EFFECT OF SALINITY ON ANTIBIOTIC RESISTANCE IN *STAPHYLOCOCCUS AUREUS* [+]

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Abstract:

Susceptibility of *Staphylococcus aureus* to gentamicin, nalidixic acid, ampicillin, amoxicillin and seven antiseptic agents: detol, ethanol (70 %), sodium hypochlorite, povidin iodine, iodine, hapitin, and septol, was evaluated using disc diffusion method on elevated NaCl containing media. Resistance to Ampicillin and Amoxicillin was changed to susceptibility when *Staphylococcus aureus* was cultured on this media. The diameter of the zone of inhibition has a direct relationship with the NaCl concentration in the media. Laboratory rats were infected experimentally by *Staphylococcus aureus*. Results prove that all those animals were unsuccessfully treated, since it is possible to isolate *Staphylococcus aureus* from their urine samples, by ampicillin or amoxicillin alone but they get cured when they received antibiotics in combination with NaCl injection.

المستخلص:

قيمت استجابة بكتريا *Staphylococcus aureus* للجنتامايسين و حامض النااليدكسيك و الامبيسيلين و الاموكسيسيلين و لسبعة عوامل مطهرة: هي الديتول و الايثانول (٧٠ %) و هايبوكلورايت الصوديوم و البوفيدين ايودين و اليود و الهابتين و السبتول، باستعمال طريقة الانتشار بالاقراص على وسط زرعي حاو على تراكيز متزايدة من كلوريد الصوديوم. المقاومة للامبيسيلين و الاموكسيسيلين تغيرت الى استجابة عندما زرعت على ذلك الوسط. وكان لقطر هالة التثبيط علاقة طردية مع تركيز كلوريد الصوديوم في الوسط. اصيبت جرذان مختبرية تجريبيا ببكتريا *Staphylococcus aureus*. اثبتت النتائج فشل علاج تلك الحيوانات بالامبيسيلين او بالاموكسيسيلين فقط (إذ تم عزل البكتريا من ادرارها) بينما شفيت عند علاجها بهذين المضادين مع الحقن بـ ٩% كلوريد الصوديوم.

Introduction

Staphylococcus aureus is a gram positive, catalase positive, coagulase positive, non motile coccus bacterium that causes a variety of human infection in all groups [1]. It is the major causative agent in surgical wound infections and epidermal skin diseases in newborn infants [2]. *Staphylococcus aureus* infection may also be superimposed on superficial dermatological diseases such as eczema, pediculosis and mycosis [3]. They live as commensals in anterior naves of over half population of humans [4]. The cocci are spread from these sites into the environment by the hands, handkerchief, clothing and dust. *Staphylococcus aureus* is opportunistic pathogen in the sense that it causes infection most

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commonly in tissues and sites with lowered host resistance such as individuals with diabetes, old malnourished persons and other chronic cases [5]

Attempts to control this bacterium by chemotherapy through the use of antimicrobial agents particularly antibiotics have resulted in increased prevalence of resistance to these agents [6,7]. Several investigations have been conducted to study the antimicrobial resistance pattern of *S. aureus* and it has been shown that the organism is resistant to β-lactam antibiotics, aminoglycosides and macrolides [8,9]. *S. aureus* strains carry a wide variety of multi-drug resistant genes or plasmids, which can be exchange and spread among different species of Staphylococci [10,11].

In order to overcome such resistance, this study aims to investigate the synergistic effect of salinity on antibiotics susceptibility of a bacterium that can tolerate relatively high concentration of NaCl (to exclude the effect of the Salinity). Therefore *S. aureus* was chosen since it has such tolerance property.

Materials and Methods

Antimicrobial agents and media

Four antibiotic discs(oxoid) were used and their concentrations were as follows: gentamicin (10 µg/disc), nalidixic acid (10 µg/disc), ampicillin (25 µg/disc), amoxicillin (10 µg/disc) and seven antiseptic agents: detol (5 % chloroxylenol provided by Alkeiam co. Iraq), septol (5 % chloroxylenol purchased from Pharmaline co. Lebanon), ethanol (70 %) (Merk), 12 % sodium hypochlorite (Alwazir co. Syria), 10 % povidin iodine (Pharmaline co. Lebanon), iodine (Spartam Ltd. Co. Jordan), 10 % hapitin (Alkeiam co. Iraq), and Mannitol salt agar, Nutrient agar and Brain heart infusion broth are the used media (all provided by Himedia, India).

Sample collection

Urine specimens were collected from 20 female UTI patients in Al-Yarmouk, Medicine city, and Al-Karama hospitals, their age ranged from 22 – 48 years. All of them were having previous UTI at least one time in their life. Also they had administrated penicillins, cephalosporins, or Nitrofurantoin.

Isolation and characterization of bacteria

Loopful (0.01 ml) samples were inoculated in mannitol salt agar (difco) and streaked with sterilized wire loop so as to obtain discrete colonies. The plates were incubated at 37° C for 24 hours under aerobic conditions [12]. After 24 h of incubation the culture plates were examined recording the appearance, size, colour, and morphology of the colonies [13]. Gram stain reaction, catalase test and coagulase test were carried out [12]. Isolates that were gram positive cocci, catalase positive and coagulated human plasma were considered ***S. aureus*** in this study.

Salinity tolerance of Staphylococcus aureus

Staphylococcus aureus isolates were tested for their tolerance to increased concentration of sodium chloride by inoculating each isolate on nutrient agar plates containing (1,3,5,7, 9, 11) % NaCl.

Susceptibility of isolates to various antimicrobial agents

Antimicrobial agent sensitivity test was carried out on all isolates using paper disc diffusion technique [14]. A total of 11 antimicrobial agents (gentamicin, nalidixic acid, ampicillin, amoxicillin, detol, septol, ethanol, sodium hypochlorite, 10 % povidin iodine, iodine, 10 % hapitin) were tested. A 0.1 ml of 12- h peptone water culture of the test organism was used to inoculate on six dry sterile nutrient agar plates containing (0, 1, 3, 5, 7, 9) % NaCl, respectively, in triplicates (11 % concentration was eliminated since it inhibited the growth of all *S. aureus* isolates). This was spread over the entire surface of the nutrient agar using a sterile glass spreader and allowed to dry for about 15 min. The antimicrobial agents' discs were placed on the agar using sterile forceps. Each disc was placed far from each other to avoid their zones of inhibition from coalescing into the other. The plates with the antimicrobial agents' discs were then incubated at 37° C for 24 hours to observe the zones of growth inhibition produced by the antimicrobial agent [12,14].

Preparation of the bacterial inoculation

Three to five colonies of tested isolate were added to 50 ml of Brain heart infusion broth, incubated at 37° C for 24 hours then washed three times with phosphate buffer three times, finally the residual was suspended in 5 ml of the phosphate buffer. A serial dilution of that suspension was done alongside with viable count on nutrient agar plate [12].

In vivo study

Fifteen female rats *(Rattus norvegicus*) weighted 166 – 208 grams were distributed into five groups (A to E), three animals per group. Each animal was put in a single cage; however, all animals were fed the same food and water.

Animal groups (A to D) were injected with *Staphylococcus aureus* isolate, respectively, with 0.5 ml of final concentration reached 10^9 cfu / ml, while control group (group E) was injected with 0.5 ml normal saline.

Inoculation protocol

All animals were anaesthetized with sodium pentobarbital [25 mg / kg] [15]. Urethra and surrounding area were sterilized with 75 % ethanol then a polyethylene tube (0.6 mm in diameter) was introduced to urinary bladder via urethra; the inoculum was injected with aid of this catheter. Thereafter the catheter was withdrawn immediately, animals were returned to their cages with their lower end directed upward to avoid effusion of the inoculum outside. The animals left without water for 24 hours.

Three days later 1ml urine was aspirated by suprapubic aspiration, cultured on blood agar (prepared by adding human blood to the blood agar base provided by Himedia, India. melted and cooled to 45° C, in a proportion of 5 %, as the manufacturer instructed) to confirm the infection.

Treatment protocol

As soon as the infection was confirmed, treatment with amoxicillin and ampicillin was started 1g quarter in die (q.i.d.)(four times a day) orally for one week for group A and B respectively. While group C and D was injected with 9 % NaCl (via the polyethylene tube in the same manner applied to bacterial injection) as one milliliter q.i.d. in addition to amoxicillin and ampicillin, respectively, 1g q.i.d. for one week.

Thereafter 1ml urine was aspirated by suprapubic aspiration, cultured on blood agar.

Statistical analysis

t-test and F test were uesd

Results and Discussion

All staphylococcal isolates were resistant to all studied antimicrobial agents when cultured on normal nutrient agar. Also they developed growth up to 9 % NaCl.

It was so clear that all isolates developed susceptibility (P=0.05) to amoxicillin (Figure 1) and ampicillin (Figure 2). Since the bacteria were able to grow under such high concentration of sodium chloride (9 %) normally, we may expect the reason behind such change could be that penicillin the binding protein [16] or β- lactamase activity [17] may be affected by such sodium chloride concentration, or a putative efflux pump plays a major role in the drug resistance by salt in this bacterium [18].

There was no change in resistance to gentamicin (insignificant differences (P=0.05)) (Figure 3) and nalidixic acid (Figure 4).

For the antimicrobial agents and antibiotics other than β- lactam group; such results could be attributed to the similar mechanisms (efflux or change permeability) employed for the antimicrobials and salinity resistance [15].

Coronado and her colleagues [20] studied the influence of salinity on the susceptibility of 13 moderately halophilic collection strains belonging to the genera *Chromohalobacter*, *Deleya*, *Halomonas*, *Vibrio*, and *Volcaniella* to 10 common antimicrobials and they found that the effect of salinity is moderate and dependent on both the individual strain and the antimicrobial tested.

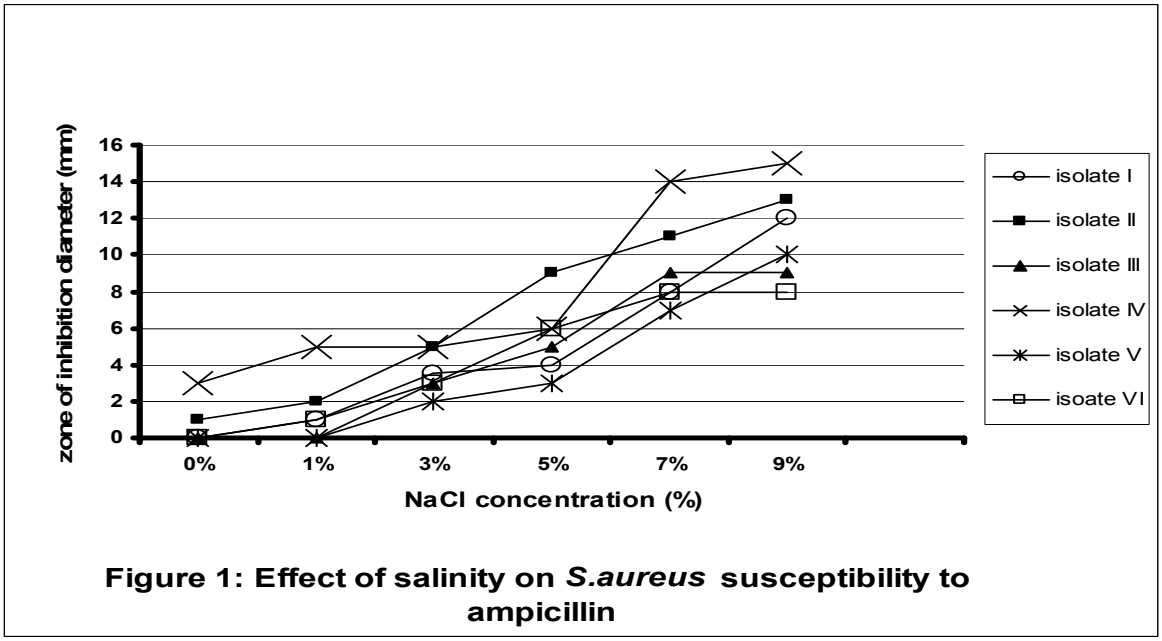

Figure 1: Effect of salinity on *S.aureus* susceptibility to ampicillin

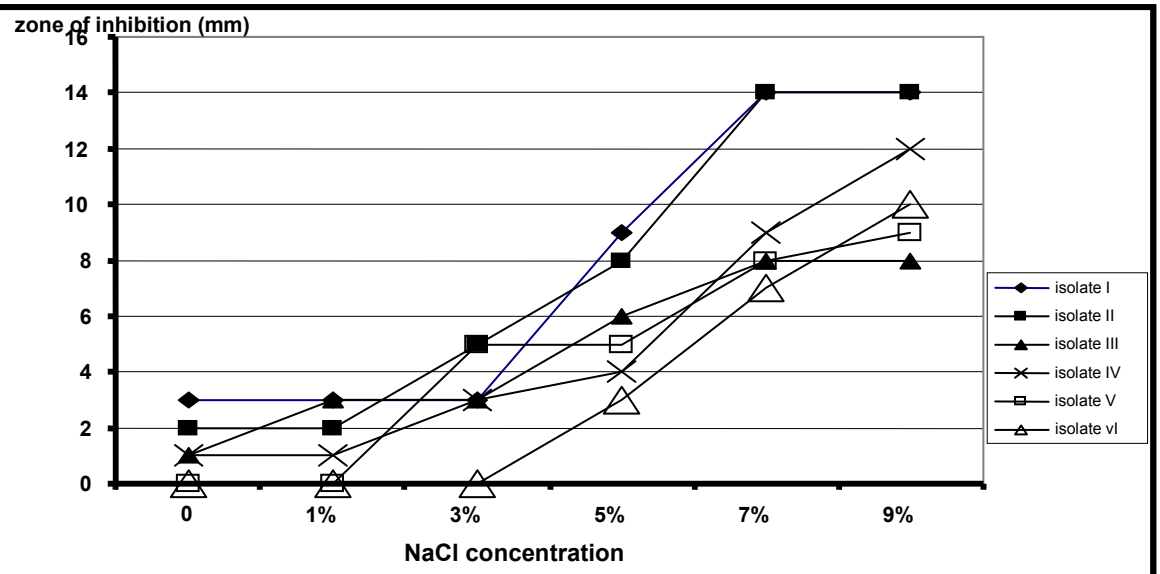

Figure 2: Effect of salinity on susceptibility of *S. aureus* to amoxicillin

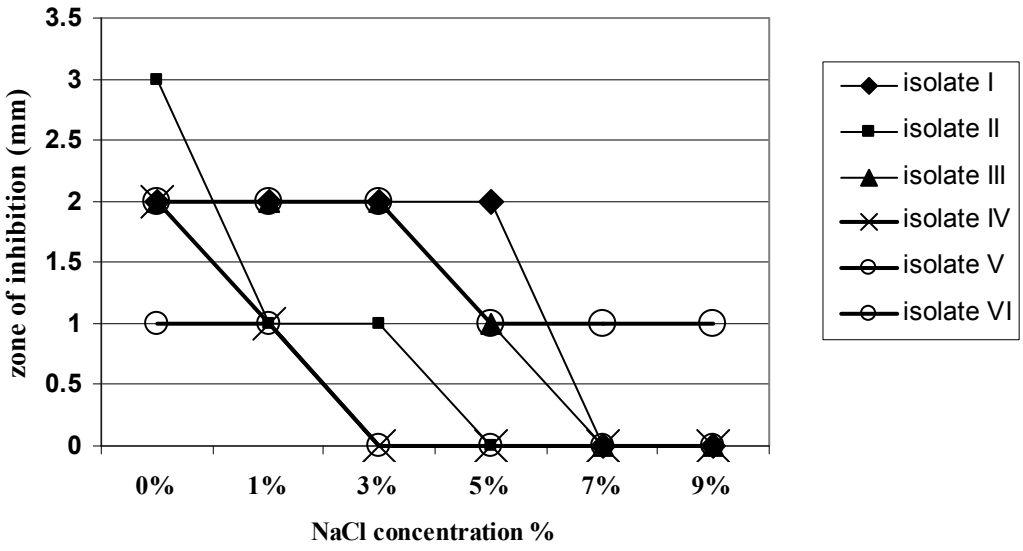

figure 3: effect of salinity on *S.aureus* susceptibility to gentamicin

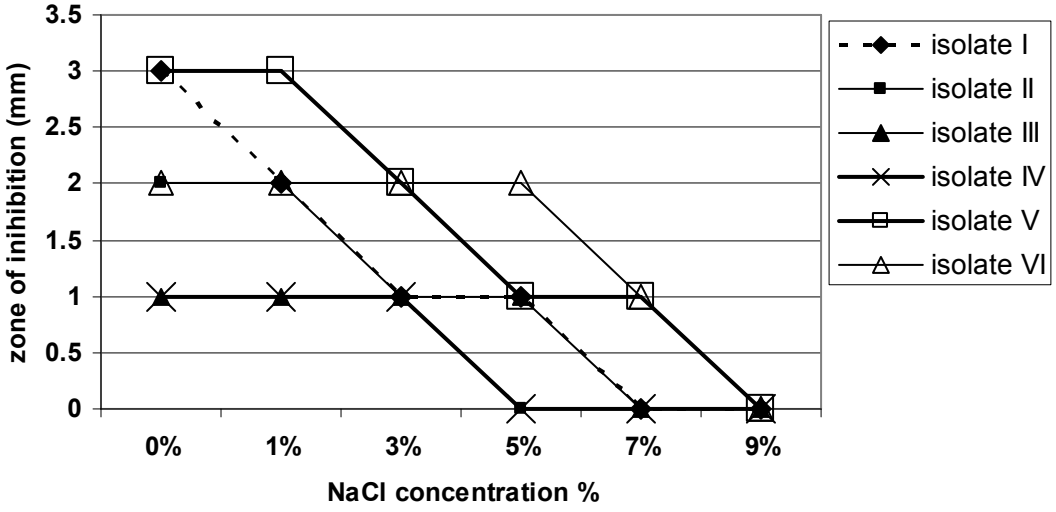

figure 4: effect of salinity on S. aureus susceptibility to nalidixic acid

Fuursted [21] points that post-antibiotic effect increased significantly in response to increased salinity. Garrod and colleagues [22] also, previously, found that monovalent cations enhance the activity of antibiotics against Staphylococci.

However, Raza *et al* [23] mentions that antibiotic resistance patterns did not correlate to NaCl, pH or $CaCO_3$ tolerance. Jorgensen *et al* [24] also found such results, when they added 2% NaCl to cation-supplemented Mueller-Hinton broth (CSMHB) was evaluated for microdilution testing of the susceptibility of staphylococci to amoxicillin clavulanate. With *Staphylococcus aureusr*, Resistance to amoxicillin clavulanate was readily determined, irrespective of the presence of added salt. Such results culd be attributed to the limited range of NaCl concentration which used in their experiments.

Even though *Staphylococcus aureus* did not show any change in sensitivity behaviour towards detol, ethanol (70 %), sodium hypochlorite, they developed susceptibility (with significant differences P=0.05) to Povidin iodine (Figure 5) and septol (Figure 6) when grown in NaCl containing media.

All animals were infected successfully by *Staphylococcus aureus*. Also results proved that all those animals were unsuccessfully treated, since it was possible to isolate *Staphylococcus aureus* from their urine samples, by ampicillin or amoxicillin alone but they get cured when they received antibiotics in combination with NaCl injection.

Due to potential interference by the NaCl to susceptibility of *Staphylococcus aureus* to amoxicillin and ampicillin we recommend doing more researches on the effect of NaCl on β-Lactamase activity and penicillin binding proteins in order to clarify such interference.

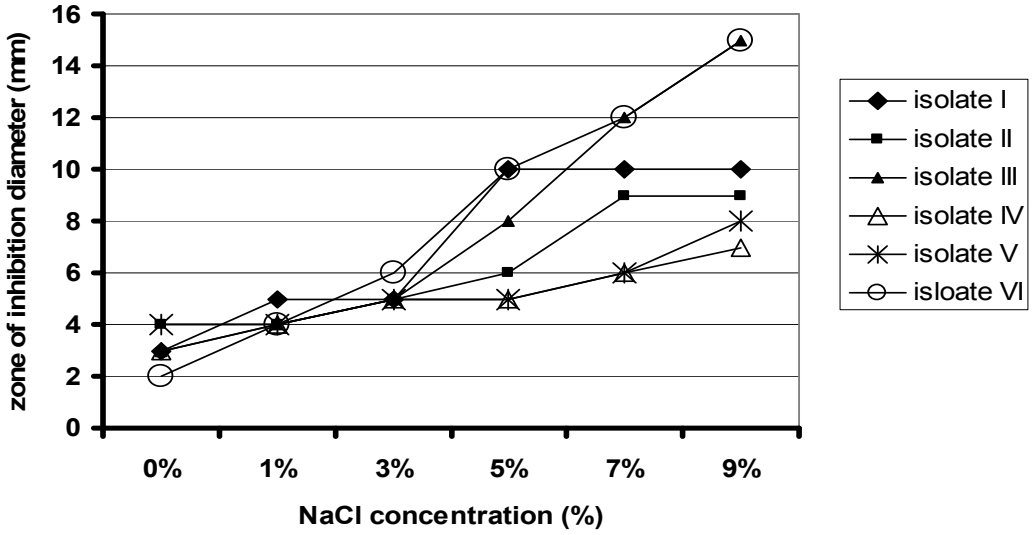

Figure 5: Effect of salinity on *S.aureus* suseptibility to povidine iodine

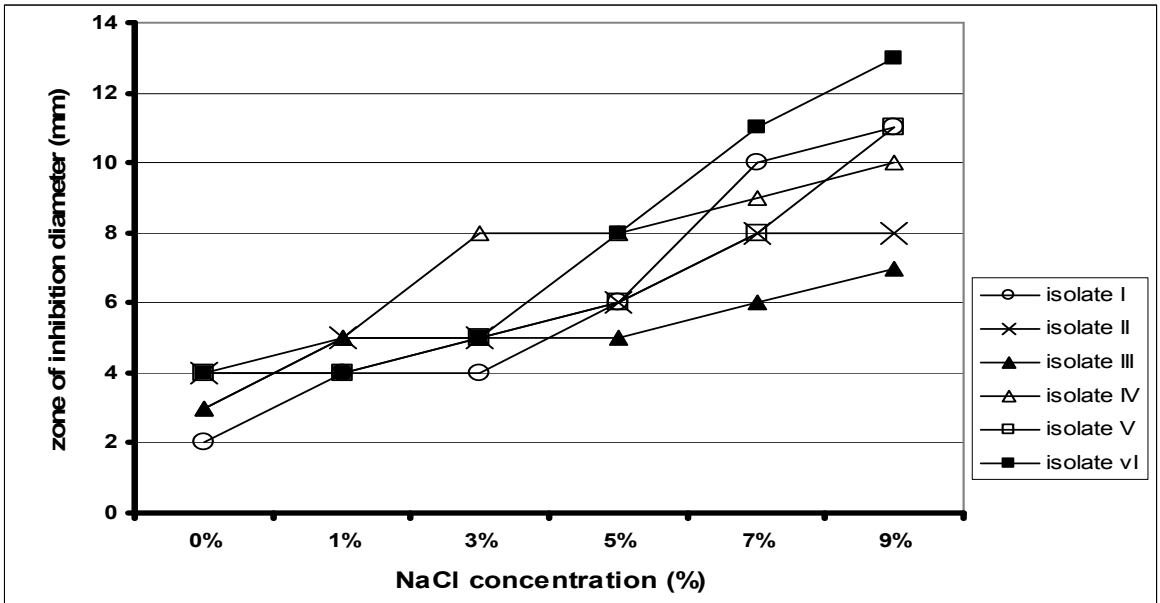

Figure 6: Effect of salinity on *S.aureus* susceptibility to Septol

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