Original Research Article

Molecular Detection of Bone sialoprotein-binding protein (*bbp*) Genes among Clinical Isolates of Methicillin Resistant *Staphylococcus aureus* from Hospitalized Orthopedic Wound Patients

ABSTRACT

Background and Objectives:
Methicillin Resistant *Staphylococcus aureus* (MRSA) is one of the bacteria that is frequently encountered in orthopedic practice causing human skeletal infections due to expression of Genes encoding Microbial Surface Components Recognizing Adhesive Matrix Molecules that aid in microbial invasion of host bone tissue. As such, this study was aimed at screening for Bone sialoprotein-binding protein (*bbp*) Genes among Clinical Isolates of ueMethicillin Resistant *Staphylococcus aureus* from Hospitalized Orthopedic Wound Patients.

Methodology
Aseptically, four hundred (400) fracture and post-surgical wound swab samples were collected from orthopedic wound patients and subjected to standard microbiological protocol for isolation and identification of MRSA using Brilliance MRSA II Agar and Double Disc Diffusion Test with inclusion Oxacillin (5µg) and Cefoxitin (30µg). MRSA strains were further screen for Penicillin binding protein 2a (*mecA*) and Bone sialoprotein-binding protein (*Bbp*) gene by PCR using specific primer.

Result
MRSA had an overall detection rate of 164 (41.0%), with an increase prevalence rate of 86 (21.5%) in post-surgical wound samples, followed by fracture wound samples (78 (19.5%). However, the isolation rate of MRSA was significantly associated with the sample source *p* value <0.05. Of the ten (10) selected MRSA strain, *mecA* gene was genetically detected 100 % comprising of 50.0 % from postsurgical wound patient (MR1-5) and 50.0% from fracture wound patient (MR6-10) while the bone sialoprotein-binding protein (*bbp*) gene was identified in 100% of MRSA strain from both post-surgical wound (MR1-5) and fracture wound (MR6-10) patients each accounted for 50.0% of the total amplified gene.

Conclusion
This study report *MecA* and *bbp* expression in hospitalized orthopedic wound patients, emphasizing that *mecA* and *bbp* detection can be used as a marker of MRSA bone invasion, especially in orthopedic wound infections. To reduce the risk of infection and to halt the clonal proliferation of these virulent strains, a proper wound care/hygiene, comprehensive and ongoing surveillance system that provides epidemiological and genetic information is required.

1. INTRODUCTION

Orthopedic wound and other wound type infection is becoming a serious global public health concern [1]. Orthopedic wound in most developing countries are associated with an increased risk of disease and morbidity for patients [2, 3,4]. Orthopedic wounds, like any other type of wound, can cause discomfort, pain, amputation or disability, financial loss, and death as a result of complications. Furthermore, due to the high cost of patient hospitalization and microbial infection control, wound infection may financially strain healthcare systems. Infection of orthopedic wounds is caused by microbial invasion and colonization, as well as the spread of virulence determinants. In the absence of proper care, pathogenic bacteria are mostly predominant at the sites of orthopedic wounds. One of the earliest and frequent colonizer of wound is Gram-positive bacteria, especially *S. aureus* strains [1, 4]. The evolution of *S. aureus* strain has been traced to the acquisition of the exogenous Penicillin binding protein 2a gene (*mec*A) and bone sialoprotein-binding protein (*bbp*) [1, 4]. The *mec*A encoding strain are often address with the lexicon 'Methicillin Resistant *Staphylococcus aureus* (MRSA). This ancillary or housekeeper gene is part of the Staphylococcal Cassette Chromosome *mec* (SCCmec) (types I–VII) and is under the control of *Mec*I (a repressor) and *Mec*R1 (a transducer) and represent the regulatory/signaling proteins of the *blaZ* system. The *mecA* gene codes for additional penicillin-binding protein (PBP2a), a peptidoglycan transpeptidase, which can confer resistance to methicillin, all *β*-lactam and other antibiotics class [1, 5] while *bbp* is microbial surface proteins that interact with the host molecule called Microbial Surface Components Recognizing Adhesive Matrix Molecules (MSCRAMMs) [6, 7]. The *Bbp* gene belongs to the Serine-aspartate repeat factors (Sdr) family, which includes several other microbial surface components that recognize adhesive matrix molecules [6]. *bbp* from MRSA strain is

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reported to be a 97-kDa protein with 76% and 96% A and B domains, respectively, compared to the corresponding domains in SdrE [6]. Furthermore, there is evidence that SdrE is not linked to bone infection, but it is known for platelet activation and immune evasion by binding to complement factor H [6, 8, 9]. However, *bbp*-encoding strains are frequently linked to septic arthritis, osteomyelitis, and orthopedic implant infections [6, 10, 11]. A hospitalized orthopedic wound infected with MRSA-dependent b*bp* gene may impair quality of life and compromise wound healing rate due to hematogenous tissue invasion. Failure to treat implies an increase in the healthcare costs, since they involve a prolonged hospitalization due to diagnostic tests, extensive antibiotic administration, and, in some cases, invasive surgery [12]. Despite, increasing concerns about MRSA virulence in orthopedic wound infection, there is a still a handful of information on Bone sialoprotein-binding protein (*bbp*) Genes among Clinical Isolates of Methicillin Resistant *Staphylococcus aureus* in Hospitalized orthopedic wound Patients.

Keyword: Orthopedic wound, Methicillin Resistant *Staphylococcus aureus*, Bone sialoprotein-binding protein

2. MATERIALS AND METHODS

2.1 Sampling procedure

This research was carried out based on a thorough knowledge of the scientific literatures, satisfactory laboratory protocols, and other relevant sources of information guiding this area of research. Every fundamental study was done in line with the World Medical Association (WMA) declaration of Helsinki on the principles for medical research involving human subjects, and identifiable human material or data [13]. The fracture and post-surgical wound samples were obtained in compliance with the Research Ethical Committee of National Orthopedic Hospital, Enugu. Patients with length of stay in hospitals for more than two months had their samples collected. When the fracture and post-surgical site was examined, it showed evidence of being deeply infected, with purulent drainage and inflammatory symptoms. Swabs were used to collect samples by gently rolling or rotating over the surface of the wound without contacting the skin around the wound. The swab was aseptically inserted in a tube containing the Amies transport medium (bioMérieux, France) and immediately transferred to the microbiological research laboratory for bacteriological analysis [5, 14].

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2.2 Bacterial isolation and identification

Aseptically, four hundred (400) fracture and post-surgical wound swab samples were suspended separately in a sterilized Brain-heart infusion broth (bioMérieux, France), and incubated at 37^{o}C for 24 hrs. The suspended swab from the turbid bacterial growth was seeded onto Brilliance MRSA II Agar (bioMérieux, France) and incubated at 37^{o}C for 18-24 hrs. MRSA colonies were identified based on colony characteristics on Chromogenic agar Brilliance MRSA II Agar (bioMérieux, France) as blue colored, round, convex, opaque colonies were indicative of MRSA and colonies were aseptically purified by sub-culturing onto Brain-heart infusion agar (bioMérieux, France) and incubated at 37^{o}C for 24 hrs [1,14]. Methicillin resistance was further characterized by Oxacillin (5µg) and Cefoxitin (30µg) (Oxoid Uk) double disc diffusion test. Isolates showing inhibition zone ≤ 10 mm for oxacillin (5µg) and ≤ 21mm for cefoxitin (30 µg) were considered MRSA in conformity to the recommended standard of Clinical and Laboratory Standard Institute [15].

2.3 Molecular Analysis

2.3.1 DNA extraction.

A typical isolate of MRSA was cultivated in double strain 5 ml Brain-Heart Infusion broth (bioMérieux, France) for 24 h at 37˚C. The bacterial genomic DNA of MRSA strains was extracted with a ZR Fungal/Bacterial DNA MiniPrep™ (USA) as described by Edemekong *et al.* [16]. Exactly 2 mls of bacterial cells broth was added to a ZR Bashing TM Lysis tube and 750 ul lysis solution was added to it. This was secured in a bead fitted with 2 ml tube holder assembly and processed at a maximum speed for 5 minutes. The ZR Bashing Bead Tm lysis tube was centrifuged at 10,000 x g for 1 minute. Up to 400 ul of supernatant was transferred to a Zygomo-Spin TM IV Filter (orange top) in a collection tube and centrifuged at 7,000 x g for I minute. About 1,200 ul of fungal/bacterial DNA Binding Buffer was added to the filtrate in the collection tube from step 4. About 800 ul of the mixture from step 5 was transferred to a Zygomo-spinTM IIC Column in a collection Tube and centrifuged at 10,000 x g for 1 minute.

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The flow through from the collection tube was discarded and step 6 repeated. Then 200 ul pre-wash buffer was added to the Zymo-Spin TM IIC Column in a new tube collection and centrifuged at 10,000 x g for 1 minute. About 500 ul of Fungal/Bacterial DNA wash Buffer was added to the Zymo-Spin TM IIC Column and centrifuged at 10,000 x g for 1 minute. The Zymo-Spin TM IIC Column was transferred to a clean 1.5 ml micro-centrifuge tube and 100 ul (35 ul minimum) DNA was added directly to the column matrix. Centrifuge was done at 10,000 x g for 30 seconds to elute DNA; subsequently, 300 µL was transferred to a new microtube and stored at -20 ^{O}C until use [16].

2.3.2 Detection of Penicillin binding protein 2a (*mecA*) and Bone sialoprotein-binding protein (*bbp*) Genes

Specific primers (NEB™ , England) for *mecA* and *bbp* were selected. The PCR reaction included 12.5 ul of Taq 2x Master Mix from New England Biolbs (M0270); 1ul each of 10 ul M forward and reverse primer (*Mec A*- F: AAA ATC GAT GGT AAA GGT TGG C: *Mec A*-R: AGT TCT GCA GTA CCG GAT TTG C; *bbp*- F: CAAAAGAAAAGCCAATGGCAAACG; *bbp*-R: CCGTTGGCGTGTAACCTGCTG), 2 ul of DNA template and then made up with 8.5 ul Nuclease free water. All the PCR reagents were provided by NZY tech, Lisbon, Portugal. The amplification was developed in a Mastercycler®Pro (Eppendorf) thermal cycler. PCR products (10 µL) were detected by electrophoresis on 1.3% (w/v) agarose gel in 1xTAE (Tris 40 mM, acetic acid 20 mM, EDTA 1 mM) buffer at 90 V for about 60 min and visualized by UV trans-illumination. A 50bp DNA ladder (NEB™ , England 50bp DNA Ladder, U. SA) was used as molecular weight marker [17, 18].

2.4 Statistical analysis

Differences in the distributions of the studied determinants were tested by Chi square (SPSS software, version 2.1, IBM, NC, USA). A P value of 0.05 was considered to indicate statistical significance [1].

3. RESULT AND DISCUSSION

Distribution of Methicillin Resistant *Staphylococcus aureus* isolates from hospitalized fracture and post-surgical wound patients is presented in Figure 1. MRSA accounted overall detection rate of 164(41.0%) comprising of increase prevalence rate of 86(21.5%) in post-surgical wound sample followed by fracture wound sample 78(19.5%) while MSSA strain accounted 139(34.8 %) comprising of 63(15.8%) and 76(19.0 %) against post-surgical and fracture wound samples respectively. However, the isolation rate of MRSA was significantly associated with the sample source *p* value <0.05. Occurrence of *mecA* and *bbp* gene among clinical isolates of MRSA is shown in Figure 2. Of the ten (10) selected Methicillin Resistant *Staphylococcus aureus, mecA* gene was detected 100 % comprising of postsurgical wound patient (MR1-5) 50.0 % and fracture wound patient (MR6-10) 50.0%. *mecA* gene were detected in isolate MR1, MR2, MR3, MR4, MR5, MR6, MR7, MR8, MR9 and MR10 at 766bp ladder (Figure 2). Genes encoding Microbial Surface Components Recognizing Adhesive Matrix Molecules; *bbp* gene were detected in 100% of MRSA strain. Both postsurgical wound patient (MR1-5) and from fracture wound patient (MR6-10) accounted 50.0% respectively. The molecular size marker of 50bp from NEB^{TM} was used for amplification of *bbp* gene detected in all isolate; MR1, MR2, MR3, MR4, MR5, MR6, MR7, MR8, MR9 and MR10 at 650bp ladder (Figure 3).

Figure 1: Distribution of Methicillin Resistant *Staphylococcus aureus* isolates from Hospitalized Fracture and post-surgical wound Patients

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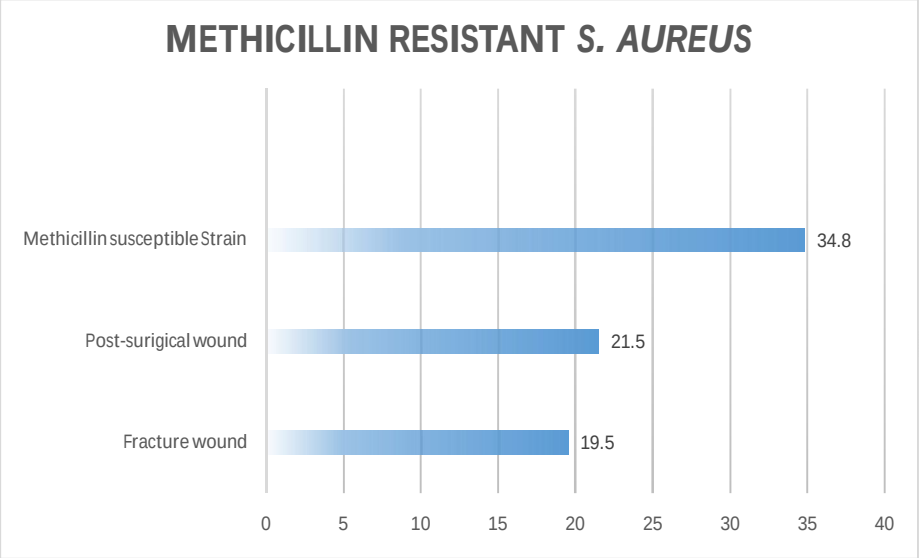


Figure 2: Occurrence of *mecA* and *bbp* gene among Clinical Isolates of Methicillin Resistant *Staphylococcus aureus*

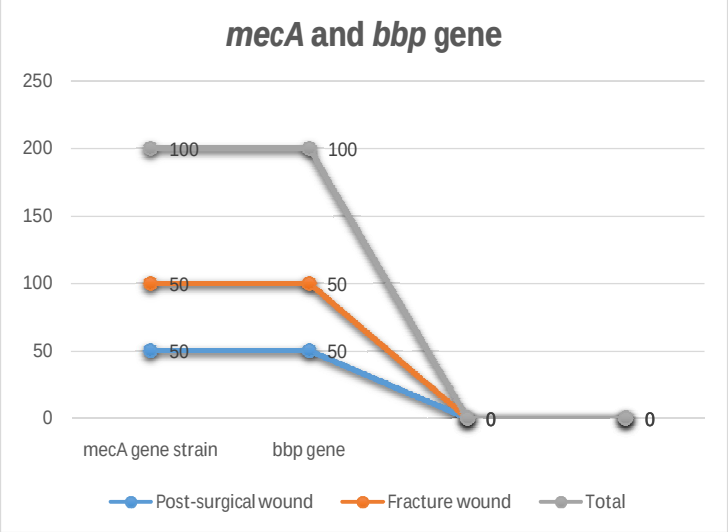


Figure 3: Amplification of MRSA *mecA* gene

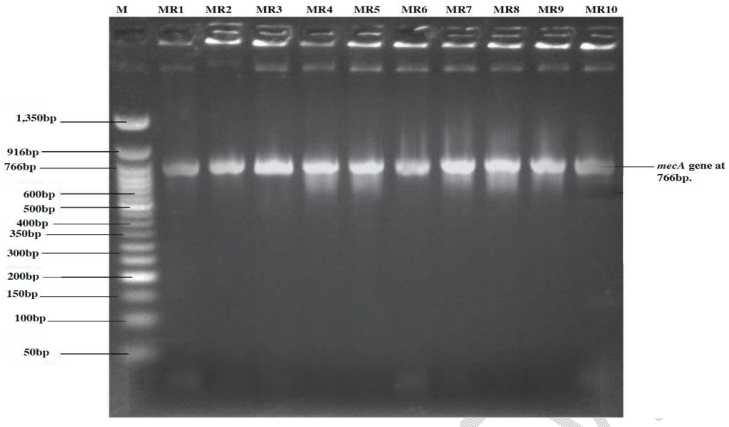
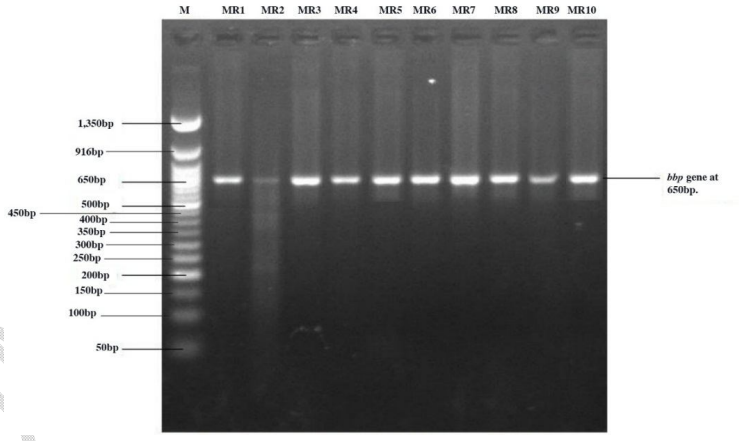


Figure 4: Amplification of *bbp* associated gene



4. DISCUSSION

Phenotypic MRSA strains selected for PCR analysis harbored *mecA* in this study other researcher has reported absence of *mecA* gene in isolates; Gaire *et al*. [19] obtained similar results, with 53.3% of MRSA isolates containing mecA gene while only 33.3% of the MRSA isolates from orthopedic patients genetically harbor the mecA gene, according to Oche *et al.* [20]. Similar findings reported *mecA* gene in *S. aureus* isolates 36.5 % in Pakistan and 29.1 % in Kathmandu, Nepal [21, 22] while in contract, another study found no *mecA* gene in phenotypically identified MRSA isolates; in Nigeria, the absence of the *mecA* gene was discovered in 36 *S. aureus* isolates that were

phenotypically MRSA positive but hyper-producers of the β-lactamase enzyme [23]. In Sudan, earlier study also reported 61.5 % *Staphylococcus aureus* isolates classified as MRSA phenotypically, but did not detect presence of *mecA* gene in their genomic DNA [24]. The *mecC* gene, which also confers methicillin resistance, may be responsible for the absence of the *mecA* gene in isolates phenotypically identified as MRSA. Hyper-production of β-lactamase by these isolates is another crucial factor that may be considered for the methicillin resistance. MRSA, which causes septic arthritis and osteomyelitis, is the most common bacterium in human skeletal infections due to expression of virulence determinant. MRSA strains that carry the housekeeper gene *mecA* are frequently MDR, with truncated antibiotic efficacy, towards vancomycin (drug of last resort) and others agent [1]. Although, the use of conventional antibiotics is still recommended to stall their invasion of wound, failure of most antibiotic agent could be the mainstay of prolong hospitalization among this patients due to expression of *mecA*.

Other studies have reported high and low prevalence of the *bbp* gene in biofilm forming MRSA isolates [17, 25, 26, 27] similar to this current finding. It's important to note that the *bbp* gene has been linked to human osteomyelitis and arthritis and has a strong affinity for certain extracellular adherences as well as the ability to modulate inflammatory response [28]. One of the adhesin genes that is strongly linked to human hematogenous tissue infections is the bone sialoprotein binding protein (*bbp*) [29]. The pathogenesis of *bbp*-dependent strain involve the interaction with bone sialoprotein (BSP), an essential component of bone and dentine extracellular matrix [30]. It's possible that *bbp* gene may act in two ways: as an important factor in bone tissue colonization and as a contributing factor in MRSA-induced hematologic diseases [31]. Earlier *in vivo* animal model showed that BSP binding capacity was found in all *Staphylococci* producing septic arthritis [32]. Moreover, the serum from patients suffering from bone and joint infection contained antibodies that reacted with the fusion protein of the BSP-binding protein, indicating that the protein is expressed during an infection and is immunogenic [6,7]. Because of its invasive nature, clinical samples used in this investigation were from orthopedic patients, which may have contributed to the 100% detection rate of *bbp*. Although the absence of data on the immune status of the patient may not precludes any firm conclusions being drawn that an inadequate local immune response brought on by diabetes and other underlying diseases may be the cause of increase expression of *bbp* in MRSA wound tissue infections in orthopedic patients.

5 CONCLUSION

This findings shows that *MecA* and *Bbp* expression is associated with orthopedic infection. We draw the conclusion from this study that MecA and *Bbp* detection can be used as a marker of MRSA bone invasion, particularly in orthopedic wound infections. To reduce the risk of infection and the clonal proliferation of these virulent strains, a thorough and ongoing surveillance system that offers epidemiological and genetic information of other Genes encoding Microbial Surface Components Recognizing Adhesive Matrix Molecules that aid in microbial invasion of host tissue through next generation molecular technique is crucial to facilitate diagnosis of wound patient. In the presence of proper wound care, pathogenic bacteria colonization and proliferation at the sites of orthopedic wounds could be stall.

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5.1. LIMITATIONS

. The current study has some limitations that should be recognized, which it was conducted in one hospital in the state, which could not reflect the epidemiology of this gene in different hospital in the states or different geographical areas.

ETHICAL APPROVAL

In compliance with international standard or university standard written ethical approval has been collected and preserved by the author(s).

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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