



Effect of the Pyridoxine Supplements on the Minimum Inhibitory Concentration of the Co-Ampicillin-Cloxacillin against *Staphylococcus Aureus*: In Vitro Study

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Abstract

Background: *Pyridoxine or vitamin B6 is a water-soluble vitamin that is naturally present in many foods, added to others, and available as a dietary supplement. Microorganisms synthesized pyridoxine and played a role in their virulence.*

Objectives: *This study aims to evaluate the effect of synthetic (exogenous) pyridoxine against Co-ampiclox susceptible and resistant Staphylococcus aureus.*

Materials and Methods: *This study was done in the Department of Microbiology, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq from November 2014 to January 2015. A total number of thirty Staphylococcus aureus isolated from infected wounds were obtained from the Laboratories of Al-Yarmouk Teaching Hospital. The antibacterial effect of Co-ampiclox (25µg/ml), pyridoxine (at serial concentrations 1-64µg/ml) or a combination were examined in vitro using broth dilution technique.*

Results: *Four out of thirty isolates were resistant to 25 µg/ml ampiclox. Pyridoxine per se at low concentration (1µg/ml) inhibits the growth of the susceptible isolates by 18.7% while at higher concentrations failed to exert any antimicrobial effect and it reduced the antimicrobial effects of Co-ampiclox against the susceptible Staphylococcus aureus. Pyridoxine at low concentration (1µg/ml) inhibits the growth of the resistant isolates by 13%. while its combination with Co-ampiclox at concentration 4µg/ml inhibit the mean growth of bacteria by 18.3%.*

Conclusions: *Exogenous pyridoxine exerts antibacterial effect as demonstrated in this study while the endogenous pyridoxine that synthesized by the bacteria is necessary for their resistance and this indicated that pyridoxine exerts dual effect against the growth of the microorganisms.*

Keywords: *Pyridoxine, ampiclox, antimicrobial, Staphylococcus aureus.*

Introduction

Pyridoxine or vitamin B6 is a water-soluble vitamin that is naturally present in many foods, added to others, and available as a dietary supplement and play essential role in many aspects of macronutrient metabolism, neurotransmitter synthesis, histamine synthesis, hemoglobin synthesis and gene expression^[1]. The uses of this vitamin are associated with improvement of maternal health and fetal development during pregnancy, reduced the risk of cardiovascular diseases and intestinal malignancies^[2-4]. There is evidence that pyridoxine is utilized for synthesis of phosphonium salts which exerted antibacterial effect against *Staphylococcus aureus* and *Staphylococcus epidermidis* strains^[5]. On the other hand, Pyridoxal and pyridoxal-5-phosphate, the derivatives of pyridoxine played a role in the inhibition a number of enzymes in gram negative microorganisms which ultimately down-regulated the cellular proliferation^[6]. A new synthetic compounds, pyridoxine-bis-phosphonium salts, are novel compounds that exerted antibacterial activity against gram positive microorganisms including *Staphylococcus aureus* and *Staphylococcus epidermidis* with a minimum inhibitory concentrations (MICs) ranged between 1-1.25 µg/ml^[7]. Moreover, nutritional supplements that contained pyridoxine are useful in the management of inflammatory acne^[8]. In respect to these controversy effects of pyridoxine on the microorganism, it is worth trial to investigate the effect of pyridoxine on the minimum inhibitory concentration of antibiotics against susceptible and resistant strains.

Therefore, this study aims to evaluate the effect of synthetic pyridoxine against Ampiclox resistant and susceptible isolates of *Staphylococcus aureus* that isolated from patients.

Materials and Methods

This study was done in the Department of Microbiology, College of Medicine, Al-Mustansiriya University in Baghdad, Iraq from November 2014 to January 2015. The study approved by the Institutional Scientific Committee. A total number of thirty isolated from infected wounds were obtained from the Laboratories of Al-Yarmouk Teaching Hospital. The swabs that obtained from the patients were aerobically cultured on the different media including blood agar, mannitol salt agar and incubated at 37°C for 24 hours. The isolates were diagnosed according to well-known established microbiological methods that principally based on the morphological characteristics, Gram stain method and conventional biochemical testing (Forbes et al., 2007). Preparation of bacterial suspension achieved with a sterile wire loop. The top surface of 3-5 isolated colonies of the *Staphylococcus aureus* to be tested were picked from the original culture and introduced into a test tube containing 10 ml of sterile Muller Hinton broth and the turbidity was compared and adjusted with the turbidity standard using McFarland tubes as prescribed by Vandepitte et al (1991).

Experiment Design

Both ampiclox (vial, 500 mg) and pyridoxine ampoule (100mg/2ml) were purchased from the

local markets. They diluted in distilled water and different concentrations were prepared in the appropriated volume (25 μ l for each concentration) to be appropriate to the volume of microtitre plate. The cut-off level of ampiclox concentration that discriminate the susceptible and resistant isolate is 25 μ g/ml). To each well of plain microplate, a final volume of 200 μ l of broth suspended with bacterial growth was added in one series. In the other series, Distilled water (served as a control) or Co-ampiclox (at a 25 μ g/ml concentration) or pyridoxine (at a serial concentrations ranged from 1-64 μ g/ml) or a combination of Co-ampiclox and pyridoxine. The Minimum inhibitory concentrations of Co-ampiclox and pyridoxine were determined using the reader of the Enzyme Linked Immuno-Sorbent Assay by measuring the absorbance at 630 nm taking in consideration the absorbance of bacterial growth in absence or presence of distilled water or pyridoxine, ampiclox or the combination of pyridoxine and Co-ampiclox. The results are expressed as number, percent, mean and median.

Results

Four isolates out of thirty are resistant to the co-ampi-cloxacillin (25 μ g/ml). The percent of inhibition of the susceptible isolates ranged between 7.2 to 77.1% (Table 1). Pyridoxine *per se* at low concentration (1 μ g/ml) inhibits the growth of the susceptible isolates by 18.7% while higher concentrations the percent of inhibition ranged between 3.2 and 16%. Figure 1 shows that pyridoxine supplementation to the Co-ampiclox interfered with the antimicrobial effects of Co-ampiclox against the susceptible *Staphylococcus aureus*. Combination of Co-ampiclox and pyridoxine at concentration 2 μ g/ml inhibit the growth of susceptible bacteria by 20.6% while the median inhibition of Co-ampiclox alone is 50.9% (Table 1). Pyridoxine *per se* at low concentration (1 μ g/ml) inhibits the growth of the resistant isolates by 13.0% while higher concentrations failed to exert any antimicrobial effect (Figure 2). Combination of Co-ampiclox and pyridoxine at concentration 4 μ g/ml inhibit the mean growth of resistant bacteria by 18.3% (Figure 2). There is a variation in the percent of growth inhibition in respect to the isolate (Table 2).

Table 1. The percent inhibition of the bacterial growth achieved by 25 μ g/ml Co-ampiclox

Isolate No.	1	2	3	4	5	6	7	8	9	10	11	12	13
Inhibition (%)	16.5	42.0	41.1	41.8	51.0	50.6	34.9	19.2	52.2	7.2	71.8	70.0	77.1
Isolate No.	14	15	16	17	18	19	20	21	22	23	24	25	26
Inhibition (%)	59.4	66.7	48.8	44.0	34.5	10.7	75.5	40.9	44.7	74.7	44.5	44.6	43.0

Table 2 The percent of growth inhibition in the presence of pyridoxine supplementation to the Co-ampiclox

Isolate No.	Pyridoxine concentration(µg/ml)	Inhibition (%)
1	8	21.8
2	32	32.9
3	2	7.8
4	64	18.5

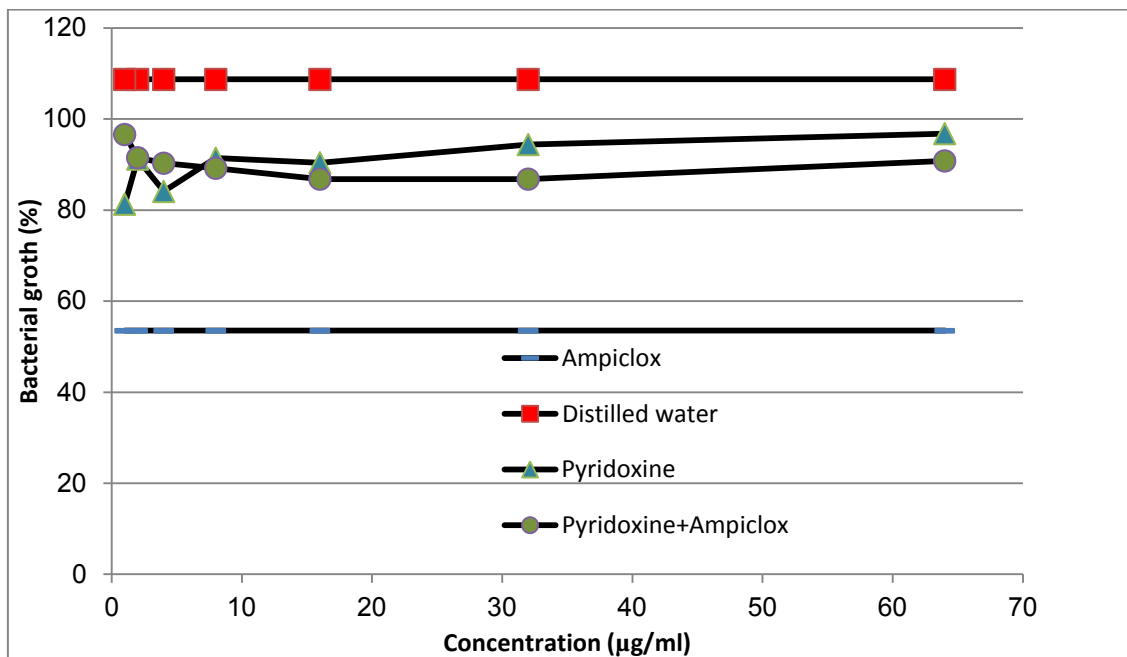


Figure 1. Effect of pyridoxine at different concentration on the ampiclox-susceptible *Staphylococcus aureus*

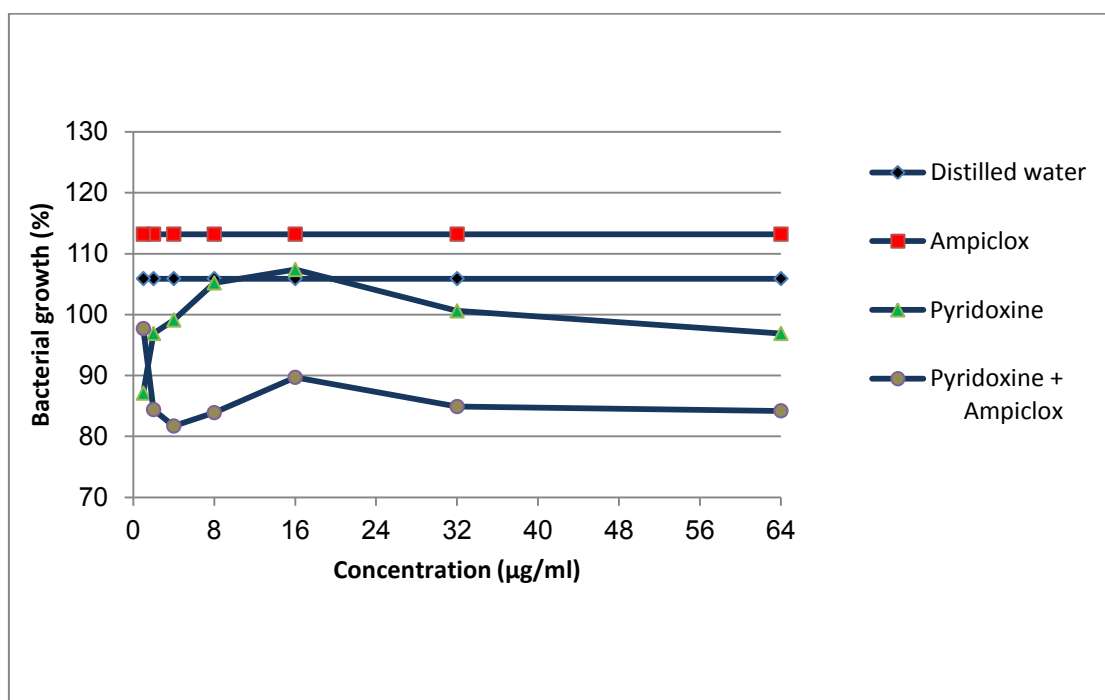


Figure 2. Effect of pyridoxine at different concentration on the ampiclox-resistant *Staphylococcus aureus*

Discussion

The results of this study showed that pyridoxine at a low concentration shows antibacterial effect against susceptible (18.7% inhibition) and resistant (13%) isolates. Its combination with ampiclox does not offer significant additive or synergistic effect. The antibacterial effect of exogenous pyridoxine that reported in this study is considered as a dual effect because it is well known that the microorganisms synthesized the vitamin B6 through different pathways and it is necessary for their growth ^[9]. There is evidence that The ampiclox resistance isolates are of plasmid-borne or chromosomal-borne and the plasmid bore resistant possessed difficulty in treatment for clinicians ^[10]. This explained that pyridoxine failed to produce synergism or additive effect when supplemented with ampiclox. It is expected to observe that pyridoxine interferes with the antibacterial effect of ampiclox because pyridoxine is used by some bacteria like *Helicobacter pylori* for the synthesis of glycosylated flagella and for flagellum-based motility, i.e. it is necessary for motility and virulence ^[11]. Moreover, Dick et al (2010) reported that pyridoxine biosynthesis is required for survival of *M. tuberculosis* in vivo ^[12]. The question that highlighted that is the exogenous pyridoxine is utilized by the microorganisms or the microorganisms synthesized endogenous pyridoxine for their virulence. Moussa et al (1982) reported that chemical complexes involving pyridoxine inhibited the growth of *E. coli* as well as the biosynthesis of RNA, DNA, and protein ^[13]. The inhibitory effect is related to the chemical

complexes and pyridoxine acts as a carrier or co-factor. It concludes that exogenous pyridoxine exerts antibacterial effect as demonstrated in this study while the endogenous pyridoxine that synthesized by the bacteria is necessary for their resistance and this indicated that pyridoxine exerts dual effect against the growth of the microorganisms.

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