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Clinical Isolates of *Staphylococcus aureus* Show Variation in β-Lactamase Production and Are More Susceptible to Antibiotics Conjugated with β-Lactamase Inhibitors

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Abstract

β-Lactam antibiotics are a cornerstone in the treatment of bacterial infections on account of its high therapeutic index and selective toxicity—they act by inhibiting the biosynthesis of peptidoglycan, a key component in bacterial cell wall. Ninety (90) clinical specimens obtained from the microbiology unit Specialist Hospital Bauchi were screened for *S. aureus*, positive isolates were examined for β-Lactamase expression by using two Penicillin G concentrations (5000 IU/ml and 25,000 IU/ml) in acidometric agar technique with phenol red as indicator, and the susceptibility pattern of the isolates to β-Lactam antibiotics was also determined. *S. aureus* prevalence of 31% (28/90) was obtained, of which 96% (27/28) of strains were β-Lactamase positive in the standard test, while 63% (17/27) were able to hydrolyze penicillin G concentration of 25,000 IU/ml (5X the concentration in the standard test), and a strain was found to be β-Lactamase negative. The resistance to five β-Lactams, ampicillin, cephalexin, amoxicillin, cloxacillin and flucloxaillin, were 100%, 96%, 89%, 74% and 56% respectively. When ampicillin and amoxicillin were conjugated to β-Lactamase inhibitors sulbactam and clavulanic acid respectively the resistance to ampicillin decreased to 21% and to amoxicillin to 15%. The antibiotic susceptibility profile revealed β-Lactamase elaboration to be the major mechanism of resistance to the β-Lactams. β-Lactam utilization as therapeutic option would thus require the search for sensitive irreversible β-Lactamase inhibitors for the β-Lactamase enzymes or agents to block the release of β-Lactamase by strains.

Keywords

β-Lactamase, Peptidoglycan, Transpeptidation, Haemolysis, Resistance, Antibiotics

1. Introduction

β-Lactam antibiotics are a group of antibiotics with a two-membered ring: a Nitrogen-containing four-membered ring (the *β*-Lacatm ring) and a Sulphur-containing five-membered ring (as in penicillins) and a six-membered ring (as in cephalosporins). They act by inhibiting the biosynthesis of peptidoglycan in bacterial cell wall through: the irreversible inhibition of transpeptidation reaction; release of an inhibitor of autolytic murein enzymes. Enzymatic destruction of peptidoglycan architecture with autolysins and finally lysis is achieved due to high internal osmotic pressure [1]. They are the most widely used antimicrobial with the most spectacular modifications which lead to an enhanced and a broader spectrum of activity [2].

Resistance mechanisms to *β*-Lactam antibiotics by bacteria involve the production of inactivating enzymes—*β*-Lactamases—which is capable of hydrolyzing the *β*-Lactam ring leading to loss of activity. Over 200 different *β*-Lactamases are known, whose production is either induced by *β*-Lactams or constitutively expressed [1]. The classification of *β*-Lactamases is complex: based upon the genetics, biochemical properties and substrate affinity for a *β*-Lactamase inhibitor-clavulanic acid [3]. Other factors which contribute to bacterial resistance to *β*-Lactam antibiotics are the affinity of the drug to the *β*-Lactamases in competition to the affinity to the penicillin-binding proteins, and the amount of *β*-Lactamase produced [2]. *β*-Lactamase overproduction is associated to borderline susceptibility due to a partial and slow hydrolysis of methicillin and other penicillinase resistant penicillins (PRPs) [4].

Overcoming resistance due to *β*-Lactamases is achieved by the conjugation of *β*-Lactam antibiotics with *β*-Lactamase inhibitors such as clavulanic acid, sulbactam and tazobactam, which have a high affinity for and irreversibly bind some *β*-Lactamases (such as penicillinases of *Staphylococcus aureus*), but are not hydrolyzed by the *β*-Lactamase [3]. These inhibitors protect simultaneously present hydrolysable penicillins such as ampicillin, amoxicillin and tircacilin from destruction. Certain penicillins such as cloxacillins also have a high affinity for *β*-Lactamases (Brooks *et al.*, 2004). We sought to isolate *S. aureus* from clinical specimens and to qualitatively determine the level of *β*-Lactamase production in these isolates. We also sought to examine whether commonly used *β*-Lactams could be effective against these isolates even in the presence of *β*-Lactamase production.

2. Materials and Methods

2.1. Isolation and Identification of *S. aureus*

Ninety (90) clinical specimens were obtained from the microbiology unit pathology department specialist hospital Bauchi Nigeria from. The specimens were inoculated on 5% (v/v) Human blood agar (nutrient agar supplemented with human citrated blood) and McConkeyagar (Oxoid, Basingstoke, UK) plates, incubated aerobically and anaerobically for 24 - 48 hours at 37˚C. Isolates were identified as *S. aureus* based on colonial morphology, Gram's stain reaction, haemolytic pattern coagulase reaction and fermentation of mannitol in mannitol salt agar (Oxoid, Basingstoke, UK) [5]. Identified

discrete colonies were labeled and preserved on nutrient agar slant for later used.

2.2. β-Lactamase Screening of *S. aureus* Isolates

Inoculum was obtained from nutrient agar (Oxoid, Basingstoke, UK) slant streaked to purity on 5% (v/v) human blood agar nutrient agar supplemented with 5% 9v/v) human blood) plates and incubated for 24 hours at 37˚C. A single discrete colony was touched from the blood agar plate above and streaked unto nutrient agar (pH 8.5 - 9.0, adjusted with IN NaOH) plates containing penicillin G (Sigma-Aldrich Germany) at a final concentration of 5000 IU/ml and 0.0008 (w/v) phenol red (Sigma-Aldrich German) and incubated at one hour at 35˚C [6]. The same colony was also touched and streaked unto the surface of a nutrient agar plate containing penicillin G at a final concentration of 25,000 IU/ml + 0.0008 (w/v) phenol red (Sigma-Aldrich, Germany) and incubated for one hour to overnight at 35˚C. β-Lactamase-positive colonies appeared yellow after incubation while β-Lactamase-negative colonies remain colourless. Experiments were repeated twice and results recorded.

2.3. Antibiotic Susceptibility Profile of β-Lactamase-Positive Isolates to β-Lactam Antibiotics

The modified disk diffusion method of Kirby-Bauer 1966 was adopted [7]. 2 - 3 discrete colonies from an overnight culture plate were emulsified in sterile phosphate buffer saline and compared to 0.5 McFarland turbidity standards. A sterile swab was dipped into the suspension, drained by pressing against the wall of the tube containing the inoculum Mueller-Hinton agar (Fluka, Germany) plate was streaked to obtain confluent growth the plate was rotated three times at 60˚ to ensure even spread of the inoculum. The β-Lactam antibiotics discs were placed on the inoculated agar. The plates were incubated aerobically at 37˚C for 24 hours. The β-Lactam antibiotics (Titan Biotec Ltd., Rajasthan, India) impregnated disk with potency were: ampicillin (10 µg), ampicillin + sulbactam (20 µg), amoxicillin (10 µg), amoxicillin + clavulanic acid (20 µg), cloxacillin (10 µg), flucloxacillin (5 µg) and cephalexin (5 µg). The zone of inhibition was measured to the nearest millimetres in two direction and the results averaged. A stock culture of *S. aureus* ATCC 25922 was used as control. The experiment was carried out twice and the results averaged and interpreted according to CLSI 2004 interpretative criteria.

3. Biostatistics

One-way ANOVA was used to assess the variability between and within groups-the antibiotics and strains ($p < 0.05$ was considered significant). The means of the zone of inhibition to the different antibiotics were compared using Duncan Multiple Range Test.

4. Results

The overall prevalence of *S. aureus* from the clinical specimens (as seen in Table 1) examined was 31% (28/90). Wound and high vaginal swabs yielded identical prevalence

for *S. aureus* for number of samples tested 53%. The skin and mucous membrane represent important reservoir for *S. aureus* and a major source of endogenous infections by this bacteria. The isolated and identified *S. aureus* were screened for β-Lactamase production, firstly to identify β-Lactamase positive strains and secondly to qualitatively examined hyperproduction of β-Lactamase by exposing the strains to five times (25,000 IU/ml) the concentration of penicillin G compared to the standard test (5000 IU/ml). Table 2 shows 96% (27/28) of the strains isolates to be β-Lactamase-positive and 4% (1/28) to be β-Lactamase-negative. The β-Lactamase-positive strains were further screened to examined for β-Lactamase hyperproducers, we observed 63% (17/ 27) of these isolates to fit to the description of β-Lactamase hyperproducers.

Table 1. Prevalence of *Staphylococcus aureus* among clinical specimens.

Clinical Specimen	Total No. Tested	Total No. *S. aureus* Positive (%)	Total No. *S. aureus* Negative (%)
Endocervical Swab	15	3 (20)	12 (80)
High vaginal Swab	15	8 (53)	7 (47)
Sputum	10	2 (20)	8 (80)
Seminal Fluid	5	1 (20)	4 (80)
Urethral Swab	10	2 (20)	8 (80)
Urine	20	7 (35)	13 (65)
Wound Swab	15	8 (53)	7 (40)
Total	90	28 (31)	62 (69)

Table 2. Screening for β-Lactamase positive and β-Lactamase hyperproducers among *S. aureus* isolates.

Clinical Source of Strains	Total No. Screened	β-Lactamase Reaction	
		Positive Reaction 5000 IU/ml (%)	Positive Reaction 25,000 IU/ml (%)
Endocervical Swab	3	3 (100)	2 (67)
High vaginal Swab	8	8 (100)	6 (75)
Sputum	2	2 (100)	1 (50)
Seminal fluid	1	1 (100)	0 (0)
Urethral swab	2	2 (100)	0 (0)
Urine	7	6 (86)	4 (57)
Wound swab	8	8 100)	6 (75)
Total	28	27 (96)	17 (63)

We do not know how diverse our strains were but we sought to know whether β-Lactam antimicrobials could still be active against our isolates or the presence of β-Lactamase inhibitors conjugated with β-Lactam could restore susceptibility and highlight the critical role of β-Lactamase as the major mechanism of resistance to β-Lactams in these isolates. The antibiotic susceptibility profile of the isolates to seven [7] β-Lactams were determined (the results were as shown in **Table 3**). Resistance to ampicillin, cephalexin, amoxicillin, cloxacillin and flucloxacillin was 100%, 93%, 89%, 71% and 57% respectively. While resistance to β-Lactams conjugated with β-Lactamase inhibitors ampicillin + sulbactam and amoxicillin + clavulanic acid was 21% and 14% respectively.

5. Discussion

The widespread use of penicillin is said to have accounted for the high frequency of penicillin resistance among the staphylococci by the late 1950s a situation which still exists. At the introduction of penicillin for clinical use only rare strains of *S. aureus* had the capacity to produce β-Lactamase [8]. *B*-Lactamases now have been described for many species of Gram positive and Gram negative bacteria [3]. Most of the *S. aureus* isolates produce an inducible β-Lactamase. The proportion of the total β-Lactamase liberated into a culture depends on the strain and on the conditions of growth [9]. Dyke 1979 reported that isolates endemic in hospitals usually produce large quantities of β-Lactamase and release 40% to 60% of it into the medium [10].

A significant reduction in resistance seen in β-Lactams conjugated with β-Lactamase inhibitors clearly suggest the role of β-Lactamases in the earlier mentioned resistances to the β-Lactams. Statistical analysis of the zone of inhibitions obtained showed the isolates do not differ significantly ($p > 0.05$) while the activity of the antimicrobials against the isolates differ significantly ($p < 0.01$). When the means of the zone of inhibitions against the antimicrobial were ranked no significant difference was observed between ampicillin + sulbactam and amoxicillin + clavulanic acid but the above antimicrobials differ significantly to flucloxacillin, cloaxicillin, ampicillin, amoxicillin and

Table 3. Antibiotic susceptibility profile of β-Lactamase-producing *S. aureus* strains to β-Lactam antimicrobials.

Antibiotics (Disk Potency)	Total No. of Strains Tested	Proportion Susceptible (%)	Proportion Resistant (%)
Ampicillin (10 μg)	27	0 (0)	27 (100)
Ampicillin + Sulbactam (20 μg)	27	21 (79)	6 (21)
Amoxicillin (10 μg)	27	3 (11)	24 (89)
Amoxicillin + Clavulanic acid (20 μg)	27	23 (85)	4 (15)
Cloxacillin (10 μg)	27	7 (26)	20 (74)
Flucloxacillin (5 μg)	27	12 (44)	15 (56)
Cephalexin (5 μg)	27	1 (7)	26 (96)

cephalexin. This agrees with the reports of McDougal and Thornsberry 1986 of little difference between clavulanic acid and sulbactam in their effectiveness in reducing the MIC of β-Lactam antimicrobial agents [9]. Investigations into the relative stabilities of cloxaillin and flucloxaicillin to staphylococcal β-Lactamase have yielded conflicting reports [11] [12].

6. Conclusion

This study establishes: the prevalence of β-Lactamase positive *S. aureus*; β-Lactamase inactivation as a major mechanism for the resistance to β-Lactams and that most strains of *S. aureus* could be induced to hyperproduced β-Lactamase and to elaborate the enzyme into the culture medium. Sourcing for newer β-Lactamase inhibitors or agents to block the release of the β-Lactamase by the bacterium is veritable tools to restore the usefulness of β-Lactams as therapeutic options.

References

[1] Kayser, F.H., Bienz, K.A., Eckert, J. and Zinkernagel, R.M. (2005) Medical Microbiology. Thieme, Stuttggart, 198, 202.

[2] Wieldemann, B. (1986) Gene Alterations Leading to Resistance to β-Lactam Antibitotics. In: Levy, S. and Novick, R.P., Eds., *Antibiotic Resistance Genes*: *Ecology*, *Transfer and Expression*, Banbury Report, 24th Edition, Cold Spring Laboratory, Colorado, 347.

[3] Massidda, O., Mingoia, M., Fadda, D., Whalen, M.B., Pia, M.M. and Varaldo, P.E. (2006) Analysis of the β-Lactamase Plasmid of Borderline Methicillin Susceptible *Staphylococcus aureus*: Focus on *bla* Complex and Cadmium Resistance Determinants *cadD* and *cadX*. *Plasmid*, **55**, 114-127. http://dx.doi.org/10.1016/j.plasmid.2005.08.001

[4] Brooks, G.F., Butel, J.S. and Morse, S.A. (2004) Medical Microbiology. 23rd Edition, McGraw Hill, Boston.

[5] Kloos, W.E., Schlafer, F.H. and Gotz, F. (1992) The Genes *Staphylococcus*. In: Balows, A., Truper, H.G., Dwarkin, M., Harder, W. and Scheilfer, K.H., Eds., *The Prokaryotes*, 2nd Edition, Vol. 2, Springer-Verlag, New York, 1369-1420.

[6] Ayello, G., Bupp, C., Elliot, J., Facklam, R., Knapp, J.S., Popovic, T., Wells, J. and Dowell, S.F., Eds. (2003) Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. CDC/WHO, 175

[7] Bauer, A.W., Knieger, W.F. and Simon, J.H. (1966) Antibiotic Susceptibility Testing by a Standard Single-Disk Method. *American Journal of Clinical Pathology*, **45**, 493-494.

[8] Witte, W. and Hummel, R. (1986) Antibiotic Resistance in *Staphylococcus aureus* Isolated from Man and Animals. In: Levy, S. and Novick, R.P., Eds., *Antibiotic Resistance Genes*: *Ecology*, *Transfer and Expression*, Banbury Report, 24th Edition, Cold Spring Laboratory, Colorado, 95-105.

[9] Dyke, K.G.H. (1979) β-Lactamase of *Staphylococcus aureus*. In: Hamilton-Miller, J.M.T. and Smith, J.T., Eds., *Beta-Lactamase*, Academic Press, Inc., New York, 291-310.

[10] McDougal, L.K. and Thornsberry, C. (1986) The Role of β-Lactamase in Staphylococcus Resistance to Penicillinase-Resistant Penicillins and Cephalosporins. *Journal of Clinical Microbiology*, **23**, 832-839.

[11] Basker, M.J., Edmondson, H.A. and Sutherland, H. (1980) Comparative Stabilities of Peni-

cillins and Cephalosporins to Staphylococcal β-Lactamase and Activities against *Staphylococcus aureus*. *Journal of Antimicrobial Chemotherapy*, **6**, 333-341. http://dx.doi.org/10.1093/jac/6.3.333

[12] Lacey, R.W. and Stokes, A. (1977) Susceptibility of the "Penicillinase-Resistant" Penicillins and Cephalosporins to Penicillinase of *Staphylococcus aureus*. *Journal of Clinical Pathology*, **30**, 35-39. http://dx.doi.org/10.1136/jcp.30.1.35