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Molecular Characteristics of *Staphylococcus aureus* from Military Hospital in Abidjan, Côte d'Ivoire

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ABSTRACT

A broad variety of infections, ranging from minor infections of the skin to post-operative wound infections can be caused by Staphylococcus aureus (S. aureus). The adaptive power of S. aureus to antibiotics leaded to the emergence of methicillin-resistant S. aureus (MRSA). MRSA strains is characterized by its potential to express many virulence factors, frequently the Panton-Valentine Leukocidin (PVL). Thus MRSA is one of the important pathogens implicated in hospital acquired infection. The main objectives of this study was to find the prevalence of MRSA, the frequency of the PVL and nasal carriage rate in healthy hospital staff. A total of 47 S.aureus strains were isolated, 31 from clinical specimens and 16 from anterior nares swabs from healthy hospital staff. All isolates were tested for the genes of MecA (mecA) and PVL (lukS)using a multiplex PCR (nested PCR). The genes mecA and luks were confirmed respectively in six (19.3%) and twenty- four (77.4%) of clinical isolates. Of the 150 nasal swabs sticks from 30 health-care workers were collected and 16 cultures were positive for S. aureus. The gene mecA was detected in two isolates giving a MRSA nasal carriage level of 1.3%. The luks gene in nasal strain occurred in one case.
The results obtained emphasize the continuous monitoring of meticillin susceptibility pattern of S.aureus isolates and the need for high standards of infection control in primary care to prevent MRSA transmission in either direction between healthy hospital staff and patients.
Key-words *Staphylococcus aureus, méticilline, leucotoxine of Panton-Valentine, pus.*

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a leading cause of diseases such as skin and soft tissue infections, pneumonia, bloodstream infections, osteomyelitis and endocarditis, as well as toxin-mediated syndromes like toxic shock and food poisoning [1].

This organism is one of the important pathogens in hospital acquired infection. It has developed resistance to a wide range of antimicrobial drugs, which complicates the treatment of infections. In particular, methicillin-resistant *S. aureus* (MRSA) has become a notorious etiologic agent for a wide variety of infections and it is one of the most important nosocomial pathogens worldwide [2]. Methicillin-susceptible *S. aureus* (MSSA) become MRSA through the acquisition and insertion into their genomes of a large DNA fragment known as staphylococcal chromosome cassette *mec* (SCC*mec*), which contains the methicillin resistance determinant, *mecA* [3].

S. aureus is characterized by its potential to express many virulence factors. The Panton-Valentine leucotoxine PVL is a major virulence factor associated with necrotic lesions of the skin and subcutaneous tissues (e.g., furuncles) and also with community-acquired severe necrotic pneumonia [4]. There exists a relationship between antibiotic resistance genes and some virulence genes in the emergence of strains of MRSA from hospital or even community acquired infection. Frequently, MRSA strains carry the gene for Panton- Valentine Leukocidin (PVL) [5].

Some risk factors are implicated in the transmission of MRSA in hospitals such as the antibiotics selection pressure, use of invasive procedures and ecological niches. In humans, colonization of *S.aureus* is found in the anterior nares. Nasal carriage of these organisms in hospital staff provides a

source for infection in hospitalized patients and elimination of nasal carriage has been reported to cause reduction in the incidence of *S. aureus* infections [6].

The antimicrobial resistance profile of nasal *S. aureus* is of great public health concern especially in developing countries where health facilities are inadequate. The aim of this study was to determine the prevalence of *S. aureus*, to estimate the frequency of methicillin resistance gene (*mecA*), the prevalence of the virulence factor gene (*lukS*) and to assess the carriage rate of *S.aureus* in healthy hospital staff

MATERIALS AND METHODS

Population Study

The study was conducted in the Military Hospital which is a National Reference Hospital for Abidjan from June to December 2011. This study was conducted at the Department of Bacteriology-Virology, Pharmacy, University of Abidjan. The *S. aureus* strains were isolated from clinical samples (pus of suppurative disease) of patients hospitalized. The nasal isolates of *S. aureus* were collected from nasal labeled sterile swabs sticks of asymptomatic health care personnel.

Bacterial strains

The specimens collected were taken to the laboratory within 2hrs of collection for inoculation on Chapman media. From colony mannitol+, species identification of *S. aureus* was carried out by standard microbiology methods. The phenotypic identification focused on cooci Gram+, catalase+, desoxyribonuclease (DNase)+ and cogulase+, the morphology of bacteria to the Gram stain, the presence of catalase, and an agglutination to Slidex Staph Plus test (BioMerieux ®) which simultaneously detects the clumping factor, protein A and capsular antigens of *S. aureus*.

Antibiotic sensitivity test

Susceptibility to methicillin was determined by using Kirby-Bauer disk diffusion method according to the Comité d'Antibiogramme de la Société Française de Microbiologie guidelines [7]. The antibiotic tested was cefoxitin (30 µg). Quality control was performed with the reference strain of *S. aureus* ATCC 29213.

Detection of mecA and the PVL genes

To prepare DNA extracts, the strains of *S. aureus* were cultured on blood agar (5% from sheep blood) up to for 24 hours. Bacterial DNA was extracted by thermal shock. Briefly, a loop of typical colonies was removed and resuspended in 400 µl of sterile distilled water. The suspension was frozen at -20 °C for 20 minutes and then the frozen suspension was incubated for 20 minutes. in a heating block preheated to 100 ° C after centrifugation at 13,000 RPM for 10 minutes, the supernatant containing bacterial DNA was recovered and stored at -20 ° C for gene amplification.

To amplify genes, a multiplex PCR (nested PCR) was performed. The following genes: 16S ran specific to the genus *Staphylococcus*, *femA* to *S. aureus* specie, *mecA* gene encoding PBP2a and *lukS* encoding production of Panton Valentine leukocidin were detected. Sequences of the primers used as shown in Table 1 have been published by Al-Talib et al [8]. The PCR (nested PCR) was performed as follows: a reaction carried out in a final volume of 50 µl containing: 10 mM Tris-HCl (pH 8.9), 50 mM of KCl, 3 mM of MgCl 2, 200 µM of each desoxynucleotide triphosphate (dNTPs), 0.6 pmol of primer 16S rRNA, 0.8 pmol of primer femA, 1 pmol of primer mecA , 0.6 pmol of primer lukS, 1U of Taq DNA polymerase (Promega, Madison, USA) and 5 µl of DNA extract. Amplification was carried out in a thermocycler GeneAmp Perkin Elmer 9700 (Applied Biosystems, Courtaboeuf, France). Initial denaturation of DNA at 94°C for 5 minutes was followed by another 30 cycles at 94°C about 30 seconds, hybridization pause for one minute at 60°C and elongation for one minute at 72°C. The amplification is completed by a final elongation at 5 minutes at 72°C.The size of PCR products (amplicons) was verified by agarose gel electrophoresis (1.5% w/v) containing ethidium bromide (EtBr) of 0.5 µg / ml and revealed on an ultraviolet table (UV).

RESULTS AND DISCUSSION

Of the 305 clinical samples tested, 147 were positive in which 31 cultures were positive for S. aureus (21%).The isolates were obtained from hospitalized patients, including 28 from surgical services and 3 from medical service.

All isolates were positive for 16S rRNA and *femA* genes. The MRSA were confirmed by detection of *mecA* gene in six (19.3%) clinical strains. The first isolation of methicillin-resistant *S. aureus* (MRSA) was reported in 1960 and since then the prevalence of MRSA has increased in all scrutinized regions, with different figures even within the same country. A multicenter study (SENTRY) conducted in 15 European countries reported a prevalence of 25% [9]. In USA there was progressive development of resistance to methicillin from 5% (1981) to 52 % (2005). In the same study, high regional variation was found from 12.5 % to 100% [10]. In Africa, a multicenter study has been detected 15% of MRSA in hospitals infection [11]. The results obtained were higher to those described previously by Ivoirian authors [12]. In contrary the MRSA rate obtained was lower than those published by Akoua-Koffi et al. [13] in Abidjan (25%).

Our study emphasizes the need for continuous monitoring of antimicrobial resistance development in *S.aureus* isolates including MRSA.

Twenty- four of clinical isolates (77.4%) expressed the leukotoxin gene l*ukS.* In 2 cases (6.4%), the *mecA* and *lukS* genes were associated. Generally, the potent PVL toxin is epidemiologically associated with furunculosis, abscesses, and skin lesions but is absent from isolates causing impetigo, blisters, or staphylococcal scalded skin syndrome [4, 14]The *lukS* gene was detected from 30 to 97% among isolates causing SSTIs. A multicenter study conducted in four African countries showed that the rate of detection of the PVL gene was 57%, especially among MSSA strains [15]. Sila et al [16] reported more prevalence of the PVL gene in MSSA strains (3%) compared to MRSA (0%). In China, MSSA isolates were more likely to carry PVL gene (25.5%) than MRSA strains (21.7%)[17]. The study on virulence factors produced by strains of *S. aureuss* isolated from urinary tract infections initiated in Benin reported that 21.5% isolates produced PVL. Six of 14 (43%) PVL-positive isolates were methicillin-resistant [18]. In China, the rate of PVL gene was higher in community-acquired infections (27.1%) than in hospital infections (20.6%) [17]. The data of Antri et al [5] showed that 36% of MRSA carried PVL gene. The results obtained showed that the gene *lukS* encoding for the production of the Panton Valentine leukocidin (PVL) was detected in 19 cases (67.7%) of isolated analyzed. This gene was associated with the gene MecA in 6.4% of cases In striking contrast, the prevalence of *luk-PV* among colonizing *S. aureus* strains was below 1%, which, again, is in agreement with the findings of previous studies [19]. The results obtained emphasized that not all pus associated strains harbored the PVL gene, implying that additional factors, either host or pathogen derived, affect the development staphylococcal suppurative disease.

Indiscriminate use of antibiotics and prolonged hospital stay are contributing factors in the emergence of multidrug resistant strains. Transmission of isolates of epidemic meticillin-resistant *S. aureus* (MRSA) has traditionally been associated with hospital facilities. Some of the studies indicate that the epidemiology of MRSA is changing and hospitalization is no longer necessarily a risk factor [20]. The role of health-care workers in sporadic, epidemic, and endemic MRSA transmission has been extensively described. Health-care workers are at the interface between hospitals, long-term care facilities, and nursing homes on the one hand and the community on the other, they may serve as reservoirs and vectors of MRSA cross-transmission Thus MRSA infections are treatable but there is a need to prevent the spread of MRSA in community and hospital settings. The best way to prevent the spread of *S.aureus* and MRSA in hospital settings is to screen health care takers for the presence of these organisms. The present study made an attempt to screen hospital staff for the *S. aureus* organisms. Of the 150 nasal swabs sticks from 30 health-care workers were collected and 16 cultures were positive for *S. aureus* giving a nasal carriage level of 10.6 %.Two isolates were positive for *mecA* gene giving a MRSA nasal carriage level of 1.3%. Mean nasal MRSA carriage in health-care workers was 4·1% (range 0—59%; 95% CI 0·3—7·9%)[21]. The results obtained are contrary to other studies where high carriage rate of 19-38% for MRSA is reported [22] In Abidjan, the carriage rate of MRSA was 17,8% in the health care personnel [13].

This is the first report on MRSA to investigate MRSA isolates both in clinical isolates and nasal carried *S.aureus* by healthy hospital staff in Abidjan. Although This study recorded 1.3 % of MRSA among colonizing from 30 healthy hospital staff is very limited in numbers to draw a definite conclusion, our study also suggests this as far as MRSA is concerned.

Table 1: Sequences of primers used for multiplex PCR

Product genes	Primer name	5' – 3'	Size (pb)	Reference
16S rRNA	16S rRNA-F 16S rRNA-R	GCAAGCGTTATCCGGATTT CTTAATGATGGCAACTAAGC	597	Al-Talib, H. et al. [8]
*fem*A	*fem*A-F *fem*A-R	CGATCCATATTTACCATATCA ATCACGCTCTTCGTTTAGTT	450	
*mec*A	*mec*A-F *mec*A-R	ACGAGTAGATGCTCAATATAA CTTAGTTCTTTAGCGATTGC	293	
*Luk*S	*Luk*S-F *Luk*S-R	CAGGAGGTAATGGTTCATTT ATGTCCAGACATTTTACCTAA	151	

Table 3 Distribution of methicillin resistance and virulence genes detected in S*. aureus*

Genes	Clinical isolates (n=31)	nasal isolates (n=16)
mecA	6(19.3%)	2
lukS	24(31.1%)	1
mecA+lukS	2(6.4%)	0

Table 2 Frequency of *S. aureus* according to the origin of samples

Origin of samples	Positive Culture (n)	*S. aureus* positive culture (n)	Frequency %
clinical	147	31	21
health care personnel	150	16	10.6

CONCLUSION

The results obtained showed that methicillin resistant *S. aureus* strains were isolated concomitantly in clinical isolates and nasal swab of healthy hospital staff. These results emphasize the continuous monitoring of methicillin susceptibility pattern of *S.aureus* isolates and the need for high standards of infection control in primary care to prevent MRSA transmission in either direction between healthy hospital staff and patients in Abidjan.

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