# Effect of methylene blue on the growth of bacteria isolated from patients with Atopic Dermatitis

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## Abstract

The study aimed to determine the bacteriological profile and effect of antibiotic and methylene blue on bacteria isolated from atopic dermatitis patients . volume of samples were 100 patients from two hospitals in Baghdad province. After the swabs had been cultured on different media, conventional bio chemical tests to identify bacterial isolates and testing effect of antibiotic and methylene blue on growth of bacterial isolates.

Out of the one hundred patients with eczema in all study groups , results showed that *Staphylococcus aureus* isolated at a highest percentage 20 (57.14%), followed by *Staphylococcus haemolyticus* 7 (20%), and *Pseudomonas fluorescens* 5 (14.28%) and two isolates of *Enterobacter aerogenes* (5.7%) ,and one isolate of *Staphylococcus warneri*(2.8%).

All isolates lost ability to growth at concentration 10mg/ml of methylene blue, the MIC of the methylene blue against *S. haemolyticus* and *P.fluorescens* were (1) mg/ml, while against *S .warneri* and *S.aureus* were (10) mg/ml,

Isolates showed high resistance to cloxacillin and Augmenten ,but more sensitive to Nitrofurantin.

## INTRODUCTION

Atopic dermatitis ,or Eczema,is acommon skin disease that is associated with other disorders,such as allergic rhinitis and asthema1.Thae hallmarks of atopic dermatitis are achronic ,relapsing form of skin inflammation ,adisturbance of epidermal –barrier function that culminates in dry skin and IgE mediated sensitization to food and enviromental allergene[1].

The dominant mechanism of atopic dermatitis in lesional skin are governed by Th2 cell-related cytokines such as IL-4 and IL-13, and chemokines such as TARC and exotoxin .Th1 cells producing IFN-γ and IL-12 are reportedly dominant in the chronic stage4. In an eczematous

lesion of atopic dermatitis, antimicrobial peptides (defensins, cathelicidins, etc.) are inhibited from being expressed by keratinocytes[2].

A wide variety of etiological and exacerbating factors has been proposed, with the importance level of each varying among individual patients. In addition, inflammation associated with this disease will be elucidated by both allergic and non-allergic mechanisms. Etiological and exacerbating factors vary among age groups. While the dominant factors in the first half of childhood include foods, sweating, physical irritation(including scratching, environmental factors, microbes_fungi,).the dominant factors in the second halfof childhood to adulthood include environmental factors ,sweating, physical irritation (including scratching), microbes -fungi, contact allergens, stress, and food[3].

Allergens such as mites and house dust, pollen allergensin specific seasons, and organic solvents such as formaldehyde and toluene can become problematic[4].Being sensitized to mites in infancy is reportedly a marker for the development of asthma . Periocular pathological changes are often observed during airborne pollen seasons[5].

## Subject and Methods

A total of 100 patients of eczema (57 contact dermatitis ,35 atopic dermatitis ,5 disco dermatitis and 3 neuro dermatitis) in two hospitals (AL-Sadeer hospital and AL-Imam Ali hospital) in Baghdad .The period of study was during November2013 to March 2014.

All swabs were taken from patients at direct contact ,with used disposable gloves and a protective gown ,and by transport swabs ,after that cultural in the laboratory on 5% blood human agar and MacConkey agar plates and incubated over night at 37c aerobically.

Bacterial pathogens were identified by Biochemical methods according to the standared microbiological technique[6].Antimicrobial susceptibility was performed on Muller Hinton agar by standered disk diffusion method according to standered institute (CLSI) [7].

To determined effective concentration of methylene blue incubation isolates in plane tube contained different concentration of this stain over night in 37c after that transfer to nutrient agar plates after incubation over night in 37c observed the bacterial growth .

## Results and discussion

Bacterial isolates were found in 35 samples of patients .tested with 10 antibiotics (Amickacin ,Augmentin ,Azethromycin,Ceftriaxone ,Cloxacillin ,Gentamycin, Imipenem, Neomycin, Nitrofurantoinand Trimethoprime).

The results showed in table(1) that all Isolates Resistance to Augmintin and Cloxacillin and all were Sensitive to Nitrofurantoin

*Pseudomonas fluorescens* resistance to eight of antibiotics and sensitive to two (Neomycin ,Nitrofurantoin).

*Staphylococcus aureus* resistance to sex antibiotics and sensitive to three (Amikacin, Nitrofurantoin, Imipenem) and intermediat to Neomycin.

*Staphylococcus warneri* sensitive to eight and resistance to two (Augmentin ,Cloxacillin). *Staphylococcus haemolyticus* resistance to seven and sensitive to three (Amikacin, Nitrofurantoin, Imipenem).that determined according to CLSI(2010).Development of antibiotics resistance are therapeutic problems that explaining according to some hypothesis .resistance to Augmenten may be due to secreting of β-lactamase enzymes from bacteria that inhibited the work of antibiotic by breaking betalactam ring of antibiotic [8].The resistance to Gentamycin by converting antibiotic by (adenylating ,Phosphorylating and Acetylating ) enzymes or by chromosomal mutation in gene which coding of protein in ribosomal sub unit 30s which prevented anti biotic to binding with target protein and reduce cell pearmability to antibiotic [9]. Antibiotics are also commonly used in food animals to prevent, control, and treat disease, and to promote the growth of food-producing animals . The use of antibiotics for promoting growth is not necessary, and the practice should be phased out [12].The other major factor in the growth of antibiotic resistance is spread of the resistant strains of bacteria from person to person, or from the non-human sources in the environment[12].

*Pseudomonase* spp. become dominant and important by resistant to many anti microbial agents [12].this resistance may be explained to repeat exposure of gram negative bacteria to antibiotics [13].second reasone to this resistance who transfer plasmids from one cell to another in planktonic state and coded to resistance to different antibiotics[ 14].other reasone was quorum sensing and biofilm which protecting bacteria to contact with antibiotic attack (15,12,18,20). *Staphylococcus aureus* is carried as a nasal commensal in 30% of population and has been linked to common skin infaction and multi drug resistant pathogen [15] .in addition formation biofilm which protection bacteria aginst various antibiotic treatment [16].

Methylene Blue (MB) is one of non toxic dye [22] and has been used for avariety application [23].This dye has been approved for use by the united states food and drug administration (FAD) for treatment of methemoglobinemia in a non photodynamic mode [24].use of MB has been limited by its lack of activity when used in vivo [25].

The ability of MB to kill bacteria in suspention because MB is able to generate sufficient $O_2$ that can diffuse out of the matrix ,perforate adjacent cell membranes and kill cells [26].

Table( 1) the resistance of bacterial isolates to antibiotics

| Antibiotics | Concentration disk | *P. fluorescence* | *S. warneri* | *S. haemolyticus* | *S. aureus* |
|---|---|---|---|---|---|
| **Amikacin** | AK/30 | R | S | S | S |
| **Azethromycin** | AZM/15 | R | S | R | R |
| **Augmentin** | AMC/30 | R | R | R | R |
| **Neomycin** | N/30 | S | S | R | I |
| **Nitrofurantoin** | F/300 | S | S | S | S |
| **Gentamycin** | CN/10 | R | S | R | R |
| **Imipenem** | IMP/10 | R | S | S | S |
| **Cloxacillin** | CX/1 | R | R | R | R |
| **Trimethoprem** | TMP/5 | R | S | R | R |
| **Ceftriaxone** | CRO/30 | R | S | R | R |

Table (2) ability of bacteria to growth in different Concentration of methylene blue

| Isolates | 10mg/ml | 1mg/ml | 0.1mg/ml |
|---|---|---|---|
| ***P. flurecense*** | - | - | - |
| ***S. auruse*** | - | + | + |
| ***S .haemoliticuse*** | - | - | + |
| ***S .warneri*** | - | + | + |

+Growth

- No growth

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