British Microbiology Research Journal
17(5): 1-7, 2016; Article no.BMRJ.27848
ISSN: 2231-0886, NLM ID: 101608140

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Extended Spectrum β-lactamase Detection and Antibiotic Susceptibility Profile of *Staphylococcus aureus* Strains Isolated from Surgical Wounds

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Authors' contributions

This work was carried out in collaboration between all authors. Author NT designed the study and wrote the first draft of the manuscript. Authors NT, OTO and AA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/27848
Editor(s):
(1) Ng Zhi Xiang, Department of Biomedical Sciences, Faculty of Medicine, MAHSA University, Malaysia.
Reviewers:
(1) Anonymous, University of Jordan Hospital, USA.
(2) Teresita Sainz-Espuñes, Universidad Autónoma Metropolitana-Xochimilco, Mexico.
(3) Akobi Oliver Adeyemi, Federal Medical Centre, Bida, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/16878

Original Research Article

Received 22nd June 2016
Accepted 19th September 2016
Published 11th November 2016

ABSTRACT

Aims: The study aims to determine the antibiotic susceptibility pattern, incidence of β-lactamase and extended-spectrum β-lactamase (ESBL) production in *Staphylococcus aureus* isolated from surgical wounds.
Study Design: A cross-sectional study designed to evaluate the incidence of antibiotic resistance and ESBL production in *S. aureus* recovered from surgical wound patients was conducted.
Place and Duration of Study: The study participants included hospitalized patients who were presented with surgical wounds at the Obafemi Awolowo University Teaching Hospital Complex (OAUTHC), Ile-Ife, Osun-State, Nigeria between April-December, 2013.
Methodology: Wound swabs were aseptically collected from one hundred and ten in-patients that have been hospitalized for more than 48 hr in a tertiary teaching hospital. The antibiotic susceptibility typing was conducted using Kirby-Bauer disc diffusion method. The β-lactamase was assayed with the acidometric method while the ESBL was screened with the double disk approximation method. The SHV and TEM resistance genes were detected with polymerase chain reaction (PCR) based technique.

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Results: Forty seven (42.7%) *S. aureus* isolates identified were all multi-resistant to the antibiotics tested, but 42 (89.4%) of the isolates were susceptible to imipenem. β-lactamase was detected in 26 (55.3%) of the *S. aureus* isolates and ESBL was phenotypically expressed in two isolates. However, sulfhydryl variant (SHV) gene was detected in 5 (19.2%) of the β-lactamase producing strains while the TEM gene was not detected.
Conclusion: This study suggests a continuous screening and surveillance of MRSA among the patients in the hospital setting due to the high prevalence of these microorganisms in Osun state, Nigeria.

Keywords: Antibiotics; resistance; extended spectrum β-lactamase; Staphylococcus aureus.

1. INTRODUCTION

Colonization provides a reservoir from which *Staphylococcus aureus* can be introduced when host defenses are breached, and it clearly increases the risk for subsequent infections [1-2]. It is one of the major nosocomial pathogen and hospitalized patients have shown to be more at risk in-hospital mortality compared with in-patients without the infection [3]. It is a frequent etiological agent of human diseases and exhibits resistance to a growing number of therapeutic agents.*S. aureus* is one of the most frequently isolated organisms from wounds. It is also the leading cause of bloodstream, lower respiratory tract and skin/soft tissue infections [4-6]. The development of such infections causes patient's discomfort, delayed healing, anxiety, longer stays at hospitals and adds to cost of healthcare services significantly [7-8].

Beta-lactam antimicrobial agents are the preferred drugs in the treatment of *S. aureus* infections. However, *S. aureus* has developed mechanisms to render these drugs ineffective. The predominant mechanism of resistance to several major classes of antimicrobial agents is the β-lactamase inactivating enzyme [9]. It has been shown that the incidence of β-lactamase production in *S. aureus* is high [10-11]. Beta-lactamase encodes resistance to β-lactam antibiotics, by inactivating the cleavage of the beta-lactam ring, thus facilitating invasion of viable strains into the host [12-13]. It has been reported to spread to most clinical isolates of *S. aureus* as well as other species of Staphylococci [14].

In addition, extended spectrum beta-lactamase (ESBL) capable of hydrolyzing penicillins, broad spectrum cephalosporins and monobactams in Enterobacteriaceae [15-16] are often located on plasmids that are transferable from strain to strain and between bacterial species [17]. Extended spectrum beta-lactamase (ESBL) producers have continued to draw attention globally with their attendant clinical failure to new generation antibiotics and nosocomial spread [18]. In addition, rapid changing over time in ESBL has been observed with variations within geographic areas.Clinical outcomes data indicate that ESBL are clinically significant and when detected, suggest the need for the use of appropriate antibacterial agents.Hence, this study is designed to isolate *S. aureus* from surgical wounds, evaluate the incidence of β-lactamase and ESBL using molecular tools.

2. MATERIALS AND METHODS

2.1 Identification of *S. aureus* Isolates

Ethical approval from the Obafemi Awolowo University Teaching Hospitals' Complex (OAUTHC) ethical research committee and verbal patient consent were obtained prior to sample collection. Wound swabs were collected with sterile cotton-tipped applicators from 110 surgical wound patients that were hospitalized for more than 48 hr at the Obafemi Awolowo University teaching hospital complex Ile-Ife, Nigeria. The samples were each inoculated into nutrient broth and incubated aerobically at 37℃ for 18-24 hr. Thereafter, the cultures were streaked on mannitol salt agar (MSA) (Lab M Ltd) and incubated at 37℃ for 18-24 hr. [19]. The isolates were identified as *S. aureus* using standard biochemical, coagulase tests [19] and Pastorex Staph-Plus (BioRad, USA) Latex agglutination test.

2.2 Antibiotic Susceptibility Testing

Antibiotic susceptibility of the *S. aureus* isolates was done using the Kirby-Bauer disc diffusion method on Mueller Hinton agar and interpreted according to the recommendations of the Clinical and Laboratory Standards Institute [20]. The inoculum was standardized to match the 0.5

McFarland turbidity standards and ATCC 25923 was used as the control strain. The impregnated susceptibility disc included erythromycin (5 µg), amoxicillin (25 µg), augmentin (30 µg), chloramphenicol (30 µg), cloxacillin (5 µg), cotrimoxazole (25 µg), gentamicin (10 µg), tetracycline (10 µg) (Abtek Biologicals, UK) while aztreonam (30 mcg), cefoxitin (30 mcg), ceftriaxone (30 mcg), imipenem (10 mcg), oxacillin (1 mcg), penicillin G (10 iu) were products of Oxoid (UK). The susceptibility of each antibiotic was determined from measurement of the zone of inhibition of growth. The data was analyzed using simple percentage. Multi-antibiotic resistance was analyzed using the multiple antibiotic resistance (MAR) index which was defined as a/b where 'a' represent the number of antibiotics to which the isolate is resistant to and 'b' the number of antibiotic to which the isolate is exposed. The isolates with MAR index values higher than 0.2 were considered multiple resistant [21].

2.3 β-Lactamase Enzyme Assay

The acidometric agar plate method was used as described [22]. In 1000ml of distilled water containing 0.2 ml of 0.5% phenol red solution to yield a concentration 5000 µ/ml, 1.5g of agar was added. The solution was sterilized, poured into sterile petri-dishes and allowed to set. The test isolates were placed in discrete spots on the plate. Positive control (penicillin resistant *S*. *aureus*) was placed on the plate. The plates were incubated at 35℃ for 1 hr. β-lactamase positive strains produced bright yellow zones around the colonies due to the release of penicilloic acid whereas, the colour of the area around negative strains remain unchanged.

2.4 Determination of Extended Spectrum β-lactamase (ESBL) Production

The detection of ESBL in the *S. aureus* strains was conducted using the double disc synergy method. Test strains were inoculated into nutrient broth and incubated at 37℃ for 18-24 hr. The inoculums were standardized to an optical density of 0.5 McFarland turbidity standardsand inoculated into Mueller-Hinton Agar plate by swabbing with sterile cotton-tipped applicators. Two antimicrobial disks were placed 30 mm apart (center to center) with one of the discs containing amoxicillin / clavulanic acid and the other an extended-spectrum cephalosporin (ceftriaxone) purchased from Mast Diagnostics (UK). Enhancement of the zone of inhibition > 5mm in between the disks after 24 hr incubation indicated a positive result [23].

2.5 Deoxyribose Nucleic Acid (DNA) Analysis

DNA was extracted using the (NorgenBiotek, Canada) DNA extraction kit. Prior to DNA extraction, proteinase K was reconstituted with 0.6 ml of PCR grade water. The wash solution was also prepared by the addition of 96% ethanol (42 ml) to the wash solution provided by the manufacturer. Lysate was prepared by transferring some of the bacteria culture in a Luria broth grown at 37℃ for 18 hr into 1.5 µl microfuge tubes and centrifuged for 1 min at 14,000 rpm to pellet the cells. The amplification of the *bla*SHV and *bla*TEM gene was done using primers obtained commercially from the manufacturer (NorgenBiotek, Canada). The primer sequence was forward 5'- GGG TTA TTC TTA TTT GTC GC 3' and reverse primer, 5'- TTA GCG TTG CCA AGT GCTC 3' and 5'- ATA AAA TTC TTG AAG AC 3' and reverse primer, 5'- TTA CCA ATG CTT AAT CA 3' respectively. The PCR mix was done according to standard protocol. The PCR for SHV gene detection was carried out according to the following conditions: initial denaturation temperature of 95℃ for 2 min (1 cycle), denaturation at 95℃ for 30 s, annealing at 55℃ for 30 s, extension at 72℃ for 1 min all run for 40 cycles to amplify 500 bp as enumerated (18). The PCR for *bla*-TEM was carried out according to the following conditions: initial denaturation temperature of 95℃ for 2 min (1 cycle), denaturation at 95℃ for 30 s, annealing at 55℃ for 30 s, extension at 72℃ for 1 min all run for 40 cycles to amplify 400 bp as enumerated [18].

3. RESULTS

A total of 47 (42.7%) *S*. *aureus* isolates were cultured and the *in-vitro* antibiotic susceptibility profile of the isolates showed all the isolates were resistant to the common β-lactam antibiotics which included penicillin, cloxacillin and amoxicillin (Table 1). However, 42 (89.4%) of the isolates were susceptible to imipenem (Table 1).

The frequency of antibiotic resistance revealed all the 47 *S. aureus* isolates tested were multi-drug resistant (Table 2).

Beta-lactamase production was detected in 26 (55.3%) of the isolates and phenotypic

expression of ESBL was observed in only two (7.7%) of the β-lactamase producing isolates. The SHV gene was detected in 5 (19.2%) of the isolates (Fig. 1) while no detection of the TEM gene was observed.

Table 1. Antibiotic susceptibility profile of the *Staphylococcus aureus* isolates

Antibiotics	Susceptible n (%)	Intermediate n (%)	Resistant n (%)
Amoxycillin	0	0	47 (100)
Augmentin	13 (27.7)	0	34 (72.3)
Aztreonam	12 (25.5)	11 (23.4)	24 (51.1)
Cefoxitin	11 (23.4)	0	36 (76.6)
Ceftriaxone	20 (42.6)	7 (14.8)	20 (42.6)
Chloramphenicol	6 (12.8)	5 (10.6)	36 (76.6)
Cloxacillin	0	0	47 (100)
Cotrimoxazole	1 (2.1)	1 (2.1)	45 (95.7)
Erythromycin	0	6 (12.8)	41 (87.2)
Gentamicin	6 (12.8)	4 (8.5)	37 (78.7)
Imipenem	42 (89.4)	1 (2.1)	4 (8.5)
Oxacillin	4 (8.5)	0	43 (91.5)
Penicillin	0	0	47 (100)
Tetracycline	0	0	47 (100)

Table 2. Multiple antibiotic resistance of the *S. aureus* isolates

No of antibiotic (b)	No of antibiotics to which the isolate is resistant to (a)	MAR index a/b	Freq of MAR (%)
	7	0.5	1
	8	0.6	1
	9	0 .6	11
	10	0.7	7
14	11	0.8	6
	12	0.9	8
	13	0.9	11
	14	1	2
Total			47 (100%)

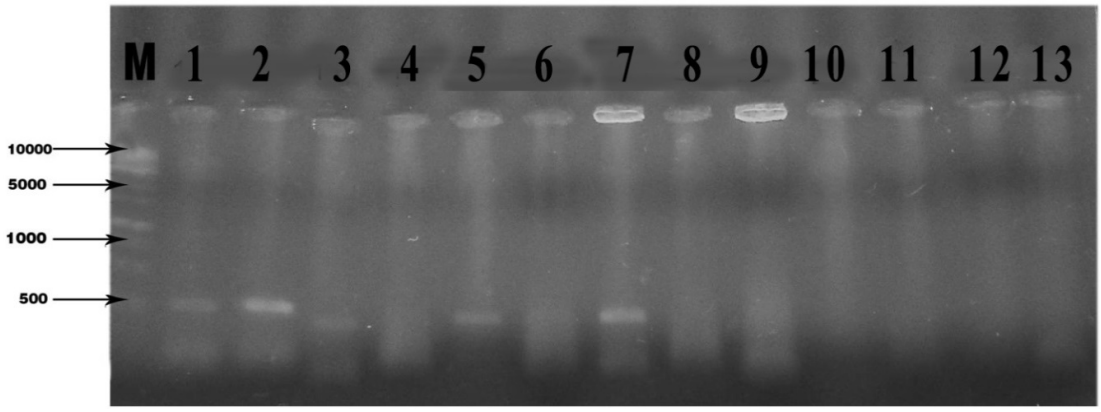

Fig. 1. PCR detection of the SHV gene

Lane M = 1 kb Molecular Weight Marker; Lane 1 = SA1; Lane 2 =- SA2; Lane 3 = SA3; Lane 4 = SA4; Lane 5 = SA5; Lane 6 = SA6; Lane 7 = SA7, Lane 8 = SA8; Lane 9 =SA9, Lane 10 =SA10; Lane 11 =SA11; Lanes 12 and 13 = Negative Control without DNA

4. DISCUSSION

Staphylococcus aureus isolated from surgical wound accounted for 47 (42.7%) of the 110 samples cultured. The study revealed a high resistance to penicillin and other common β-lactams which is in agreement with previous studies [24-25]. The emergence of multi-drug resistance in bacteria has global health implications and resistance of *S. aureus* to multiple antibiotics make infections difficult to treat [26-27]. In this study, the MAR index revealed that 100% of the isolates were multi-drug resistant which is higher compared to the report of other studies [28-29]. This result reflects the predominance of resistant strains in the study area, a serious threat to public health, which agrees with previous reports of other investigators [11,25]. The role of β-lactamase in the development of resistance to β-lactam antibiotics in *S. aureus* has been well documented. It was observed that the production of β-lactamase is low at 26 (55.3%) when compared to high rate (70-80%) previously reported at this center [10-11,25]. This contrast may be as a result of the mono-nature of the samples collected as compared to the various clinical samples analyzed in previous studies.

This study presents the first baseline information on ESBL detection in *S. aureus* in this environment. ESBL has been widely observed in Gram negative *Enterobacteriaceae* and increasing association between ESBL production and fluroquinolone resistance has been reported (17). In *S. aureus* resistance to methicillin confers resistance to all β-lactam antibiotics including cephalosporins and carbapenems [30]. Besides, the detection of ESBL expression in 2 of the *S. aureus* isolates tested could suggest an emerging acquisition of ESBL resistance characteristic in the hospital setting. It has been shown that ESBL is transmitted by plasmids [17]. This may indicate possible transmission of ESBL from nosocomial enterobacteriaceae since the subjects involved were observed to have been hospitalized for more than two weeks. It has been reported that since ESBL genes are transmissible, it is important that it is tested for in other organisms in hospital and long term care facility patient populations where ESBLs are encountered [31]. It was however regretted that plasmid analysis was not conducted in order to ascertain the transmissibility of ESBL from Gram negative organisms to the *S. aureus* isolates, though plasmid borne resistance could either be lost or gained.

The *bla*SHV gene was detected in some of the isolates including the two phenotypic ESBL isolates indicating a possible non-phenotypic expression of ESBL gene carriage. It has been reported that inactivated ESBL genes exist in non-ESBL producing antibiotic-sensitive *Klebsiella pneumoniae* strains which could have the potential to transform into ESBL phenotype if an inappropriate application or overdose of antibiotics is implemented during clinical management [32].

5. CONCLUSION

The *mec*A gene present in methicillin resistant *S. aureus* which confers undue advantage for resistance to vast array of antimicrobial agents was first reported in nosocomial *S. aureus* strains and was subsequently observed in community strains without exposure to antibiotic selective pressure in the hospital. The detection of extended spectrum β-lactamases (ESBLs) in nosocomial *S. aureus* strains is a thought for concern. Considering the fact that *S. aureus* can cause disease in both hospital and community and it is also asymptomatically harbored in the anterior nares of healthy population. Most of the *S. aureus* isolates in this study were multi-resistant to different antibiotics tested *in vitro* suggesting the prevalence of multiple resistant strains in the study area. The study suggests aggressive surveillance system in hospitals for early detection of ESBL carrying *S. aureus* and also for MRSA among the patients in the hospital setting due to the high prevalence of these microorganisms in Osun state, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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