The Kinetics of β-lactamase Positive and Negative Staphylococci Species to Ampicillin in The University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria.

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Abstract

β- Lactamase production by some bacteria is a cause of decreased clearance of bacteria in infected tissue by β–Lactam antibiotics like ampicillin and cephalosporin's. A total of two hundred and fifty clinical isolates of staphylococcus aureus (n=180) and coagulase negative staphylococci (n=70) from different urine samples were obtained from the microbiology laboratory of the University of Benin Teaching Hospital, Benin City had their kinetics to ampicillin tested using different concentrations of the drugs. This study comparatively assessed the effectiveness of ampicillin on β-lactamase negative and positive staphylococcus. We also determined the minimum therapeutic dose of ampicillin through the MIC against β-Lactamase positive and negative staphylococci. Finally, the study also determined the pattern of kinetics of ampicillin against the various staphylococcus species. This study revealed that the minimum inhibitory concentration (MIC) of ampicillin was 10µg/ml for β-Lactamase negative staphylococci. However, there was no complete inhibition of the β-Lactamase producing staphylococci when subjected to ampicillin. There was a reduction of 58% from the initial colony forming unit per ml (cfu/ml) of $4.8 \times 10^{\beta}$ to $2.05 \times 10^{\beta}$ in 10hours at a concentration of 25µg/ml of ampicillin for β-Lactamase producing strains. Conclusively, the β-Lactamase negative staphylococci were more sensitive to ampicillin than the β-lactamase positive staphylococci.

INTRODUCTION

The genus staphylococcus is a member of the family micrococcacea in the order eubacteriales [1] . Staphylococci are gram positive, coagulase, toxin producing coccospherical bacteriae measuring 0.8-1cm in diameter [2] . They are non-spore forming, non-capsulating, facultative anaerobes [3] . Staphylococcus is a common cause of disease in man and animals worldwide [4] . Such infections caused by Staphylococcus pose a serious threat to the lives of the affected individuals and communities [5] .Hence, the treatment of staphylococci infections can be challenging and the associated mortality ranges from 20-25% in spite of availability of highly active antimicrobial agents [6,7] . Treatment of staphylococci infections was formally based on ampicillin. However, clinical failures with delayed sterilization of the affected tissue or the environment have been reported with the penicillins [8] .

In the 1960s, the first case of resistance to the penicillins was identified among the β-Lactamase producing staphylococcus aureus [9] . The idea of prescribing β-Lactam antibiotics as an empirical treatment may need to be considered if staphylococcus strains that are resistant to β-Lactam antibiotics are becoming significant pathogens.

According to a report by the National Nosocomial Infection Surveillance System staphylococcus was identified in purulent materials by Robert Koch in 1878 and was cultivated two years later by Loius Pasteur [10] .They are ubiquitous and some are members of the natural floral of the skin and upper respiratory tract of man [11] . They are therefore commonly found or isolated in clinical specimens from skin, nose, throat, wounds, burns and bed soars where their presence may become very significant [12] . They are a major cause of deep seated infections such as osteomyelitis, bronchopneumonia and a wide range of abscesses [13] . They are also implicated in some cases of blepharitis, impetigo, skin graft rejection and cavitatory pneumonia [14] . Currently, the most widely encountered staphylococcus species are staphylococcus aureus, staphylococcus saprophyticus and

staphylococcus epidermidis. .Staphylococcus aureus is the most important medically, because it is mostly associated with human diseases. However, staphylococcus epidermidis and saprophyticus occasionally cause human infection [15] .

Ampicillin is penicillin which is effective against a wide range of gram positive and negative bacteria. Several of these bacteria produce an enzyme, β-Lactamase (Penincillinase) which can inactivate penicillin by opening the β-Lactam ring. However, some penicillins are more resistant to the enzyme than others and consequently, may be extremely valuable in the treatment of infections caused by β-Lactamase producing bacteria. Ampicillin is similar to penicillin –G which is also destroyed by β-Lactamase. However, it is acid stable and more active against gram negative bacteria.

MATERIALS AND METHODS

A total of two hundred and fifty (n=250) clinical isolates of staphylococcus species from different urine samples were obtained from the microbiology laboratory of the University of Benin Teaching Hospital. The isolates were identified using catalase test and the tube coagulase test in accordance with procedure described by Cowan and Steal [16] . Gram stain was done on suspected colonies as described by Stokes [17] . Gram stain slide was examined under the microscope using oil immersion of (100x objective). Those that appeared as blue black cocci in the cluster were suspected to be staphylococcus species. Generally, when gram stain is applied on a slide, gram positive bacteria appears blue black while gram negative are pink on microscopy.

Control experiments were set up using known catalase positive and negative staphylococcus aureus. β- lactamase production by staphylococcus species was detected using the iodometric method [18] . The MIC (Least concentration of antibacterial agent that inhibits the visible growth of bacterial after incubation for 24hours) [19] was carried out on the different strains of staphylococci using a range of dilution of ampicillin of 1mg/ml made in nutrient broth to form concentrations of 5, 10, 15, 20 and 25µg/ml. The nutrient agar plates were labelled with different concentrations of the ampicillin dilutions. The nutrient broth bottle containing ampicillin and organism were incubated for 24hours on nutrient agar plates. The plates were then withdrawn and colonies counted at intervals of 2,4,6,8 and 10hours respectively. The MIC was taken as the lowest drug concentration that inhibits the visible growth of the staphylococci.

RESULTS

Table 1 shows the prevalence of staphylococcus aureus and coagulase negative staphylococcus in different urine samples. Staphylococcus aureus (n=180) was 72% and coagulase negative staphylococci (n=70) was 28% respectively.

Figure 1

Table 1: The incidence of Staphylococcus aureus and coagulase negative staphylococci

Staph. Aureus	CNS	TOTAL
180(72%)	70(28%)	250(100%)

* CNS = Coagulase negative staphylococci

In table 2, the incidence of β-Lactamase producing strains of staphylococcus aureus and coagulase negative staphylococci were 176(98%) and 12(17%) respectively.

Figure 2

Table 2: Incidence of β-lactamase production amongst Staphylococcus aureus and coagulase negative staphylococci isolates.

Staph. aureus (n=180)	CNS(n=70)
176(98%)	12(17%)

* CNS = Coagulase Negative Staphylococci

n = number tested

The results of table 3 show the susceptibility of β- lactamase negative staphylococci isolates to ampicillin. At 0hour, the inoculums $4.8x10^{6}$ cfu/ml gradually reduced until there was no visible count 10hours after incubation. The MIC was 10ug/ml after 10hours of incubation.

Figure 3

Table 3: The Kinetics of ampicillin on β-lactamase negative staphylococci

TIME IN HOURS	Staphylococci count(cfu/ml) for Ampicillin at µg/ml concentration					
	25	20	15	10	5	0
0	4.8×10^6	4.8×10^6	4.8×10^6	4.8×10^6	4.8×10^6	4.8×10^6
2	1.0×10^6	2.1×10^6	2.5×10^6	4.0×10^6	N	N
4	0	1.2×10^6	1.3×10^6	3.1×10^6	N	N
6	0	0	0	1.2×10^6	4.0×10^6	N
8	0	0	0	0.6×10^6	3.0×10^6	N
10	0	0	0	0	2.2×10^6	N

* N = Numerous

µg/ml = Microgram per millilitre

Table 4 shows that there was no complete inhibition of β-lactamase positive staphylococci isolates 10hours after incubation. At 5ug/ml the viable bacteria count remained the same. However, at 10ug/ml and 10hours after incubation, 75% of the total counts were still viable. Furthermore at 25ug/ml the total count reduced to 42% 10hours after incubation.

Figure 4

Table 4: The kinetics of Ampicillin on β-lactamase positive staphylococci

Time Hours	Staphylococci count(cfu/ml) for Ampicillin at µg/ml concentration					
	25	20	15	10	5	0
0	4.8×10^6	4.8×10^6	4.8×10^6	4.8×10^6	4.8×10^6	4.8×10^6
2	4.2×10^6	4.50×10^6	4.6×10^6	4.7×10^6	N	N
4	4.10×10^6	4.30×10^6	4.55×10^6	4.65×10^6	N	N
6	3.60×10^6	3.95×10^6	4.0×10^6	4.10×10^6	N	N
8	3.0×10^6	3.65×10^6	3.9×10^6	3.85×10^6	N	N
10	2.05×10^6	3.35×10^6	3.45×10^6	3.65×10^6		

N = Numerous

µg/ml = Microgram per millilitre

DISCUSSION

The worldwide emergence of staphylococcus aureus resistance to antibiotics has changed its chemotherapy. It is obvious from this research analysis that the efficacy of the ampicillin for most staphylococci infection can no longer be assumed. There is a clear evidence of clinical failure amongst the β-lactamase positive staphylococci in which there was no MIC despite the increased drug concentration and duration of treatment. This research finding corroborates the work of many researchers like Anadiotis et al, who demonstrated that there was a delayed sterilization of affected tissue by ampicillin as a result of β-lactamase inactivation of the β-lactam ring of ampicillin [20].

This has translated to the increase mortality and morbidity seen with the use of ampicillin in suspected staphylococcus Infections. The use of ampicillin is on the rise because of its easy affordability over the counter and the poor social economic status of patients who cannot afford to pay for their medical care in the rural areas especially. We therefore recommend that ampicillin alone should not be prescribed for patients who are suspected to have infections caused by β-lactamase positive infections. However, we hope to carry out more research work on combination of cheaper drugs like Gentamycin and ampicillin, in order to improve the present situation of patients who cannot afford expensive drugs or appropriate medical care especially in our rural areas.

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