* and *Streptococcus pyogenes*, Micrococcus spp. and Diphtheroid spp.,

Proteus spp. and Diphtheroid spp. showed lower rates of isolation (2 out of 17).

While *Staphylococcus aureus* and Micrococcus spp., Bacillus spp. and Diphtheroid spp., *Escherichia coli* and Micrococcus spp., *Escherichia coli* and Diphtheroid spp., Proteus spp. and Bacillus spp., *Pseudomonas aeruginosa* and *Staphylococcus aureus* showed the lowest rate of isolation (1 out of 17) (Table-5).

Bacterial Antigen Studies:

Patients with positive culture from both age groups showed more positive ELISA test for IgE specific for *Staphylococcus aureus* antigen, *Staphylococcus epidermidis* antigen and *Staphylococcus aureus* (standard antigen) than that of the negative culture group (P < 0.005) (Table- 6).

Males from both age groups with positive and negative cultures showed more positive test for (three bacterial antigens) than that of females (Table-6).

Number of positive ELISA IgE test specific for the prepared crude *Staphylococcus aureus* local isolate antigen was (14) which was more than that of *Staphylococcus epidermidis* local isolate antigen that was (11) and standard *Staphylococcus aureus* antigen which was (8). Only (10) patients showed positive ELISA IgE specific for the three antigens which were from positive culture group.

While patients who showed positive ELISA IgE specific for the prepared crude *Staphylococcus aureus* local isolate antigen and that of *Staphylococcus epidermidis* were (2) patients, (1) patient was from the positive culture group and (1) patient was from the negative culture group (Table-6).

Patients who showed positive ELISA IgE specific for the prepared crude Staphylococcus aureus local isolate antigen and *Staphylococus aureus* (standard antigen) were (2) patients who were from the positive culture group (Table-6).

Eosinophil absolute counts:

The mean values of eosinophil absolute counts in peripheral blood of the positive culture group in both age groups were higher than that of the negative culture group (P < 0.05). Adult males showed higher mean values of eosinophil absolute counts in both positive and negative culture groups, the same thing was true for young males (Table-7).

Test groups showed higher mean values of eosinophil absolute numbers than that of control individuals (P < 0.001) (Table-7).

Total IgE values IU / ml:

Patients within age group (3-17) years males and females with negative culture group showed higher total IgE mean values than that of positive culture group, while patients of age group (18-70) years males and females with positive culture group showed higher total IgE mean values than that of negative culture group (P < 0.05).

Males of both age groups with positive and negative cultures showed higher total IgE mean values than that of females (P < 0.05).

Patients with positive and negative culture groups showed higher total IgE mean values than that of control groups (Table-8) (P< 0.001).

Discussion

Age and sex distribution:

The rate and severity of eczema were found to be high in females in age group (3-17) years and adult males. This is in accordance with that of other workers (19,20).

Females within age group (3-17) years showed higher rate of eczema than that of males. This might be due to sex hormones (21). Furthermore, females are

more affected by sensitive substances in kitchen like soaps, detergents and chlorides. While adult males showed higher rate of eczema than that of females (22).

This might be due to the fact that males are more affected by sensitive factors by different jobs and occupations (22,23).

Skin Swabs Investigations:

Direct smear examination:

Gram stain:

Gram stained smears were used to follow up cultural results. Skin swabs positive culture depended on the predominance of suspected pathogenic microorganism or presence of actual pathogen (24), so results of Gram stain were helpful in the identification of infection due to Gram negative bacilli or Gram positive cocci.

Cultural Results:

Bacterial isolate distribution showed that *Staphylococcus aureus* was with the highest rate of isolation followed by Diphtheroid species. This was consistent with the observations of (25,26).

The role of bacterial infections in eczema was variable, it might be due to induction of IgE specific for bacterial antigens (7,9) and this was seen in the present study.

Number of patients who showed positive skin swab cultures for Staphylococcus aureus was logical due to *Staphylococcus aureus* that was pathogenic bacteria containing different pathogenic antigens and may completely or partially induce humoral immunological response to produce total and specific IgE.

IgE specific for *Staphylococcus aureus* crude antigen and to that of *Staphylococcus aureus* standard antigen was detected in the sera of patient. This was

consistent with the findings of (27) who showed that *Staphylococcus aureus* specific IgE was the frequent.

Patients with positive culture showed more positive IgE specific for *Staphylococcus aureus* crude antigen prepared from bacterial isolates (Isolated from patients) and for *Staphylococcus aureus* standard antigen. This might be due to presence of shared antigens between *Staphylococcus aureus* (isolated from patients) and *Staphylococcus aureus* (isolated from patients) and *Staphylococcus aureus* standard isolates. So results of the present study indicated that children and adults showed eczema associated with infection (62.5 %), others had eczema with no association of infection (37.5 %) (Table-3). Moreover, eczema associated with infection seemed more severe as indicated by tested parameters and difficulty in diagnosis and treatment (26,28).

Patients with positive culture showed positive IgE specific for *Staphylococcus epidermidis*. This indicated the presence of bacterial antigens in the same bacteria that induce allergic reaction although this bacteria was present in normal form in some individuals (5,29,30), many studies showed that this bacteria causes many injuries such as skin injuries (burns and wounds) (31,32).

Patients with positive culture showed positive IgE specific for *Staphylococcus aureus* (standard strain). This indicated the presence of shared antigens between *Staphylococcus aureus* (standard strain) and bacterial isolates *Staphylococcus aureus*, *Staphylococcus epidermidis*. This represents evidence for the appearance of positive specific IgE for three antigens in some patients during the study.

Patients with negative culture showed positive IgE specific for *Staphylococcus aureus*. This refers to either presence of this bacteria in the form of carrier in patients (not in enough number to give growth on culture media), there are references that refer to presence of normal carriers for *Staphylococcus aureus*

(healthy individuals) (32) or that the positive results in patients with negative culture were due to shared antigens between *Staphylococcus epidermidis* (that present in normal form on the skin but not in enough number to give growth on culture media) and *Staphylococcus aureus*, *Staphylococcus epidermidis*. This leads to the appearance of positive IgE specific for three antigens although this bacteria is not isolated from patients.

Isolation of *Staphylococcus aureus* from normal carrier young male was consistent with findings of (30).

Eosinophil counts:

Eosinophil infiltration seemed a prominent feature of eczematic inflammation. These induction and infiltration were due to many factors such as complement fragment (C5a) platelet activating factor PAF, Eotaxin and leukotrine B4 LTB4 (33).

So patients showed higher eosinophil absolute numbers especially patients with positive culture and these observations were in accordance with the results of (34).

Serological Tests:

IgE:

Total IgE values increased in sera of patients with positive culture and others with negative culture. These results were in accordance with that of (5,6,35).

Some patients with negative culture group showed more IgE than patients with positive culture which might be due to the potency of allergens (may be environmental allergens) involved with eczema (36).

Patients within age group (18-70) years with positive culture showed more total IgE mean values. This is due to bacterial or environmental antigens or both.

Males of both age groups with positive and negative cultures showed higher total IgE mean values than that of females. This might be due to the fact that males were exposed to sensitive factors more than females by different jobs and occupations (2,3,37).

Patients with positive and negative cultures showed higher total IgE mean values than that of control groups due to allergic reaction in patients because of the bacterial or environmental antigens (5,6,35,38).

Increased IgE enabled bacterial antigens in patients with positive culture and other allergens in patients with negative culture to release inflammatory mediators from mast cells, basophils and other cells involved with eczema pathogenesis (39).

Increased IgE titers were ought to the eosinophils recruitment by IL-5, IL-4, IL-8 and IgE release by (Th2 activation) (40).

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Table 1: Age and sex distribution of the eczema patients (80 patients)

Age group years) Total Σ Œ 16:80 (20%) 12 ଛ ଛ 28 12: (15) 23.75 %) 9 19:80 22 35:80 (43.75 %) 45:80 56.25%) **Total** 8

Table 2: Patients distribution according to culture results

Patients	Age group	(3 - 17)	Age group	(18 - 70)	Total
group	M	F	M	F	
Positive	8:12	11:16	18:33	13:19	50:80
culture	(66.66 %)	(68.75 %)	(54.54 %)	(68.42 %)	(62.5 %)
Negative	4:12	5:16	15:33	6:19	30:80
culture	(33.33 %)	(31.25 %)	(45.45 %)	(31.57 %)	(37.5 %)

Table 3: Number of bacterial isolates from positive culture samples in patients

cuitui	Samp	ics in p	aticita
Age group (years)	M	Ŧ	Total
(3 - 17)	13:65	14:65	27:65
	(20 %)	(21.5 %)	(41.54 %)
(18 - 70)	23:65	15:65	38:65
	(35.3 %)	(23.07 %)	(58.46 %)
Total	36:65	29: 65	65:65
	(55.3 %)	(44.6 %)	(100 %)

Table 4: Distribution of bacterial types among patients and control

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		Ī	patier	nts				coı	ntro	ol
Bacterial spp.	Age group 1	(3-17)	Total	Z anoso seV	(18-70)	Total	Age group 1	(3-17)	Age group 2	(18 - 70) Total
	M	Ł		M	H		M	F	M	Ŧ
Staph. aureus	3:8 (37.5 %)	4:11 (36.3 %)	7:19	13: 18	9:13	22:31 (70.9 %)	1	0	0	30
Staph. enidermidis	3:8 (37.5%)	1:11 (9.1%)	4:19	1:18 (5.5 %)	3:13	4:31	0	0	0	0 8
St. faecalis	0:8 (0%)	0:11 (0%)	$0:19 \ (0\%)$	1:18 (5.5 %)	0:13	1:31	0	0	0	0 1
St. nvovenes	1:8	0:11	1:19	2:18	1:13	3:31	0	0	0	0 4

Ē	Pseudomona	Escherichia	Proteus	Bacillus	Micrococcu	Diphtheroid
Total	s aeruoinosa	coli	.aas	.aas	s sun.	species
13:65	8:0	1:8	1:8	8:0	2:8	2:8
(20%)	(% 0)	(12.5 %)	(12.5 %)	(% 0)	(25 %)	(25 %)
14:65	0:11	1:11	1:11	1:11	2:11	4:11
(21.5%)	(0%)	(9.1%)	(9.1%)	(9.1%)	(18.1%)	(36.3 %)
27:65	0:19	2:19	2:19	1:19	4:19	6:19
(41.5%)	(%0)	(10.5 %)	(10.5 %)	(5.2 %)	(21.05 %)	(31.5%)
23:65	1:18	0:18	0:18	0:18	1:18	4:18
(35.3 %)	(5.5%)	(%0)	(0%)	(% 0)	(5.5%)	(22.2%)
15:65	0:13	0:13	1:13	0:13	0:13	1:13
(23.07 %)	0 % 0	(%0)	(% 9.2)	(%0)	(%0)	(2.6 %)
38:65	1:31	0:31	1:31	0:31	1:31	5:31
(58.4 %)	(3.2 %)	(%0)	(3.2 %)	(0%)	(3.2 %)	(16.1%)
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
	0	0	0	0	0	0
99	1	2	3	1	v	11

Table 5: Distribution of bacterial types (mixed infection) among patients

Mixed	Age 1	1(3-		2 (18 70)	Total
Mi	M	F	M	F	Tc
Staph . aureus and Diphtheriod spp.	1:8 (12.5%)	0:11 (0%)	2:18 (11.1%)	0:13 (0%)	3:50
St . pyogenes and Diphtheriod spp.	(% 0) 8 : 0	0:11 (0%)	2:18 (11.1%)	0:13 (0%)	2:50 (4%)
Staph . aureus and Micrococcus spp.	0:8	0:11 (0%)	1:18 (5.5%)	0:13 (0%)	1:50 (2%)
Staph. epidermidis and St. pyogenes	1:8 (12.5 %)	0:11 (0%)	0:18	1:13 (7.6%)	2:50 (4 %)

Proteus spp . and Bacillus spp .	Proteus spp .and Diphtheriod spp.	E . coli and Diphtheriod Spp .	E . coli and Micrococcus spp .	Bacillus spp . and Diphtheriod Spp .	Micrococcus spp.and Diphtheriod spp.
0 : 8	1:8	0 : 8	1:8	(% 0)	1:8 (12.5%)
8 : 0	(12.5 %)	(0 %)	(12.5%)	8:0	
1:11	0:11	1:11	0:11	1:11	1:11
(9.1 %)	(0%)	(9.1 %)	(0%)	(9.1%)	(9.1%)
0:18	0:18	0:18	0:18	0:18	0:18
(0%)	(0%)	(0%)	(0%)		(0%)
0:13	1:13	0:13	0:13	0:13	0:13
(0%)	(7.6 %)	(0%)	(0%)	(0%)	(0%)
1.50	2:50	1.50	1.50	1:50	2:50
(2 %)	(4%)	(2%)	(2 %)	(2%)	(4%)

Table 6: Specific IgE in patient's sera with positive and negative culture

	-	negativ			~	1
		Pati	ents		Con	trol
		itive		ative		
	cult	ure	cult	ure		
Antigens	Age 1 (3 - 17) years	Age 2 (18-70) years	Age 1(3-17) years	Age 2 (18-70) years	Age 1(3-17) years	Age 2 (18-70) vears
	M	M F	M F	M F	M	M
Staph . aureus crude Ag	2:4	5:10 5:7	0:2 0:3	1:13	QN QN	ON ON
Staph epidermidi s crude Ag	2:4	4:10	0:2	1:13	ON ON	ON ON

Staph . aureus Standard Ag	2:4	1:3	3:10	2:7	0:2	0:3	0:13	0:5	QN	QN	QN	UN
Staph . aureus crude Ag & Staph. aureus Standard Ag	b :0	$\epsilon:0$	1:10	1:7	z:0	$\epsilon:0$	0:13	5 : 0	QN	QN	QN	UN

ND: Not detected

Table 7: Eosinophil Absolute numbers in peripheral blood of studied samples

Eosino Absol			Pati	ents			L
No Cel	•	Posi cult		Nega cult		Con	trol
mm	3.	M	F	M	F	M	F
Age (3-17) y	Mean	730.125	581.18	454.62	330.7	124.125	106.35
Age (18- 70)y	Mean	608.02	501.2	491.13	441.91	122.1	108.2

Table 8: Total serum IgE values (IU /ml) in patients and control $\,$

0			Pati	ients			Contr	ol	
Group		. ,	1 (3- years	Age 2 70) y	2 (18- years		1 (3- years		e 2 -70) ars
		M	F	M	F	M	F	M	F
Positive culture	Min Max Mean	32 150 102	33 120 67.6	5 500 187. 8	30 270 144. 2	8 20 11.6	7 15 17.3	5 30 19. 7	6 22 10
Negative culture	Min Max Mean	10 335 172. 5	70 300 171. 6	40 350 141. 5	6 150 128. 2	8 20 11.6	7 15 17.3	5 30 19. 7	6 22 10

تأثير البكتريا على الاكزما: الأوجه البكتريولوجية والمناعية

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الخلاصة

هدفت هذه الدراسة إلى معرفة البكتريا الهوائية الشائعة ولها علاقة بالاكزما إضافة إلى محاولة استخلاص المستضد الجرثومي من العزلة الجرثومية الأكثر شيوعا و قياس الضد JgE)E الكلى والخاص بالمستضد الجرثومي باستخدام اختبار الاليزا أعطى خمسون مريضا (50) من العدد الكلى ثمانون (80) نتائج موجبة لزر وعات مسحات الجلد .كانت عزلات المكورات العنقودية الذهبية هي الغالبة من كلى الجنسين والفئات العمرية (85%) . وجاءت الوتديات غير الخناقية Diphtheroids بالمرتبة الثانية (22%). بعدها جاءت المكورات السبحية البيضاء (16 %) وكذلك المايكروكوكس (10 %) . المرضى ذوى المسحات الجلدية الموجبة للزرع الجرثومي أعطوا نسب أعلى من الضد الخترة القياسية لنفس الجرثومة. (65.5 %) من المرضى من الجنسين والفئات العمرية الذين كانت الاكزما لديهم مصحوبة بالخمج. كما وبينت اختبارات الدم إن المرضى ذوى الزرع الموجب لمسحات الجلد لديهم أعداد مطلقة أعلى للخلايا الحمضية من غيرهم. ختاما يمكن القول إن الاخماج الجرثومية المصاحبة للاكزما تجعلها أكثر شدة من خلال تحفيز بعض العوامل منها الضد E في الدراسة الحالية وعوامل أخرى محتملة. جاءت المكورات العنقودية الذهبية بالمرتبة الأولى بنسب العزل.