Biosciences, Biotechnology Research Asia Vol. **5**(2), 629-633 (2008)

Antibacterial sensitivity kinetics of urinary Staphylococcal isolates in Benin city; A case for Synergy?

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(Received: August 20, 2008; Accepted: November 08, 2008)

ABSTRACT

Two hundred and fifty isolates of Staphylococci were obtained from urine samples of patients presenting with urinary symptoms at University of Benin teaching Hospital microbiology laboratory. The isolates were identified and the 180 isolates of *Staphylococcus aureus* were classified on the basis of β-lactamase production and subjected to dilution antibacterial sensitivity tests and minimum inhibitory concentration (MIC) determination using ampicillin and gentamicin. As expected ampicillin was active against β-lactamase negative organisms but was resisted by the positive organism however there was significant reduction in bacterial count at high doses. Gentamicin showed activity with MIC at 5 µg/ml for the negative organism and 10µg for positive organisms. The above finding support the growing recognition of *Staphylococcus aureus* as a urinary pathogen and suggest the possibility of cheap effective chemotherapy by synergistic combination of ampicillin and gentamicin in management of Urinary infection, especially where *Staphylococcus aureus* is expected.

Key words: β-lactamase, minimum inhibitory concentration (MIC), Synergism, Malaria, Quinine, *Plasmodium flaciparum*, Drug resistance.

INTRODUCTION

The genus staphylococcus was identified in a purulent material by Robert Koch in 1878 and later cultivated by Louis Pasteur(NNISS,2002).They form the natural floral of human skin and mucous membrane(Iyer, 2002). Staphylococcus is a member of the family Micrococcacea in the order Eubacteriales(Mandell *et al,* 1990 and 1995). They are gram positive, Coccospherical in shape. The most widely encountered *staphylococci* are *staphylococcus aureus*, epidermidis and saprophyticus. *Staphylococcus aureus* is the most important medically because of its association with human diseases. *Staphylococcus aureus* is one of the most versatile and dangerous human pathogen and have been implicated as a cause of urinary infections. (Lowy, 1998). Resistance to penicillin, which was the mainstay of anti-staphylococcal chemotherapy, was identified among the β-lactamase producing staphylococcus aureus in the 1960s(Naimi et al 2003)hence, the treatment of staphylococcus can be challenging and the associated mortality ranges from 20-30% despite the availability of highly active antimicrobial agents (Mourin *et al,* 2001)

Based on this understanding,the idea of prescribing the penicillins alone may be reconsidered, since staphylococcus strains resistant

to the β-lactam antibiotics have led to the use of several potent antibiotics like vancomycin which are largely unavailable in Nigeria and when obtainable prohibitively expensive or the quinolone which are availablebut expensive so difficult to obtain in rural hospitals. Some authors have reported good activity against staphylococci by the aminoglycoside, gentamicin.

This study sought to determine the MIC and pattern of kinetics of ampicillin and gentamicin on both β-lactamase positive negative urinary isolates of staphylococci.

MATERIAL AND METHODS

A total of two hundred and fifty clinical isolates of staphylococcus species from urine samples of patients with urinary symptoms were obtained from the medical microbiology laboratory of the university of Benin Teaching Hospital .

The isolates were identified using colony characteristics, gram stain, catalase test and the tube coagulase test in accordance with procedure described by Cowan and Steal (1985). (Stokes, 1980). Control experiments were set up using stock culture of *Staphylococcus aureus* and *Staphylococcus epidermidis* (coagulase negative *Staphylococcus*). β-lactamase production by Staphylococci was detected using the iodometric method(Lennette *et al,*1980) and the β-positive isolates were separated from the negative isolates.

The MIC was carried out on the different species and 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 for β-lactamase positive and negative staphylococci isolates. The same dilution was obtained for gentamycin 1mg/ml but at concentrations of 2.5,5,10,15 and 20µg/ml. The MIC defined as the least concentration of antibacterial agent that inhibits the visible growth of bacterial after

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

Table 2: Incidence of â-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

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

Time in	**Staphylococci count(cfu/ml) for Ampicillin at µg/ml concentration**					
hours	**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** = Number µg/ml = Microgram per milliliter

incubation for 24hours was assesed (Hugo *et al,* 1990). The nutrient agar plates were labelled with different concentrations of the ampicillin and vancomycin. The nutrient broth bottles containing ampicillin, gentamicin and organisms were incubated for 24hours on nutrient agar plates. Within this time the plates were 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.*

Table4: **The kinetics of Ampicillin on β-lactamase producing staphylococci**

Time	Staphylococci count(cfu/ml) for Ampicillin at µg/ml concentration					
hours	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	N

* N= Numerous µg/ml = Microgram per milliliter

Table 5: The kinetics of Gentamycin on β-lactamase negative staphylococci

Time in	Staphylococci count(cfu/ml) for Ampicillin at µg/ml concentration					
hours	20	15	10	5	2.5	5
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	1.8×10^6	4.0×10^6	4.2×10^6	4.6×10^6	4.6×10^6	N
4	0.41×10^6	2.2×10^6	3.4×1^{06}	3.9×10^6	4.6×10^6	N
6	0	0	1.95×10^6	3.1×10^6	3.9×10^6	N
8	0	0	0	1.2×10^6	3.9×10^6	N
10	0	0	0	0	2.7×10^6	N

*** N** = Numerous µg/ml = Microgram per milliliter

Table 6: The kinetics of Gentamycin on β-lactamase positive staphylococci

Time	Staphylococci count(cfu/ml) for Ampicillin at µg/ml concentration					
hours	20	15	10	5	2.5	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	2.5×10^6	4.0×10^6	4.20×10^6	4.60×10^6	4.70×10^6	N
4	1.65×10^6	3.20×10^6	3.90×10^6	3.95×10^6	4.65×10^6	N
6	0	1.75×10^6	2.10×10^6	3.20×10^6	3.75×10^6	N
8	0	0	0	1.85×10^6	3.25×10^6	N
10	0	0	0	0	2.95×10^6	N

* N = Numerous µg/ml = Microgram per millilitre

RESULTS AND DISCUSSION

The emergence of resistance to antibiotics of, Staphylococci have led to the emergence of new drugs and new approaches to chemotherapy. The increasing recognition of *Staphylococcus aureus* as a urinary pathogen especially in this part of the world makes the consideration of appriopriate chemotherapy important for the management of urinary tract infections. staphylococci isolates. As expected, the β-lactamase Staphylococci resisted ampicillin in this study, however at high doses (equivalent of ≥ 2 grammes per dose), there was significant reduction in bacterial count. The increasing resistance of β-lactamase producing staphylococci isolates to penicillins has rejuvenated the need to investigate alternatives which are cheap and effective. Cephalosporins, quinolones and vancomycin are excellent alternatives but rather expensive for example

1 ampoule of 500mg ampicillin cost.....40 naira
1 ampoule of 1000mg cefotaxime cost.....800 naira
1 ampoule of 1000mg ceftriazone cost.....1800 naira
1 ampoule of 200mg ciprofloxacin cost.....500 naira
1 ampoule of 280mg gentamicin cost.....50 naira
Vancomycin is hardly available in Nigeria.

Scott *et al* demonstrated that β-lactamase producing organisms were susceptible to gentamycin.Nobert et al also reported the antibacterial action of gentamycin against certain gram positive and gram negative bacteria except bacteroids. The result of this present study shows that gentamycin had an excellent antibacterial activity against staphylococci isolates irrespective of β-lactamase production. This may occur because gentamycin is not a β-lactam antibiotics and its mode of action is by inhibition of protein synthesis of bacteria. Contrarily, Bint *et al* 1977 reported an outbreak of gentamycin resistant staphylococcus aureus. Recently,other researchers have reported good results with the use of gentamycin. The result of this study collaborates that of Linhua *et al* 2004 who revealed that staphylococcus aureus and epidermidis were susceptible to gentamycin and other antibiotics like vancomycin, ciprofloxacin, amikacin etc. This present study also agrees with the work of Lelievre et al who reported that the emergence and spread in French hospital of methicillin resistant staphylococcus aureus had an increasing susceptibility to gentamycin and other antibiotics. The MIC of gentamycin were 5µg/ml and 10µg/ml in the â-lactamase negative and positive staphylococci respectively. The lower MIC of gentamycin on the â-lactamase negative staphylococci shows that they were easily inhibited and more susceptible to gentamycin. The outcome of this study is in agreement with the work of Turano et al (1994) who emphasised that gentamycin was more active against coagulase negative than coagulase positive staphylococci isolates. Furthermore, Lemaitre *et al* (1998),reported an unusual heterogeneous staphylococci resistance to methicillin and tobramycin in the USA which were susceptible to gentamycin.In addition, the findings of Kalsoom et al (2006) shows that gentamycin was the second most effective drug against staphylococci isolates after vancomycin.she also noticed that the resistant pattern observed in gentamycin was less significant.In conclusion, gentamycin can be prescribed as a substitute for ampicillin in staphylococci infections that are resistant to the penicillins.We propose a case the synergistic combination of ampicillin and gentamycin in the management of methicillin or ampicillin resistant staphylococci isolates.

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