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Molecular Detection of Beta-Lactam Resistance Genes in Staphylococcus Aureus Isolated From Women in Nasarawa State, Nigeria

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Abstract

Resistance to antimicrobials by pathogenic microorganisms has raised serious global clinical concerns in recent times. The present study aimed at detection of β-lactam resistance genes in *S. aureus* isolates from women with symptomatic and asymptomatic cases of urinary tract infections in Nasarawa state, Nigeria. A total of 200 non-repetitive midstream urinal samples were analysed and 50 (29%) bacterial isolates were identified as *S. aureus*. The susceptibility profile of the bacterial isolates to tested antibiotics was Nitrofurantoin (74.1%), Gentamicin (72.4%), Ciprofloxacin (65.5%), Ofloxacin (56.9), Augmentin (36.2%), Cotrimozazole (29.3%), Ampicillin (27.6%), Erythromycin (25.8%), Ceftazidine (20.7%) and Cefurozime (10.3%). Thirteen bacterial isolates were found to be resistant to all β-lactam antibiotics tested, out of which 7 were confirmed β-lactamase producers using the acidometric and iodometric methods. The detection of β-lactamase genes (blaZ, blaI and blaR1) was carried out and only five of the isolates were found to be expressing the blaI genes. This research finding suggests that β-lactam resistance by *S. aureus* may not be dependent only on the blaZ, blaI and blaR1 genes.

Keywords: Staphylococcus aureus; β-lactamase; Urinary tract infection; Antibiotic susceptibility.

1. Introduction

Staphylococcus aureus belongs to the family Micrococcaceae and is part of the genus *Staphylococcus*, which contains more than 30 species such as *S. epidermidis*, *S. saprophyticus* and *S. haemolyticus*. Among the staphylococcal species, *S. aureus* is by far the most virulent and pathogenic for humans [1]. *S. aureus* is a Gram-positive cell that in the laboratory may be observed as single cells, in pairs or as grape-like irregular clusters. It is characterized as coagulase and catalase positive, non-motile, non-spore-forming and facultative anaerobe. It grows as yellow colonies on nutrient rich media and is referred to as the yellow Staphylococci [2].

S. aureus has emerged as one of the most important human pathogens and has over the past several decades, been a leading cause of hospital and community acquired urinary tract infections which may be symptomatic or asymptomatic most especially in women [3, 4]. *S. aureus* is a coagulase positive opportunistic pathogen known to cause series of pyogenic infections and intoxications in immunocompetent and immunocompromised individuals [1]. *S. aureus* used to be dismissed as colonization, since most patients do not show the classical symptoms of urinary tract infection [1, 5]. Most strains of *S. aureus* possess the ability to produce beta-lactamases, an enzyme that can open beta-lactam rings in Cephalosporin and Penicillin. Some acquire resistance genes from the environments and/or from other bacteria and thus may exhibit resistance to antibiotics in other classes. The commonest resistance genes code for the expression of beta-lactamase enzymes in *S. aureus* resistance to beta-lactam antibiotics earlier reported are *blaZ, blaI* and *blaR1* genes [6]. There is paucity of information on the beta-lactamase resistance genes in Nasarawa State, Nigeria. Hence, this study is aimed at molecular detection of beta-lactamase resistance genes in *S. aureus* isolated from women in Nasarawa State, Nigeria.

2. Materials and Methods

2.1. Study Area

This research work was carried out in Nasarawa State, Nigeria. Nasarawa State has a central location in the middle belt region of Nigeria. The state lies between latitude 7° and 9° 25'N of the equator and between longitude 7°

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and 9° 37'E of the Greenwich Meridian. It shares boundary with Kaduna state in the North, Plateau state in the East, Taraba and Benue states in the South while Kogi and the Federal Capital Territory flanks in the West [7].

2.2. Sample Collection

Sterilized universal bottles were used to collect urine samples from pregnant and non-pregnant women of child bearing age attending General hospitals in Nasarawa State, Nigeria after educating them on how to collect the mid-stream urine samples. The samples were labelled and transported in ice packs to the Microbiology Laboratory of Nasarawa State University, Keffi for analysis. A total of 200 midstream urine samples of (5 to10ml) were collected and were examined within 6 hours of the collection time [8].

2.3. Isolation and Identification of *Staphylococcus aureus*

Standard microbiological procedures were used for the isolation and identification of *S. aureus* [9].

2.4. Antibiotics Susceptibility Test

The antibiotics were tested against the isolates using standard procedures [10].

2.5. Detection of Beta Lactamase Enzymes Producing *Staph. aureus*

The protocol for β-lactamase enzyme production for selected multiple antibiotics resistant *S. aureus* isolates was adopted with some modifications [11].

2.6. DNA extraction

2.6.1. Genomic DNA Preparation by Boiling Method

The *S. aureus* isolates were grown in a Luria Bertani (LB) Broth at 37^0C for 24h. Then 1ml of the liquid culture was transferred into 1.5 ml volume Microfuge tube. Bacteria cells were harvested by centrifugation at 12,000 Xg for 5min. The supernatant was discarded and the pellet washed twice with Ultra pure water and re-suspended in 1ml Ultra pure water. The bacteria suspension was boiled for 10min to lyse the cells and release the DNA followed by a 'cold shock' treatment in ice for 10min. The suspension was then centrifuged at 12,000 g for 5min and the clear supernatant containing the DNA was transferred to a new microfuge tube and used directly in specific PCR to detect and confirm the Genus and species of isolates and detection of mutant genes [12].

2.6.2. PCR Amplifications and Cycling Conditions

PCR amplifications were performed on a thermocycler (A and E Laboratories, UK Model Cyl-005-1). The primer pairs used is as shown in table 1.

The reaction volume was 25 µl and consisted of 10X PCR buffer, 25 mM $MgCl_2$, 10 mM dNTP's mixture, 5 U/µl of Taq DNA polymerase (Fermentas, USA),10pmol of each primer set, and 5ng of extracted bacterial DNA. Amplifications were performed following an initial denaturation temperature at 95^0C for 5 minutes, followed by 35 amplification cycles of denaturation for 30 seconds at 94^0C, annealing for 30 seconds at 56^0C, polymerization for 30 seconds at 72^0C, and a final extension cycle for 5 minutes at 72^0C. *Staphylococcus aureus* ATCC 29213 was used as quality control strains.

Table-1. Primer sequences for beta-lactamase resistance genes in *S. aureus*

Primer	Sequence (5' – 3')	Reference
blaZ-F	*GATAAGAGATTTGCCTATGC*	[12]
blaZ-R	*GCATATGTTATTGCTTGACC*	
blaI-F	*GCAAGTTGAAATATCTATGG*	
blaI-R	*GAAAGGATCCATTTTCTGTACACTCTCATC*	
blaR1-F	*CATGACAATGAAGTAGAAGC*	
blaR1-R	*CTTATGATTCCATGACATACG*	

2.6.3. PCR Protocol

PCR products were viewed using Agarose gel electrophoresis following standard protocol [13].

2.7. Statistical Analysis

The data obtain from this study on isolation rate of *S. aureus* were analysed using Chi square using Smith statistical package (SSP) Version (2.80), the significance was determine at 95% confidence interval or 5% probability level (i.e P= 0.05).

3. Results and Discussion

Table-2. Isolation Rate of *Staphylococcus aureus* From Urine of Women in Respect to Some Risk Factors in Nasarawa State, Nigeria

Risk Factors	No of Samples	No. (%) Isolated	Chi Square (χ^2)	p-value
Age (Years)				
14-24	68	23 (33.80)	**0.5334**	**0.9115**
24-34	62	18 (29.00)		
34-44	55	12 (21.80)		
44-54	15	5 (33.30)		
Pregnancy status				
Pregnant	93	19 (20.40)	**1.5076**	**0.2194**
Non pregnant	107	39 (36.40)		
Marital status				
Married	92	23 (25.00)	**0.6846**	**0.7101**
Single	88	31 (35.20)		
Widow	20	4 (20.00)		
Educational status				
Primary	28	16 (57.10)	**3.2447**	**0.3553**
Secondary	97	23 (23.70)		
Tertiary	69	10 (14.50)		
Uneducated	11	9 (81.80)		
Type of toilet facility				
Pit latrine	91	39 (42.80)	**11.7739**	**0.0026**
Water closet	109	19 (17.40)		
Open field	0	00		
Total	**200**	**58 (29.00)**		

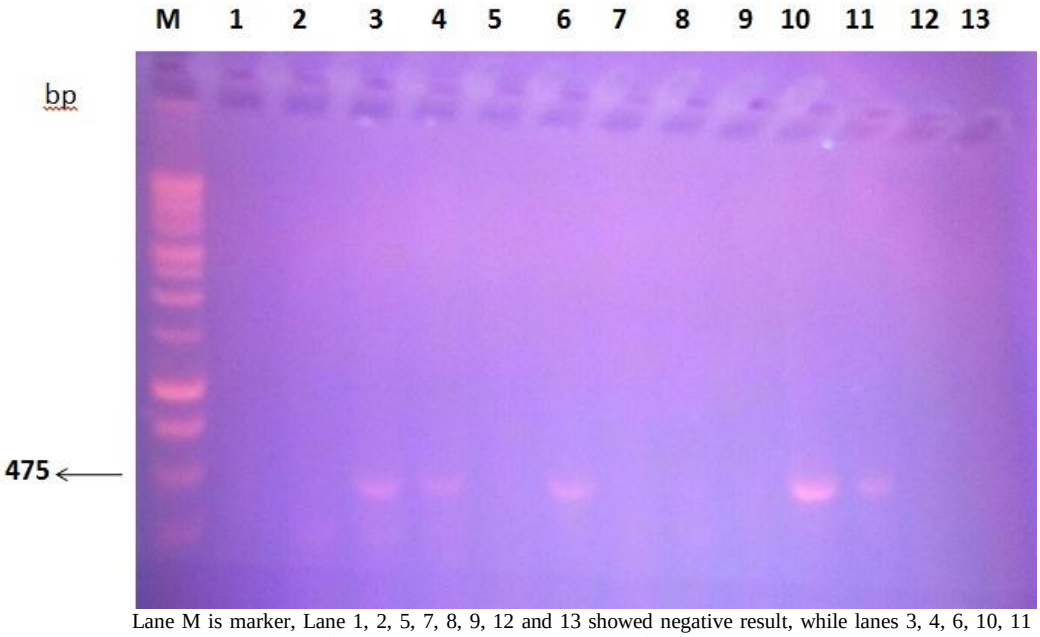
Table-3. Antibiotics Susceptibility Profile of *S. aureus* isolates to test antibiotics

Antibiotic	Disk content (mg)	No. (%) susceptibility of *S. aureus* (n=58)
Ceftazidine	30	12(20.70)
Cefuroxine	30	6(10.30)
Gentamicin	30	42(72.40)
Contrimoxazole	10	17(29.30)
Eythromycin	30	15(25.80)
Ciprofloxacin	5	38(65.50)
Ofloxacin	5	33(56.90)
Augmentin	30	21(36.20)
Nitrofurantoin	30	43(74.10)
Ampicillin	30	16(27.60)

Table-4. Production of Beta lactamase by Beta lactam resistance *S. aureus* from urine of women of child bearing age in Nasarawa State

S/No.	Acidometric	Iodometric
1	++	++
2	--	--
3	++	++
4	++	++
5	--	--
6	++	++
7	--	--
8	--	--
9	--	--
10	++	++
11	++	++
12	--	--
13	++	++

Plate-1. Agarose gel electrophoresis



Lane M is marker, Lane 1, 2, 5, 7, 8, 9, 12 and 13 showed negative result, while lanes 3, 4, 6, 10, 11 showed positive amplification corresponding to BlaI gene of *Staphylococcus aureus.*

Staphylococcus aureus is a documented etiologic agent of urinary tract infections (UTIs) and has become one of the most successful adaptable human pathogens [14]. *S. aureus* has been reported by various researchers to have remarkable ability to acquire antibiotic resistance contributes to its emergence as an important pathogen in different environment [15]. A total of 58(29 %) isolates were isolated and identified as *Staphylococcus aureus* from 200 urine samples examined which is comparable with the finding of. Stanley, *et al.* [16] in Lagos state, Nigeria which found the prevalence of *S. aureus* to be 22.9%, and 19.7% among pregnant and non-pregnant women respectively. However, Kahsay, *et al.* [17] reported a higher prevalence of 39% in a study conducted in Ethiopia. In another study, Adamu, *et al.* [18] reported a prevalence of 52% among apparently healthy women in Maiduguri, Nigeria. The isolation rate of *S. aureus* from urine observed in this study is lower than the other study conducted by Emeka, *et al.* [14] and Adamu, *et al.* [18] and this however is in agreement with previous studies which indicate that women are carrier of *S. aureus* as one of the most common etiological agent of urinary tract infections (UTIs).

From this study we also observed that *S. aureus* isolates are more susceptible to the antibiotic tested in the order Nitrofurantoin (74.1%), Gentamicin (72.4%), Ciprofloxacin (65.5%) and Ofloxacin (56.9) but less susceptibility to antibiotics such Augmentin (36.2%), Cotrimoxazole (29.3%), Ampicillin (27.6%), Erythromycin (25.8%), Ceftazidine (20.7%) and Cefuroxime (10.3%) respectively. The low susceptibility of *S. aureus* isolates to antibiotics mentioned may be due to misused and abused of antibiotics [19] and this however shows that the antibiotics mentioned may not be useful for treatment of *S. aureus* related urinary tract infections. However, Nitrofurantoin, Gentamicin, Ciprofloxacin and Ofloxacin were shown to be effective in the treatment of infections caused by *S. aureus.* The susceptibility of Gentamicin observed in this study may be possible and this may be due to the fact that such aminoglycoside antibiotics are injectable dosage forms and because of the discomfort and pains associated with injections, the abuse of such drugs may be minimal. The high susceptibility of *S. aureus* isolates to the drugs observed in this study is in agreement with other studies reported [19-21].

The antibiotic susceptibility results of isolated *S. aureus* showed a remarkably high percentage of resistance to the test antibiotics. The susceptibility of *S. aureus* isolates observed in this study to the antibiotics tested is not in agreement with the studies earlier reported by some researchers [16, 18]. The ineffectiveness of some tested antibiotics observed in the study is due to the fact that most of these drugs are in tablets form and they can easily abused or taken without medical prescription. The production of β-lactamase in *S. aureus* appears to be consistently high in Nigeria as observed in this study which is in agreement with 70-80% β-lactamase prevalence in other previous reports [4]. The spread of β-lactamase genes had been enhanced by their integration within mobile genetic elements such as plasmids and transposons which facilitate the rapid transfer of genetic materials between microbes [17].

As shown by multiplex PCR (Plate 1), few of the *S. aureus* isolates showed the presence of the blaI genes, that code for extended spectrum Beta Lactamase (ESBL) production. The Beta-lactamase locus that encode for Beta lactam resistance in *S. aureus* contains the structural gene (blaZ), a repressor (blaI) and a sensor/inducer (blaR1) gene respectively. The blaI and blaR1 system induces mecA in a few minutes whereas mecI-mecR1 system takes several hours [22]. Most of the genes identified in this studies have same base pairs from those of the primers used,

while some of the isolate shows negatives amplicons, indicating that, the resistance genes which primers were used for this study were not contained in these isolates or the amount of the genes was too small or it could also be due to the fact that the gene is not expressed at the molecular level of the primers used or this isolate may contain other resistance gene. The report of Zscheck and Murray [6] tends to support these insertions which also found DNA bands similar to those genes tested and concluded that additional resistance genes may exist but not expressed at the molecular level. The findings in this study shows that some isolates may contain only one type of ESBL genes is not in agreement with the report of Geser, *et al.* [23] that out of the 24 multiple Antibiotic resistance isolates, Eleven (11) were PCR positive for blaTem gene, Eight (8) were found to be blaI, and four (4) expressed blaR1 gene respectively.

4. Conclusion

The isolation rate of *S. aureus* was very high and the bacterium were more susceptible to Nitrofurantoin, Gentamicin, Ciprofloxacin and Ofloxacin which suggests that such antibiotics may be useful for the treatment of urinary tract infections caused by *S. aureus.* In addition, most of *S. aureus* isolates were multiple antibiotic resistant isolates and most of them were positive for Beta lactamase and only blaI genes was detected in Beta lactamase producing *S. aureus* isolates. In accumulation, the appearance of pathogens with resistance to various antimicrobial agents indicates a growing need for new antimicrobial agents, with novel modes of action, for use in the treatment of serious Staphylococcal infections.

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Disclosure of Conflict of Interest

Authors have declared that no competing interests exist.

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