International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2016): 79.57 | Impact Factor (2017): 7.296

Study about the Relationship of Some Aerobic Anaerobic Bacteria of Acne and its Resistance to Some of Antibiotic

Yasameen Hasan Ali

Assistant Professor, Basic Science Dept. College of Nursing – University of Baghdad, Iraq

Abstract: ***Acne vulgar is a common chronic skin disease involving blockage and/ or inflammation of pilosebaceous unit (hair follicle) and their accompanying sebaceous glands). Acne develops as results of an interplay of the following four factors one of them the presence and activity of commensal bacteria Propionibacterium acnes. Methods: (438) samples were collected from (312) infected patients with acnes from both sexes (132 males) and (180 female) for the period from January 2015 to February 2016 where the comedone samples were (116) and pustules samples (322) samples. Samples were distributed in different agricultural medium where identified their depending upon appearance characteristics and biochemistry tests. (12) Plant extracts were used, testing microbe sensitivity of plant extracts and all the results were analyzed by using variance analysis (ANOVA table). Moral valued at level (P< 0.05). Results: Clinical results showed that repetition of infections at females was more than the male. Domination of (P. acnes) bacteria in comedone while domination of (S. epidermidis) bacteria in pustules. it was noticed that the Antibiotic Doxycycline was the most effective in killing all micro-organism under consideration comparing with extracts of used plants. Conclusion: The study revealed that QuercesRobur L. has fatal effectiveness at rate of (100%) to all positive and negative isolates to gram stain comparing with other plant extracts. We recommend using new medical herbs not used before for the treatment of acne which must have fatal effect upon bacteria (Antibacterial Activity).***

Keywords: Acne vulgaris, Antibiotics, clinical findings, Bacteriological findings

1. Introduction

Acne vulgar is a common chronic skin disease involving blockage and /or inflammation of pilosebaceous unit (hair follicle and their accompanying sebaceous gland). Acne can present as Non-inflammatory inflammatory lesions or a mixture of both. Affecting mostly the face, but also the back and chest (1).Acne develops as result of n interplay of the following four factors:

1) Follicular epidermal hyper-proliferation with subsequent plugging of the follicle.
2) Excess sebum production.
3) The presence and activity of the commensal bacteria propion bacterium acnes and,
4) Inflammation (3).

Management

Treatment of acne vulgar should be directed towards the known pathogenic factors including follicular, hyper-proliferation, Excess sebum production acnes and inflammation. The most appropriate treatment is based on grade and severity of the acne. The following medications are used in treatment of propion bacterium acnes vulgar:

1) Retinoid – like agents (isotretinoin).
2) Antibiotic.
3) Acne products (azelaic acid).
4) Herbal therapy (4).

2. Methods

2.1 Collection of the Samples

(438) samples were collected from (312) patients suffer from acne from both sex (132 male, 180 female) for a period from January 2015 till February 2016 with different ages around (15-35 years) in which samples preparation were from (116) sample taken by sterilized comedone extractor after rubbing the face skin three times with spirit (70%). While the number of the pustule s was (322) samples were taken by pustule s tingling by sterilized disposable lancet after rubbing the face skin twice or three times with spirit (70%) with cotton swab. Both samples were put in small glass bottles with screw heads (capped bottles) called (pigot tube) filled with Thioglycolate, then transfer the samples by cooled containers to the laboratory.

***Sample Implant*:** Comedones samples were implanted after breaking them by sterilized glass balls (glass beets) were put inside glass bottle by vortex mixture upon blood agar and MaCconkey agar (separation medium) by using planning through sterilized standard implanting transport (loopful).While the pustule s samples were implanted directly from transfer medium upon enriched medium of blood and MaCconkey agar. This process carried out in case of aerobic implant. In case of anaerobic, both samples were implanted (comedone and pustule s) on the (Thioglycolate) were put already in well caped glass bottles (screw capped bottles) after adding (1%) (Tween 80) in order to motivate the bacteria (*P. acnes*). The bottles will be hugged for the period of (5-7 days).

Then transfer (loopful) from implanted to blood agar which was put in anaerobic refreshment (anaerobic jar) for (2-3)

days for separation of P. acnes. Also conduct anaerobic implant upon Brewer and anaerobic hugging in an anaerobic jars kit) for a period of (5-7) days.

Identification of the Isolate: Identification of isolate depend upon appearance characteristics of the implanted item against implanting medium including (size, color, edges and heights of the colonies and conducting bio-chemical tests according to (Bergy's Manual of determinative) (Bacteriology -1994).

***Clinical Results*:** Clinical results of the current study revealed which included (312) patients infected with acne, their ages ranging between (19-390 years in which the infections of the males was (132) at the percentage of (42.30%), while at the female was (180) infections at the percentage of (57.69%), (appendix-1).

Table 1: The relation of acne occurring with age group for both sexes

Age groups	Male%	Female%	Total number
12-17	52(39.39)	82(45.55)	134(45.55)
18-23	64(48.48)	75(41.66)	139(44.55)
24-29	12(9.09)	19(10.55)	31(9.93)
30-35	4(3.03)	4(2.22)	8(2.56)
Total	132(42.30)	180(57.69)	312

Microbiology Results

(438) samples were implanted infected by acne including (116) comedones and (132) pustules. After separation and identification of all germ isolates according to the gender and type depending upon appearance characteristics for both types, and biochemical tests, the results showed the lesion of both types illustrated in figures (2, 3) which refers to identified isolates germ from (116) samples (comedone) and (322) pustule.

Table 2: Types of isolates bacteria from (116) comedone

Isolates bacteria	Percentage
P. acnes	68.96
S. epidermidis	65.51
S. aureus	8.62

Table 3: Types of isolates bacteria from (322) pustule

Isolates bacteria	Percentage
S. epidermidis	71/32
P. acnes	37.26
P. aeruginosa	11.49
S. aureus	9.93
E. coli	7.14

It was noticed that the domination of isolation of bacterial microscope organism (*P. acnes*) at percentage of (68.96%) followed by bacterial type of (*S. epidermidis*) at isolate percentage of (65.51%), then the third grade comes negative bacteria of gram stain.(appendix No. 2, 3).

Identification of Isolates

Antibiotic sensativity Tests

(16) antibiotic discs were used in the experiment and we tasted the sensitivity of microbes towards Antibiotic discs by using Mollar – Hinton agar, then measuring Antibiotic sensitivity testing after incubation of isolates at (37°) for (18-24 hours). All clinical results subjected to statistical analysis by using analysis of Variance (ANOVA). The moral was valued at the level (P. 0.05).

3. Discussion

Clinical Results

It was noticed that clinical results under study the repletion of infection with the female more than male especially in the age between (12-17) due to sexual maturity stage and appearance of adolescence much earlier than male. Appearance of acne is the beginning of sexual maturity stage for both sexes (6).

These results agreed with international studies (7 & 8), and local study (9, 10), but differ with local studies (11). While conducting statistical analysis, we found there were moral differences at (P< 0.003) between male and female regarding the relationship of the disease with both sexes and their age categories for each.

Microbiology Results

Domination of the type (*P. acnes*) refers to oxygen pressures in comedone which helps to create suitable anaerobic environments of this type (12). Domination of the type (*S. epidermidis*) in pustules refers to high oxygen pressures and change of Hydrogen basis of the medium which lead to create suitable environment of above-mentioned type and non-suitability of other type (*P. acnes*).

Also the swept of white blood cells (Neutrophil) in infected area will lead to lessen of (*P. acnes*) which excreted enzyme of chemical attractive characterized by its low molecular of neural cells which lead to preferring the neural cells (*P. acnes*) more than other types (13).

Separation of negative bacteria of gram color from both (*P. aeruginosa*, *E. coli*) at total percentage of (17.07%) agreed with a study in this regard (14) whereas the existence of these organism very few in natural conditions, but possibility of its existence refers to world –wide using of antibiotic and long period of medication with lead to allowance of these organism to make colony and grow, and reproduction in pustules, and this case called (G-ve folliculitis) - inflammation of the hair follicle by negative microbiology with gram stain form which inflame the pustules and creates inflammatory papules more than comedone. Testing microbes sensitivity of plant extracts by using (Moller-Hinton Agar)

Test of Microbe sensitivity towards Antibiotic by using Moller-Hinton Agar

Test carried out to check the sensitivity of all bacterial isolates for (16) Antibiotic discs and the result illustrated in the table (4, 5, 6, 7, 8) in which the results showed that Doxycycline has fatal effect (100%) to all bacterial isolates under study.

The results also showed variation of Antibiotic sensitivity to different isolates under study. The results also showed Antibiotic Doxycyclinehas fatal effect (100%) to all

bacterial isolates under consideration because possess the ability of broad spectrum against different bacterial isolates of gram negative and gram positive isolates. Hence it has the ability to interfere protein synthesis by attack with bacterial ribosome prevent of connection with amino-acyl transfer RNA (t RNA) and have lipophilic activity aggregation in sebaceous gland and the kill micro-organism found in it (16).

4. Conclusion

We have noticed in the conclusions of the study that infections with the females were more than male regarding clinical study. Also we noticed that (*P. acnes*) bacteria was dominated in the samples of acnes (comedones), while (*S. epidermidis*) bacteria has dominated in (pustules samples)regarding bacteriology study. Antibiotic resistance of tetracycline to all isolates under study is due to excess of using these antibiotics systemic and typically foundation of these plasmids instability of these plasmids and transfer to another bacteria and become from (17) sensitive to resist to antibiotic and possess gram negative bacteria outer membrane that prevent antibiotic permeability inside the cell (18). Regarding the study of effect of Doxycycline noticed and is most effective antibiotics and affect all kinds of aerobic and anaerobic bacteria.

Table 4: Patterns Of Antibiotic Sensitivity For (306) ***S. epidermidis*** isolation

Percentage	Antibiotic code	Name of the antibiotic
99.01	**Do**	Doxycycline
87.58	CIP	Ciprofloxacin
84.64	N	Neomycin
81.69	Ra	Rifampicin
78.34	DA	Clindamycin
77.77	L	Lincomycin
73.85	CTX	Cefotaxime
73.2	C	Chloramphenicol
72.78	E	Erthromycin
67.64	TE	Tetracycline
53.26	Kf	Cefalexin
45.42	Fu	Fluxacillin
37.25	SXT	Methoprim
16.33	Ax	Ampiclox
13.72	Amp	Ampicillin
10.78	Amx	Amoxycillin

Table 5: Patterns Of Antibiotic Sensitivity For (180) ***P. acnes*** isolation.

Percentage	Antibiotic code	Name of the antibiotic
93.33	Do	Doxycycline
92.22	Ra	Rifampicin
91.11	DA	Clindamycin
88.88	N	Neomycin
84.44	C	Chloramphenicol
82.22	L	Lincomycin
81.11	E	Erthromycin
80	CIP	Ciprofloxacin
77.77	CTX	Cefotaxime
76.66	Kf	Cefalexin
73.33	TE	Tetracycline
51.11	SXT	Methoprim
50.55	Amp	Ampicillin
36.66	Amx	Amoxycillin

Table 6: Patterns of Antibiotic Sensitivity for (42) S. aureus isolation

Percentage	Antibiotic code	Name of the antibiotic
97.61	Do	Doxycycline
85.71	N	Neomycin
83.33	Ra	Rifampicin
80.95	DA	Clindamycin
76.19	L	Lincomycin
73.8	CIP	Ciprofloxacin
71.42	C	Chloramphenicol
69.04	CTX	Cefotaxime
66.66	TE	Tetracycline
61.9	E	Erthromycin
52.38	Kf	Cefalexin
40.47	Fu	Fluxacillin
33.33	SXT	Methoprim
21.42	Ax	Ampiclox
11.9	Amp	Ampicillin
9.52	Amx	Amoxycillin

Table 7: Patterns Of Antibiotic Sensitivity for (23) E. coli isolation

Percentage	Antibiotic code	Name of the antibiotic
91.30	Do	Doxycycline
78.28	CIP	Ciprofloxacin
73.91	N	Neomycin
69.56	CTX	Cefotaxime
65.21	TE	Tetracycline
30.34	Fu	Fluxacillin
8.69	Kf	Cefalexin
4.34	SXT	Methoprim

Table 8: Patterns of Antibiotic Sensitivity For (37) P. aeruginosaisolation.

Percentage	Antibiotic code	Name of the antibiotic	
89.81	Do	Doxycycline	.1
87.21	CIP	Ciprofloxacin	.2
81.08	N	Neomycin	.3
67.56	CTX	Cefotaxime	.4
62.12	TE	Tetracycline	.5
35.13	Fu	Fluxacillin	.6
16.21	Kf	Cefalexin	.7
13.51	SXT	Methoprim	8

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