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Full Length Research Paper

Repeats in the 3' region of the protein A gene is unique in a strain of *Staphylococcus aureus* recovered from wound infections in Lagos, Nigeria

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Bacterial strain typing has quite a lot of important applications in microbiology. In medical practice, strain typing is useful for diagnosis and determining strategies for treatment of infections. More essentially, it is considered for rapid identification of disease outbreaks and new virulent strains. In this study, Protein A gene (*spa*) short sequence repeats region sequencing was used to measure relatedness among a collection *Staphylococcus aureus* were recovered from patients presenting with different degree of wound infections at three major hospitals in Lagos, Nigeria. The strains were also compared with representative sputum and blood isolates. From the pulsed field gel electrophoresis (PFGE) fingerprinting generated, the seventeen *S. aureus* isolates was defined as belonging to diverse genotypes with isolates recovered from different individuals generally having distinctive profile. *Spa* typing distinguished five major "clones" within the three hospitals and classified the some wound strains and the sputum representative as members of the same clone (*spa 7*). The predominant occurrence of *spa* 454 suggested the propensity of the clone to be involved in outbreaks. A combination of codon giving a unique cassette was however identified in the whole set and was postulated that this might be restricted to the indigenous strains in Nigeria.

Key words: *Spa* typing, *Staphylococcus aureus*, wound isolates, Protein A gene.

INTRODUCTION

Staphylococcus aureus is a pathogen responsible for a wide variety of diseases in humans and animals, including endocarditis, osteomyelitis and wound infections. The pathogenicity of the organism is related to various extracellular protein toxins and virulence factors such as capsular polysaccharide and protein A. Staphylococcal protein A is a membrane-bound exoprotein produced by over 95% of *S. aureus* strains (Wann et al., 1999). It has been extensively characterized and well known for its ability to bind to the Fc region of immunoglobulins of most mammalian species (Alonso and Dagget, 2000). Apart from the immunobiological properties of this protein, investigations involving the basic steps in epidemic spread of strains with elevated adherence capacity have also emphasized the importance of protein A gene.

Staphylococcal protein A gene is about 2150 base pair (Frenay et al., 1994) with an X region giving approximately 300-base pair repetitive region (X_r) followed by an invariable region of 81 amino acids (X_c) (Uhlen et al., 1984). Within the last 5 years, sequencing of the X-region has become a powerful tool for epidemiological typing especially in outbreak investigation in hospitals or local settings. Although, Pulsed Field Gel Electrophoresis (PFGE) using restriction endonuclease *sma*I is often recognized as the "gold standard", it has been concluded that the technique is limited in generating reproducible and comparable images between laboratories (Mathema and Kreiswirth, 2004). Furthermore, clinical epidemiological information is needed to interpret PFGE results, and it may be required to compare strains under investigation to epidemic strains. *spa* typing is however considered less expensive, generates an unambiguous, portable dataset that simplifies information sharing between laboratories

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and makes possible the establishment of a large-scale database for studying epidemiology at both local and global levels (Shopsin et al., 1999, Shopsin and Kreiswirth, 2001). More significantly, Koreen et al. (2004) suggested that the *spa* locus could single-handedly and precisely identify strains throughout the span of the species and then correctly assign them to lineages.

Accordingly, *spa* sequencing data has been employed to define *S. aureus* clonal types and showed the genetic relatedness between MRSA and its methicillin susceptible background (Crisostomo et al., 2001). The technique has also been used to track staphylococcal outbreaks occurring in a definite time and/or location. Harmsen et al. (2003) analysed a collection of one hundred and ninety-one strains of methicillin resistant *S. aureus* at a German University Hospital over two study periods. The results revealed equally distributed ten unique *spa* types in both study periods and showed a rapid dynamics of clone circulation in the University Hospital.

So far, more than 600 numeric *spa* types have been identified and catalogued in the relational database at the Public Health Research Institute (PHRI) Tuberculosis Center in Newark, New Jersey, USA (Said-Salim et al., 2005). The number and organization of the repeats or repeat profile delineate the *S. aureus spa* type (Koreen et al., 2004). Although much of the work aimed at defining the *spa* types of *S. aureus* has been done with strains from diverse clinical sources, no studies have however demonstrated that certain *spa* variants are of particular significance to a restricted collection of isolates Towards this end, we analyze a collection of *S. aureus* from infected wounds to appraise the ability of *spa* typing to discriminate among strains of comparable origin. In Nigeria, most staphylococcal infections are associated with methicillin susceptible strains and our experience regarding the genetic analysis of indigenous strains is limited (Adesida et al., 2005). Besides, there is no indication of the use of spa typing. This is therefore the first documented report on the analysis of the polymorphic X-region of the proteinA gene of S.aureus from Nigeria.

MATERIALS AND METHODS

Bacterial isolates

Fifteen out of 54 well characterized *S. aureus* isolates obtained from wound infections were randomly selected for this study without predilection. The isolates were gotten from patients presenting with osteomyelitis, gunshot wounds, and burns of various degree at three hospitals in Lagos, Nigeria. Only one isolate per patient was included. In addition, two isolates from a sterile site (blood) and non-sterile site (sputum) were included. All isolates were confirmed to be *S. aureus* by established methods, including colony morphology, Gram stain characteristics, the presence of catalase activity, and the ability to coagulate rabbit serum (Cowan and Steel, 1993). All isolates were tested for methicillin resistance using oxacillin screen agar test as recommended by NCCLS (2000). Isolates were stored at –80°C and were routinely cultured on solid medium prior to incubation overnight at 37°C.

PFGE DNA fingerprinting

The procedure for genomic DNA fingerprinting by PFGE was performed as previously described (De Lencastre et al., 2000). The fragments were resolved on a 1% gel on a CHEF Mapper (Bio-Rad, Richmond, Calif.) apparatus with the following conditions: 6 V/cm for 22 h at 14°C with linear pulsed times of 5 to 35 s. The gels were stained with ethidium bromide, rinsed, and photographed under UV light. PFGE patterns were interpreted according to the criteria of Tenover et al. (1995).

spa typing

The procedure was performed essentially as previously described (Shopsin et al., 1999). The forward primer SpaF 832 (22): 5' – GAC GAT CCT TCA GTG AGC AAAG – 3' and the reverse primer SpaR 1232 (22): 5' – GCA GCA ATT TTG TCA GCA GTAG – 3' were commercially obtained. DNA amplification was performed in a GeneAmp PCR system 9600 (Perkin-Elmer Biosystem). The complementary sequences for the reverse sequences generated were retrieved with All-IN-ONE SEQ-ANALYZER Version 1.35 for Netscape Communicator (http://www.personal.umich.edu/~ino/blast.htm). The database maintained at PHRI was used to assign *spa* type and types with similar repeat profile are grouped together as part of the same numeric lineage.

RESULT

All the seventeen srtains were sensitive to oxacillin and displayed different PFGE fragment pattern. The *spa* typing analysis revealed a total of nine *spa* types (Table 1). Strains from wound exhibited variants that were found in those from blood and sputum. Six main clonal types spreading within the institutions were recognized. Four clones shared related spa motifs and were found among the strains belonging to spa types: 457(ZEDMGMM), 454(XMJHM), 7(YHGCMBQBLO), 449(UJGFGDMGG) (Figure 1). The predominant type was *spa* type 454 (X1-M1-J1-H2-M1) found in two hospitals. It was followed by repeat motif Y1-H1-G1-C1-M1-B1-Q1-B1-L1-O1 of *spa* 7 that was also found among isolates from two different institutions. Of the four genetically possibly related isolates, the common *spa* type, type 454 had 5 repeats and a clonal type showed 9 repeats. For strains in *spa* type 7, 10 repeats were found but of the shortest repeats, four was exhibited by strains with spa type 455. Other variants observed in sporadic cases consisted of eight, nine or ten repeats (Table 1).

The analysis also allowed the identification of a type containing new cassette of ten atypical repeat units (U1NEWFMBBKBPE) of 24 bp (Figure 2) that is unique within the data set at PHRI. There was however no relationship between the observed variation of the *spa* region and the whole genome as determined by PFGE

Table 1. *spa* repeat patterns of the seventeen strains.

BK #	Source		*spa* Type	*spa* Motif
12719	wound	Orthopedic	411	I2-Z2-E1-G1-M1-J1-H2-M1
12565	blood	Central Laboratory	449	U1-J1-G1-F1-G1-D1-M1-G1-G1
12672	wound	Orthopedic	449	U1-J1-G1-F1-G1-D1-M1-G1-G1
12590	wound	Orthopedic	207	U1-J2-G1-M1-K1-K1-P1-N1-S1-G1
12712	wound	General-Ikej	NEW	U1-NEW-F1-M1-B1-B1-K1-B1-P1-E1
12591	wound	Orthopedic	454	X1-M1-J1-H2-M1
12597	wound	Orthopedic	454	X1-M1-J1-H2-M1
12667	wound	Orthopedic	454	X1-M1-J1-H2-M1
12717	wound	Orthopedic	454	X1-M1-J1-H2-M1
12766	wound	Orthopedic	454	X1-M1-J1-H2-M1
12592	wound	Orthopedic	455	X1-M1-J1-Q1
12718	wound	Orthopedic	456	Y1-C2-M1-B1-Q1-B1-L1-O1
12631	wound	Orthopedic	7	Y1-H1-G1-C1-M1-B1-Q1-B1-L1-O1
12845	wound	Orthopedic	562	Y1-H1-G1-M1-B1-Q1-B1-L1-O1
12593	wound	Orthopedic	457	Z1-E1-D1-M1-G1-M1-M1
12595	wound	Orthopedic	457	Z1-E1-D1-M1-G1-M1-M1
12618	sputum	LaMainland	7	Y1-H1-G1-C1-M1-B1-Q1-B1-L1-O1

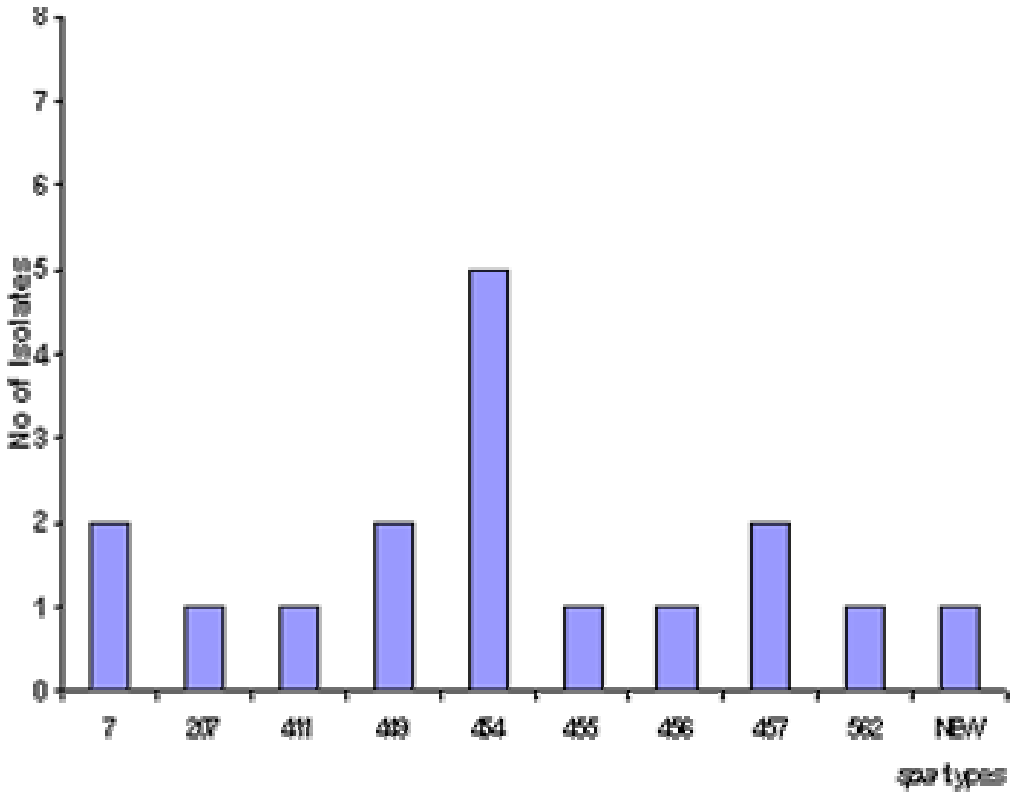

Figure 1. Distribution of the *spa* types among the seventeen isolates.

Strain 12712
Spa type: New Cassette

Motif: U1-NEW-F1-M1-B1-B1-K1-B1-P1-E1

AAATGACGCAGAANGGCGTAAAAAGCTANACACGATGCTCAAGCTCCAAAA

1	2	3	4	5	6	7	8	*spa* repeat code
GAG	GAA	GAC	AAC	AAC	AAA	CCT	GGT	U1
AAA	GAA	GAC	AGC	AAC	AAA	CCT	GGC	NEW
AAA	GAA	GAC	AAC	AAC	AAG	CCT	GGC	F1
AAA	GAA	GAC	GGC	AAC	AAG	CCT	GGT	M1
AAA	GAA	GAC	AAC	AAA	AAA	CCT	GGT	B1
AAA	GAA	GAC	AAC	AAA	AAA	CCT	GGT	B1
AAA	GAA	GAC	GGC	AAC	AAA	CCT	GGT	K1
AAA	GAA	GAC	AAC	AAA	AAA	CCT	GGT	B1
AAA	GAA	GAT	GGC	AAC	AAG	CCT	GGC	P1
AAA	GAA	GAC	AAC	AAC	AAA	CCT	GGT	E1

AAAGAAGACGGCAACGGAGTACATGTCGTTAAACCTGGTGATACAGTAAATGACAT
TGCAAAAGCAAACGGCACTATCTGCTGACAAAATTGCTAGAA

Figure 2. Repetitive unit of region X of the UNIQUE type.

fragment pattern differences. All isolates displayed different PFGE fragment pattern.

DISCUSSION

The clonal relatedness of the set of isolates used in this study was determined on the basis of the 24-bp repeat unit in the 3' region of protein A (*spa*). Most of the identified spa types are already members of the spa database maintained at PHRI, USA, which contained collections of *S. aureus* all over the world. Nine definite spa types were achieved for the seventeen strains and it appears that the Nigerian *S. aureus* population encloses a considerable extent of spa variation that exists worldwide

It was of interest that *spa* 454 strains circulated exclusively in one of the hospitals. Although the patients were not documented to be in close, it could be that this *spa*type has been adapted to the hospital. *Spa* type 7 is well established among archaic MRSA strains implicated in community-acquired infections in the US (Said-Salim et al., 2003). The present communication presents this *spa* type circulating in two centers in which one of the centers is well known for community health services. Although, this *spa* type was exhibited by methicillin susceptible strains, this finding possibly offers another line of evidence that community-acquired MRSA strains are closely related directly or indirectly to the healthcare system (Aires de Sousa and DeLencastre, 2003). Instances have been reported of isolates with the same *spa* type belonging to unrelated *spa* types. Kahl et al. (2005) showed that two isolates of five patients with different pulsotypes showed the same *spa* types. This finding corresponds to the PFGE analysis performed in this study. While it was noted that this new spa type was restricted to single hospital, it could be suggested that the production of exclusive extracellular protein A or a genetic substitution event in the X region of the protein A gene might have occurred.

Changes in the composition of the repeats are known to be consistent with deletion or duplication of repeats and point mutations (Kahl et al., 2005). In this study, a strain with a particular "novel" cassette was also documented. Although, it was noted that this new *spa* type was restricted to single hospital, it would be suggested that the production of exclusively extracellular protein A or a genetic substitution event in the X region of the protein A gene, which encodes the cell wall binding domain could have occurred. As previously hypothesized (Feil et al., 2003), it also became apparent that *S. aureus* are clonal in nature. For instance, the difference in the *spa* type 7 and a clone circulating Orthopedic is the result of a single genetic event. An entire codon in *spa* 7 has been deleted in *spa* 562. Possibly the epidemic ability of these strains arose from long-standing occurrence and adaptive activities. Mathema and Kreiswirth (2004) proposed a similar

hypothesis.

Various studies have proposed that the *spa* genotypes are good markers for short-term epidemiological studies, being able to distinguish between epidemic and sporadic strains (Montesinos et al., 2002). In study, we could not assess the epidemiological relationship between the related strains because most of the patients did not supply their addresses; nevertheless, it was observed that *spa* was able to predict the potential epidemic behavior of some of the isolates examined. There were three episodes of *spa* motifs belonging to strains from different hospitals. Interestingly, the limited available epidemiologic information revealed that the strains were isolated months apart. Although, inter-institutional transfer or a common patient source could not be demonstrated for the strains, it might be speculated that the *spa* variation among some isolates in our locality is limited. Accordingly, some strains in our environment could be highly endemic and subsequently represented in our hospitals.

There has also been controversy over the relationship between the number of repeats and strain epidemicity (Schmitz et al., 1998). Frenay et al. (1994) tried to discriminate between epidemic and sporadic strains and reported that strains with more than seven repeats in the X region of the protein A tend to be epidemic, while the presence of seven or fewer repeats was indicative of nonepidemic strains. A longer X region would allow a more favorable exposure of the Fc binding regions at the cell surface, facilitating colonization and infection of the skin or other not-yet identified sites that may be important for epidemic spread (Frenay et al., 1994; Hoefnagels-Schuermans et al., 1997). In our study, not all the potentially epidemic strains showed more than seven repeats. Some of the genetically related strains had as short as four repeats while nine or ten repeats were detected in some single strains. These observations are in concordance with the report of Shopsin et al. (1999), who also proposed that the length of the variable region might not be an accurate indicator of strain type as many of their isolates had the same number of repeats. It was suggested that multiple loci of the chromosome might contribute to the epidemic charac-ter of a strain. The number of 24-bp repeats in the X region of the protein A gene might therefore be only one of these markers (Hoefnagels-Schuermans et al., 1997).

In conclusion, the application of *spa* typing to our local strains provided practical information on their clonal relationship and also strengthens the representation of the population of *S. aureus* circulating in Nigeria. Obviously the genetic structure of a given *S. aureus* population is a very dynamic subject and ours is particularly interesting in that, clones found in the hospitals are also identified in the community. Nevertheless, comprehensive epidemiologic data and use of other genetic marker in combination with this technique is however desirable for a meaningful approach to investigating outbreak situations.

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