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Antibacterial susceptibility and Invitro effect of sodium chloride, magnesium ions, and serum on Staphylococcal-β-lactamase production in Ekpoma Nigeria

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ABSTRACT

Background: Resistance to agents of tropical infections remains a public health challenge especially in resource-poor countries despite the efforts by multiple disciplines to face the challenges.

Objectives: This study was carried out to determine the effects of sodium chloride, magnesium ion, and serum on beta-lactamase production; and the antibiotic susceptibility pattern of staphylococci strains isolated from clinical samples from Irrua Specialist Teaching Hospital, Edo State, Nigeria.

Methods: Thirty-five (35) clinical isolates were collected and screened for the presence of beta-lactamase production using standard microbiological procedures, the staphylococci isolates were subjected to antibiotic susceptibility tests using the disc diffusion method, and beta-lactamase production test was carried out using starch paper hydrolysis. The effects of various concentrations of sodium chloride (0.1 – 7.0%), magnesium tetra oxo sulphate (VI) (0.1 – 1.0%), and serum (1 – 20%) on beta-lactamase production by the staphylococci were assayed using the double indicator method.

Results: Of the 35 staphylococci, isolates identified, 23 were *S. aureus,* and the remaining 12 were coagulase-negative staphylococci. Out of all the 35 isolates, 7 isolates (20%) were beta-lactamase producers, with 4 being *S. aureus* and 3 being coagulase-negative staphylococci. The staphylococci isolates showed a high level of resistance (71.4 – 97.1%) to the antibiotics used.

Conclusion: Beta-lactamase production was enhanced by NaCl and serum at a high concentration, but when subjected to statistical analysis individually it was not significant $p>0.05$, when compared against each other it was significant $p<0.05$. Mg^{2+} was inhibitory at higher concentration of 1.0%, 5.0% and 7.0% with $p>0.05$. This study indicates that Mg^{2+} could be used as a beta-lactamase inhibitor in the manufacturing of beta-lactam antibiotics.

INTRODUCTION

Staphylococci are Gram-positive spherical cells, usually arranged in grape-like irregular clusters. They grow readily in many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia [1]. Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems [1]. Many staphylococci, and certain other bacteria species are resistant to penicillin G because they produce beta-lactamase (penicillinase). Beta-lactamases are enzymes produced by bacteria that inactivate beta-lactam drugs by hydrolyzing the beta-lactam ring of the beta-lactam molecules. Most beta-lactamases inactivate either penicillins or cephalosporins, but some can inactivate both classes of drugs [2].

Staphylococcal resistance to penicillin is mediated by *blaZ*, the gene that encodes beta-lactamase. This predominantly extracellular enzyme, synthesized when staphylococci are exposed to β-lactam antibiotics, hydrolyzes the β-lactam ring, rendering the β-lactam inactive. Genes that encode beta-lactamases can be located on the bacterial chromosome, plasmids, or transposable elements [2, 3]. Resistance to β-lactams mediated by beta-lactamases can be overcome with the use of beta-lactamase inhibitors. Beta-lactamase inhibitors are substances designed to inhibit or destroy the effectiveness of beta-lactamase enzymes. Inhibitors generally have little antimicrobial properties themselves and so are combined with a β-lactam antibiotic [4]. Staphylococcal infections have been prevalent in various communities and healthcare institutions in both developed and developing countries.

OBJECTIVE

Hence, the present study was carried out to determine the effect of sodium chloride, magnesium, and human serum on beta-lactamase production; and then to ascertain the antibiotic susceptibility pattern of clinical isolates of staphylococci from in-patients of the Irrua Specialist Teaching Hospital, Irrua, Nigeria.

MATERIALS AND METHODS

Analysis of samples: Aseptic and standard microbiological method [16] was adopted in media preparation and sample inoculation. MacConkey agar, Blood agar base, Nutrient agar, and Mueller Hinton sensitivity testing agar medium were prepared according to the manufacturer's (LAB M Limited UK) instructions. They were sterilized at 121^0C for 15 minutes holding time in an autoclave. Ten percent sheep blood agar was prepared according to the manufacturer's instruction. About 20 ml of each medium was dispensed on a sterile disposable plastic Petri dish and allowed to set. Samples were inoculated

onto MacConkey, Nutrient, and Blood agar plates respectively for bacterial isolation. They were all incubated at 37±3^{0}C for 18-24 hours. Direct Gram smear was made on a microscope slide and wet mounts prepared and examined microscopically. Significant mixed growth of bacteria colonies (more than 25 colonies per plate) was separated into single colonies by obtaining purity plates and all suspected colonies for other microorganisms and *Staph aureus* were identified according to the scheme of Cowan and Steel's Manual for the identification of medically important bacteria as revised by Barrow and Feltham [17]. All the identified organism was inoculated into nutrient agar slant and stored at -20^{0}C.

Collection of Samples

A total of 35 isolates of *Staphylococcus* species were collected from the Microbiology Department of Irrua Specialist Teaching Hospital, Irrua, Nigeria. These isolates were from clinical samples sent to the Microbiology Laboratory that was stored at -20^{0}C after identification using standard microbiology methods [5].

Characterization of Staph

To confirm the identity of all the isolates collected, they were inoculated separately onto Mannitol Salt agar and blood agar (Lab M Limited UK); then incubated at 37^{o}C for 18 - 24 hours. Gram staining and catalase test were performed on the isolates. All colonies that are Gram-positive cocci and catalase-positive were tested for coagulase production by both the slide and tube tests using human pooled plasma. Based on coagulase production, the *Staphylococcus* species were grouped as *S. aureus* (coagulase-positive) and coagulase-negative Staphylococci [5, 6].

Beta-lactamase (Penicillinase) Production Test

All the staphylococcal isolates were tested for their ability to produce beta-lactamase. Beta-lactamase test was carried out using the Starch Paper Method [7]. Strips of starch paper measuring 4 cm by 7 cm were cut, put in a glass petri dish, and sterilized using the hot air oven at 160^{0}C. The strips were soaked for 10 min in a solution of benzylpenicillin dissolved in phosphate buffer and spread evenly on sterile Petri dishes. About 18 – 24 hrs old cultures of the staphylococcal isolates grown on nutrient agar were inoculated on the surface of the test starch paper and spread over an area of 2 to 3 mm. The Petri dishes were incubated at 37^{0}C for 30 min, then Gram's iodine solution was used to flood the plate and drained off immediately. The starch paper turned uniformly black within 30 seconds of application. Colonies with decolorized zones around them were reported as positive for beta-lactamase but colonies with black background showed beta-lactamase negative.

Antibiotic Sensitivity Tests

Antibiotic susceptibility of staphylococcal isolates was determined by the Kirby-Bauer disk diffusion method as described by Clinical Laboratory Standards Institute [8]. The staphylococcal isolates were grown in nutrient broth at 37^{o}C overnight. The suspension was visually adjusted with normal saline to 0.5 McFarland turbidity standard. Each inoculum was separately swabbed across the entire surface of sterile Muller Hinton agar plates using a sterile swab stick. The commercial antibiotic disks which consisted of amoxicillin (25 mcg), tetracycline (30 mcg), cloxacillin (5 mcg), erythromycin (4 mcg), chloramphenicol (30 mcg), cotrimoxazole (25 mcg), gentamicin

(10 mcg), and Augmentin (30 mcg) were aseptically placed on the inoculated agar plates. After 30 minutes, the plates were inverted and incubated for 18 – 24 hours at 37°C. Then the zones of inhibition were measured in millimeters and the interpretation chart [8] was used to determine the susceptibility patterns of the staph isolates.

Effect of Sodium Chloride, Magnesium, and Serum on Beta-Lactamase Production

The effects of sodium chloride, magnesium, and serum on beta-lactamase production were carried out using a combination of penicillin-sensitive *Staphylococcus aureus* (NTCT 6571) and 5% starch solution in nutrient agar plate as indicators (double indicator method) to demonstrate the production of penicillinase by the isolates.

The sodium chloride and magnesium were diluted in nutrient broth to contain 0.1 to 7.0g concentration in test tubes. The serum was diluted to 1 to 20% concentration in broth, to control the experiment test tubes containing only nutrient broth was added, and all the test tubes were inoculated with 100ul of 0.5 Mac Farland standard turbidity of the test isolate from overnight broth culture, which contained 1000iu of Penicillin G and incubated at 37^0C in a water bath overnight. After overnight incubation, the tubes were centrifuged at 2500 rpm for 30 min and the supernatant was collected and stored frozen for further test.

The nutrient agar, to which 5% of starch and penicillin G (1000 iu) was added, was then inoculated by a penicillin-sensitive strain of *Staphylococcus aureus* (Oxford *Staphylococcus* –NCTC 6571). With the help of punch, about 5 – 6 punch holes (wells) were made on each plate. The thawed frozen filtrate from the nutrient broths was used to fill the wells. The plate was then incubated at 37°C for 18 – 24 hours.

The growth of the sensitive strain of *Staphylococcus aureus* was observed around the wells. This shows that the beta-lactamase in the well has been able to diffuse into the agar and inactivate the action of penicillin, thereby allowing the growth of the organism. After this observation, the plate was flooded with iodine solution and it was immediately drained off. This caused the surface of the agar containing starch to turn uniformly blue-black within 30 seconds of application. There was no blue-black coloration around the wells containing the filtrate. This indicates the action of beta-lactamase in the wells. The readings were taken by measuring the diameter across the wells of the decolorized zones, to determine the effects of different concentrations of sodium chloride, magnesium ions, and serum on β-lactamase production

RESULTS

Out of the 35 isolates of *Staphylococcus* species collected from Irrua Specialist Teaching Hospital, Irrua, 23 were Coagulase Positive (*Staphylococcus aureus*) and 12 were coagulase-negative Staphylococci. Out of the 35 isolates, 7 isolates (20%) were beta-lactamase producers, comprising of 4 *S. aureus* and 3 coagulase-negative staphylococci (Table 1).

The patterns of antibiotic resistance of the isolates recovered from different clinical sources were evaluated and are shown in Table 2. Of the 35 staphylococci isolates tested; 97.1% of the isolates were resistant to tetracycline, 94.3% to Cotrimoxazole and gentamicin, 91.4% to erythromycin, 88.6% to cloxacillin, 80% to chloramphenicol, 74.3% to amoxicillin, and the least resistance 71.4% to augmentin.

Two strains of the coagulase-positive staphylococci producing beta-lactamase and a strain of the coagulase-negative staphylococci producing beta-lactamase that were subjected to different concentrations of sodium chloride, magnesium tetra oxo sulphate (VI), and serum, revealed the effects of various concentrations of sodium chloride (0.1-7.0%) and magnesium tetra oxo sulphate (VI) (0.1-1.0%) on beta-lactamase production by the organisms as shown in Table 3 while effects of serum concentrations (1-20%) are shown in Table 4. The results revealed that beta-lactamase production was enhanced by both NaCl and serum at higher concentrations, the difference being statistically significant ($p< 0.05$) while the increase in the concentration of $MgSO_4$ 5.0% and 7.0% was inhibitory to the production of beta-lactamase.

Table 1. Production of beta-lactamase among staphylococci

Organisms	Total	No (%) of beta-lactamase positive strains	No (%) of beta-lactamase negative strains
Staphylococcus aureus	23	4 (17.4)	19 (82.6)
Coagulase-negative staphylococci	12	3 (25)	9 (75)
Total	35	7 (20)	28 (80)

Table 2. Antibiotic resistance profile of staphylococci

			No (%) resistant to							
Bacterial Isolate	No of Strains	No tested	AUG.	TET.	COT.	AMX.	GEN.	CHL.	CXC.	ERY.
S. aureus	23	23	17(73.9)	22(95.7)	21(91.3)	18 (78.3)	22(95.7)	18(78.3)	21 (91.3)	21(91.3)
Coagulase-negative Staphylococci	12	12	8 (66.7)	12 (100)	12 (100)	8 (66.7)	11(91.7)	10(83.3)	10(83.3)	11(91.7)
Total	35	35	25(71.4)	34(97.1)	33(94.3)	26(74.3)	33(94.3)	28 (80)	31 (88.6)	32(91.4)

KEY: AUG. Augmentin, COT. Cotrimoxazole, AMX. Amoxycillin, GEN. Gentamicin, CHL. Chloramphenicol CXC. Cloxacillin, ERY. Erythromycin

Table 3. Effects of sodium chloride and magnesium tetraoxosulphate VI on beta-lactamase production by staphylococci

Zone Diameter (mm) of salts on strains									
Salt in percentage	NaCl			$MgSO_4$			Control		
Strains	A	B	C	A	B	C	A	B	C
0.1	15	17	16	25	20	22	15	16	16
0.25	15	18	17	25	20	22			
0.5	16	18	18	16	20	15			
0.75	20	18	18	16	20	15			
1.0	20	20	20	16	16	15			
5.0	22	20	20	-	-	-			
7.0	22	20	21	-	-	-			

*A, B, and C designate different strains of beta-lactamase-producing staphylococci, A = *Staphylococcus aureus,* B = *Staphylococcus aureus,* C = Coagulase negative staphylococci

Table 4. Effect of serum on beta-lactamase production by staphylococci

Serum concentration (%)	Zone Diameter (mm) of inhibition produced by Staph. strains					
	serum on strains			Control		
Staph. strains	A	B	C	A	B	C

0	10	11	11	15	16	16
1	10	11	11			
5	10	11	11			
10	10	11	11			
15	15	13	15			
20	15	13	15			

*A, B, and C designate different strains of beta-lactamase-producing staphylococci
A = *Staphylococcus aureus,* B = *Staphylococcus aureus,* C = Coagulase negative staphylococci

DISCUSSION

For many years penicillin has been the most trusted and useful antibiotic in clinical practice. However, as a result of its widespread and prolonged usage, many pathogenic bacteria such as staphylococci have become resistant to it. In many cases, penicillin resistance results from the bacterium having acquired the ability to produce penicillinase (beta-lactamase)[9].

Of thirty-five strains of staphylococci that were investigated, 7(20%) were beta-lactamase producers. The prevalence rate of beta-lactamase production obtained in this study is very low compared to clinical cases where the high profile of beta-lactamase production was recorded in Nigeria which has been put between 70 and 80% [10, 11, 12]. This low rate of beta-lactamase production in this study may be attributed to the fact that *Staphylococcus* species isolates were obtained from the patient in a hospital and best practice may be one of the reasons that the transfer of plasmid within hospital strains was reduced despite the high rate of resistance to penicillin base antibiotics in this study. Antibiotic resistance, due to beta-lactamases, poses a clinical problem that may be approached by opting to use antibiotics of a different type, when possible, or using a combination of antibiotics. It has been suggested that the improvement and strengthening of existing institutional guideline about dispensing and use of antibiotics, the establishment of a surveillance group to monitor staphylococci resistance profile, and treatment of hospital personnel who are known carriers of multi-resistant staphylococci strains are among steps that could be taken to reduce the high incidence of antibiotic resistance [15]. The use of chloramphenicol in ocular, skin infection and veterinary medicine against isolates of *Staphylococcus aureus* inform its inclusion in this study since early report have documented the use of chloramphenicol in treating *Staphylococcus aureus* obtained from various animals [18], Chloramphenicol and erythromycin, used together, to delay the emergence of resistant organisms,

Was the antibiotics of choice in the treatment of pneumonia due to penicillin-resistant strains of staphylococci[19], this study recorded 80% resistance for the tested isolates.

On the effect of NaCl and serum on beta-lactamase production, the results showed that maximal beta-lactamase production was achieved at 5.0 and 7.0% NaCl showing that increasing concentrations of NaCl were accompanied by increased beta-lactamase productions, this may not be unconnected with the fact that *Staphylococcus species* grow well generally in the sodium chloride rich environment. At 1-10% serum, there was no effect on beta-lactamase production but at higher concentrations (15 and 20%), there was an increased beta-lactamase production amongst the three groups of isolates tested but was not statistically significant $p>0.05$. When we subjected the NaCl and serum to statistical analysis, there was a significant difference with $p<0.05$. From this study, it was found that Magnesium tetra oxo sulphate VI reduced beta-lactamase production as the concentration increases and completely inhibited beta-lactamase production at a concentration of 5.0g/ml and above. This shows that Mg^{2+} has an inhibitory effect on beta-lactamase production. Hence, magnesium ion could serve as a beta-lactamase inhibitor and could be combined with a β-lactam antibiotic.

CONCLUSION

This study shows the occurrence of beta-lactamase producers and high prevalence of antibiotic resistance among the staphylococci isolates investigated and underscores the need to stem this trend particularly among individuals who are vulnerable to staphylococcal infections. Also, the study has revealed the increasing induction of NaCl and serum as well as the inhibitory effect of $MgSO^4$ on beta-lactamase production.

REFERENCES

1. Brooks GF, Butel JS., and Morse SA. The staphylococci. In: Jawetz, Melnick and Adelberg's Medical Microbiology, 22nd edition. New York: McGrawHill Publica: p.197-202. 2002.
2. Coyle, M. B. *Manual of Antimicrobial susceptibility testing*. American Society for Microbiology. 2005.
3. Kernodle, DS. *Mechanisms of resistance to β-lactam antibiotics*. In: Gram-positive pathogens. VA. Fischetti, RP. Novick, JJ. Ferretti, DA. Portnoy, and JI. Rood, editors. American Society for Microbiology, Washington, D. C., U. S. A. 2000; pp. 609-620.
4. Leiker T. Beta-lactamase inhibitors. Accessed from http://www.fhsu.edu/nursing/otitis/bl_inhibit.html. 2000
5. Cheesbrough M. Biochemical tests to identify bacteria. In: *District Laboratory Practice in Tropical Countries*, Part 2: Low price edition. Cambridge University Press, United Kingdom, pp. 62-70, 2000.
6. Cruickshank R, Duguid, JP, Marmion BP., and Swain RH. *Medical Microbiology*, 12th edition. Churchill Livingstone, Pub. Co. Ltd., London. 1980; Pp. 356-366.
7. Odugbemi TO, Hafiz S. and McEntegart MG. Penicillinase-producing *Neisseria gonorrhoeae*: Detection by starch paper technique. *British Medical Journal*, 1977; 2:500.
8. Clinical Laboratory Standard Institute [CLSI]. Performance standard for antimicrobial susceptibility testing: Approved standard M2-M7, eighteenth informational supplement, CLSI document M100-S18. 2006.
9. Narayani TV, Shanmugam, JJ, Naseema, KK, Bhattacharya RN. and Shyamkrishnan, KG. Correlation between beta-lactamase production and MIC values against penicillin with coagulase-negative staphylococci. *Journal of Postgraduate Medicine*, 1989; 35: 147- 51.
10. Torimiro N, Moshood AA. and Eyiolawi SA. Analysis of beta-lactamase production and antibiotics resistance in *Staphylococcus aureus* strains. *Journal of Infectious Diseases and Immunity*, 2013; 5(3): 24-28.
11. Akindele AA, Adewuyi IK, Adefioye OA, Adedokun SA, and Olaolu AO. Antibiogram and beta-lactamase of *Staphylococcus aureus* isolated from human clinical specimens in a Tertiary Health Institution in Ile-Ife, Nigeria. *Am. Eurasian J. Sci. Res*., 2010; 5(4): 230-233.
12. Adegoke AA. and Komolafe AO. Multi-drug resistant *Staphylococcus aureus* in clinical cases in Ile-Ife, Southwest Nigeria. *International*

Journal of Medicine and Medical Sciences, 2009; 1(3): 68-72.
13. Eze EA, Agbo EC, and Eze CN. Occurrence of beta-lactamases and the antibiogram pattern of clinical isolates of *Escherichia coli* and *Klebsiella* species in Nsukka Metropolis. *American J. of Microbiology and Biotech*, 2015; 2(5): 69-74.
14. Singleton P. Man against bacteria. In: Bacteria in Biology, Biotechnology, and Medicine, 4th edition, John Wiley & Sons Ltd. pp. 1997; 325-327.
15. Ako-Nai, AK, Adeyemi FM, Aboderin OA. and Kassim OO. Antibiotic resistance profile of staphylococci from clinical sources recovered from infants. *African Journal of Biotechnology*, 2005; 4(8): 816-822.
16. Agwu E, Ohihion AA, Agba MI, et al. Incidence of Streptococcus pneumoniae Infections among Patients Attending Tuberculosis Clinics in Ekpoma, Nigeria. Shiraz Electronic Medical Journal. 2006; 7: 1
17. Barrow GI and Feltham RKA. Cowan and Steel's Manual for the identification of medical bacteria. 3rd ed. Cambridge Univ Press, 1993.
18. Joseph E. Rubin, Katherine R. Ball and Manuel Chirino-Trejo. Antimicrobial susceptibility of *Staphylococcus aureus and Staphylococcus pseudintermedius* isolated from various animals. Can. Vet J. Feb;2011; 52(2): 153-157
19. Charles V. Pryles, http://pediatrics.aappublications.org/by guest on October 26, 2017