

β -lactamase Induction in Nosocomial *Staphylococcus aureus* Strains Cultured from the Nasal Cavity and Wound of Patients

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Abstract

The study was conducted to determine β -lactamase induction in *Staphylococcus aureus* strains cultured from the nasal cavity and surgical wounds of in-patients at a tertiary teaching hospital.

Swab samples were taken from the nasal cavity -wound pair of 110 consented patients. The *S. aureus* isolates were identified and the antibiotics susceptibility tests were conducted using standard protocols. β -lactamase induction was assayed with the micro-iodometric method. The data obtained were statistically analysed.

A total of 81 *S. aureus* isolates were cultured from the nasal cavity and wounds of the 110 samples collected. β -lactamase was produced by 14 (41.2%) and 26 (55.3%) of the isolates cultured from the nasal cavity and wounds respectively. Constitutive and induced β -lactamase production was observed.

β -lactamase production in the isolates is not dependent on antibiotic exposure alone. The findings underscore the need for appropriate infection control and adequate hygiene practices to prevent the spread of *S. aureus* in the hospital settings.

Keywords: Antibiotics, β -lactam, β -lactamase, Induction, nasal cavity, resistance, *Staphylococcus aureus*, Wounds

1. Introduction

The human skin acts as an excellent barrier to infection but if this barrier is breached, microorganisms regarded as commensals on body surface may assume the role of opportunist pathogens. Wound is caused as a result of a breach in the intact skin and wound infections are an important cause of morbidity and account for 70-80% mortality in the hospital setting (Wilson et al., 2004; Gottrup, Melling, Hollander, 2005). The risk of developing surgical wound infection and the severity of complications depend mainly on the number of pathogens and site of infection (Dohmen, Gabbieri, Weymann, Linneweber & Konertz., 2009). It has been observed that *S. aureus* is the most common organism isolated from wound and is the leading cause of bloodstream, lower respiratory tract, skin/soft tissue infections in all body regions (Lowy, 1998; Gordon and Lowy, 2008). A study carried out by Emele, Izomoh & Alufohai (1999) revealed that *S. aureus* is the most frequently isolated organism in wound infection in post-operative sepsis. Gayathree and Deshpande (2011), Vijayalakshmi (2012) in their study on surgical site infections observed *S. aureus* as the most common organism isolated from surgical site infection.

Apart from the organism being an important and virulent nosocomial pathogen, it has been reported that the organism possesses the propensity to resist the action of a plethora of antimicrobial agents (Archer, 1998; Cooper et al., 2004; Torimiro, Moshood & Eyiolawi., 2013). Beta-lactam antimicrobial agents are the preferred drugs in the treatment of *S. aureus* infections. They exhibit their bactericidal effects by inhibiting enzymes involved in cell wall synthesis. The integrity of the bacterial cell wall is essential to maintaining cell shape in a hypertonic and hostile environment (Massova and Mobashery, 1998). However, reports have shown that resistance to β -lactam antibiotics is rampant. (Francis, Harold, Roger, & David, 1997; Schito, 2006; Torimiro et al., 2013).

Beta-lactamase is the most prevalent mechanism of bacterial resistance to β -lactam antibiotics and have been documented to have co-evolved with them since their introduction into clinical use (Medeiros, 1997; Rice & Bonomo, 2000). Staphylococcal β -lactamase is not chromosomal and is carried on plasmids and can be non-inducible or inducible with antibiotic contact (Maddux, 1991). This enzyme inactivates the β -lactam antibiotics (penicillin) by cleavage of the beta-lactam ring. Incidence of β -lactamase production in *S. aureus*

observed to be over 80% has been documented (Parker & Collier, 1990; Schito, 2006; Akindele, Adedokun & Olaolu, 2010; Torimiro et al., 2013).

The induction of β -lactamase synthesis is important for the resistance of staphylococci to penicillins since the drug both induces synthesis of the enzyme and is hydrolysed by it. Many new β -lactam antibiotics have been developed that were specifically designed to be resistant to the hydrolytic action of β -lactamases. However, with each new class that has been used to treat patients, β -lactamases emerged that caused resistance to that class of drug. In addition, β -lactamase has been reported to spread to most clinical isolates of *S. aureus* as well as other species of staphylococci (Bradford, 2001).

S. aureus colonises the nasal mucosa of approximately 25-30% of apparently healthy population (Lamikanra, Paul, Akinwale, Paul, 1985; Yamamoto et al., 2010). This provides a reservoir from which it can be introduced when host defenses are breached, and has been reported to increase the risk for subsequent infections (Mahdi et al., 2000; Wertheim et al., 2005). Although this may depend on an array of factors that may either be environmental or patient-related. This study aims to determine antibiotic susceptibility pattern and evaluate the induction of β -lactamase in *S. aureus* isolates

2. Materials and Methods

2.1 Study Population

Nasal and wound swabs were collected from one hundred and ten >48 h hospitalized surgical wound patients at the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Osun-State, Nigeria after due informed consent and ethical clearance were obtained.

2.2 Isolation and Identification of the Isolates

The nasal and wound samples were collected using sterile tipped applicators. The samples were transported immediately to the Microbiology Laboratory and processed within four hours of collection. The specimens were inoculated into nutrient broth (Lab M, USA) and incubated at 37°C for 24 h. The cultures were streaked onto mannitol salt agar (MSA) and incubated at 37°C for 18-24 h. The isolates were characterised using standard microbiological methods as described (Cheesbrough, 2006). Also the latex agglutination test using Pastorex Staph-Plus (Bio-Rad, USA) was done and the isolates were identified as *S. aureus* (Holt, Krieg, Sneath, Stanley & Williams, 1994).

2.3 Antibiotic Susceptibility Typing and β -Lactamase Test

Antibiotic susceptibility of the *S. aureus* isolates was done using by Kirby-Bauer disk diffusion method. The impregnated antibiotics discs of known concentrations includes amoxicillin (25 μ g), augmentin (30 μ g), cloxacillin (5 μ g) (Abtek Biologicals, UK) while cefoxitin (30 μ g), ceftriaxone (30 μ g), imipenem (10 μ g), oxacillin (1 μ g), penicillin G (10 U) (Oxoid, UK). The interpretation of the zone of inhibition was done according to the recommendations of the Clinical and Laboratory Standards Institute (2009) and AB biodisk interpretation chart. Intermediate resistance was recorded as resistant.

Acidometric agar plate method for β -Lactamase detection were conducted as described Park, Lopez & Cook (1978) as reported in Olutola, Adeyanju & Torimiro (2016).

2.4 β -Lactamase Induction Test

The induction of β -Lactamase was carried out using a modified method as described by Kraft, Prabhu, Ursinus & Hölte (1999). Cells (1 ml) from the exponential growth phase in nutrient broth were diluted with 1 ml of nutrient broth (medium) containing 1-8 μ g/ml of cefoxitin and penicillin in separate tubes. The mixture was incubated at 37°C for 30 min. Control samples received medium without cefoxitin and penicillin.

After 30 min incubation, the cells were spun down at 13,500 rpm for 20 min at 4°C in a centrifuge. The spun cells were washed once with 50 mM sodium phosphate buffer (pH 7.0), re-suspended in 1 ml of the same buffer and stored in at 4°C. 20 μ l of the resuspended cells was treated with 20 μ l of lysis buffer [400 mM Tris-HCl, 8 mM EDTA, 200 μ g/ml of lysozyme (pH 8.0)] and 40 μ l of distilled water, followed by 15 min incubation at room temperature. The protein concentration of the lysate was determined by the Lowry's method (Lowry, Rosebrough, Farr & Randall, 1951). Bovine Serum Albumin (Sigma) was used as the protein standard and the absorbance of the samples at $A_{660\text{ nm}}$ were read using a spectrophotometer (Cintral, Australia).

2.4.1 Quantification of the Lactamase Activity

Modified micro-iodometric assay for the quantification of lactamase activity according to Novick (1962) as reported by Sykes & Nordstrom (1972) was used. A 0.089 g of benzyl penicillin was dissolved in 100 ml of phosphate buffer (pH 7.2, 0.1M) and the mixture was placed on ice (substrate solution).

Exactly 1 ml starch-iodine solution and 0.9 ml of 50mM phosphate buffer were mixed in test tubes. Observation of blue colour indicated the reaction of iodine with starch. Exactly 1 ml benzyl penicillin solution (substrate solution) on ice was added to the mixture in test tubes at the point of assay. Sample solution (0.1 ml) was introduced into a glass cuvette and thereafter, the mixture in the test tube was added to the sample solution in the glass cuvette which made up to 2.9 ml. The spectrophotometer was left for 5 min for the temperature to reach 30°C and blank with phosphate buffer (control solution) at A_{620nm} before the reaction was initiated. Absorbance at A_{620nm} was then read at 30 s interval for 5 min. Rapid decolourization indicated β-lactamase production.

Units of β-Lactamase: One unit of β-lactamase is defined as the amount of enzyme that hydrolyzes benzyl penicillin at the rate of 1 μmole/min (Sykes & Nordstrom, 1972; Karmakar, Dua & Ghosh, 2016).

3. Results

3.1 Antibiotic Susceptibility Profile of the *S. aureus* Isolates

Eighty-one (73.6%) *S. aureus* strains were isolated from the nasal-wound pair of 110 wound patients. All of the 34 (42%) isolates cultured from the nasal swabs were resistant to amoxicillin, augmentin and cloxacillin but only 1 (2.9%) was resistant to imipenem (Table 1).

Forty-seven (58%) of the isolates were cultured from wounds and the antibiotics susceptibility profile as observed by Olutola et al. (2016) are as shown (Table 1).

Table 1. Antibiotic Susceptibility Profile of the *Staphylococcus aureus* strains Isolated from the nasal and wound pair

Antibiotics	Nasal Isolates	Antibiotic profile		Wound Isolates	Antibiotic profile	
		S	R		S	R
Amoxycillin		0	34 (100)		0	47 (100)
Augmentin		0	34 (100)		13 (27.7)	34 (72.3)
Cefoxitin	34	8 (23.5)	26 (76.5)	47	11 (23.4)	36 (76.6)
Ceftriaxone		18 (52.9)	16 (47.1)		20 (42.6)	27 (57.4)
Cloxacillin		0	34 (100)		0	47 (100)
Imipenem		33 (97.1)	1 (2.9)		42 (89.4)	5 (10.6)
Oxacillin		3 (8.8)	31 (91.2)		4 (8.5)	43 (91.5)
Penicillin		1(2.9)	33 (97.1)		0	47 (100)

Note, R= Resistance, S= Susceptible.

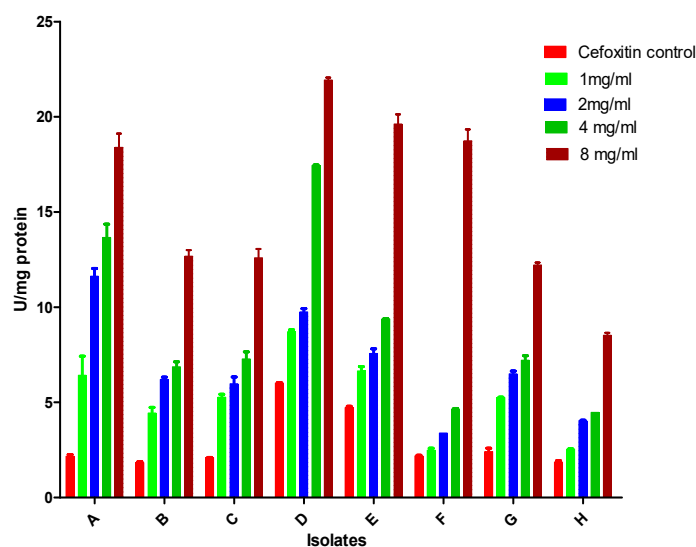


Figure 1. β- lactamase induction of Isolates at varied Cefoxitin Concentration

The rate of β- lactamase induction in isolates A- C *S. aureus* cultured from the nasal swab. D-G isolates from wound swabs increases with increase in concentration of cefoxithin; H = control strain

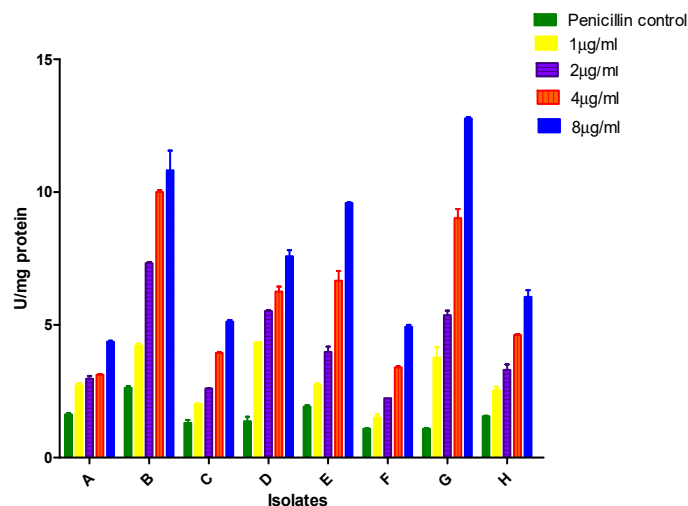


Figure 2. β - lactamase induction of Isolates at varied Penicillin Concentration

The rate of β - lactamase induction in isolates A- C *S. aureus* cultured from the nasal swab. D-G isolates from wound swabs increases with increase in concentration of cefoxithin; H = control strain

3.2 Effect of Concentration of β -Lactam Antibiotics to β -Lactamase Induction

Beta-lactamase production was detected in 48 (59.3%) of the 81 *S. aureus* strains isolated from the nasal cavity and wounds. Production of β -lactamase enzyme was observed before the addition of inducer but it was generally observed that the rate of induction increased with increase in antibiotic concentration with both cefoxithin and penicillin antibiotics respectively as shown (Figures 1 and 2).

4. Discussion

The prevalence of *S. aureus* recovered from the wound and nasal mucosa was 58% and 42% respectively. Surgical site infection by *S. aureus* has been observed to be at 45.2% prevalence rate in a study conducted in India (Vijayalashim, 2012). The study revealed a high resistance to penicillin and other β -lactams by the *S. aureus* strains which is in agreement with previous studies (Esan, Famurewa, Lin & Shittu, 2009; Akindele et al., 2010). The resistance of *S. aureus* strains to β -lactams which are the most commonly used antibiotics is well documented.

The production of β -lactamase observed in this study was 49.4% in both wound and nasal *S. aureus* strains but lower compared to previous studies in which 70-80% was reported (Akindele et al., 2010; Torimiro et al., 2013). This difference may be as a result of the collection of samples from limited specimens compared to the previous investigators who collected samples from various clinical specimens. Staphylococcal β -lactamase is not chromosomal and is carried on plasmids and can be non-inducible or inducible with antibiotic contact (Maddux, 1991). This present study revealed that the production of enzyme is both constitutive and inductive which may imply that the production of the enzyme could be an intrinsic defence of the organisms against the action of antibiotics or as a result of use of the antibiotics.

The production of β -lactamase enzyme in this study was noticed before the addition of inducer (antibiotic) but it was observed that the specific activity of β -lactamase increased with increase in antibiotic concentration. The result supports the findings of (Nordstrom & Sykes, 1973; Okonogi, Kuno, & Higashide, 1986; Raimondi, Sisto & Nikaido, 2007) who reported that the specific activity of β -lactamase increased linearly with increasing concentrations of inducer. In addition, Khan, Sallum, Zheng, Nau & Hassan (2014) in their study on rapid determination of β -lactamase and antibiotic activity detected β -lactamase in the absence of inducer however, at a lower level when compared with induction.

In conclusion, it was observed that the *S. aureus* isolates were resistant to the various antibiotics tested. It was also shown in this study, that β - lactamase production could be induced and constitutive indicating the need for improvement in infection control strategies in the hospital settings in order to reduce over dependence on antibiotics for treatment and transmission of resistant strains. In addition, there is need for the development of new classes of antibiotics to treat infections caused by *S. aureus* strains.

Competing Interest

The Authors declare there is no competing interest.

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