International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 12 (7), pp. 001-005, July, 2018. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Community acquired skin infections in children in Abidjan: Methicillin resistant *Staphylococcus aureus* and exfoliative toxin production

Kacou-N'douba, A.[1,2,3]*, Kazali, A.[1], Koffi, K.S.[1,2,3], Ekaza, E.[1], Kouablan, A.[1], Kangah, T.[3], Okpo, S.[3], Elogne-Kouamé, C.[1] and Dosso, M.[1,2]

[1]Pasteur Institute of Cote d'Ivoire, 01 BP 490 Abidjan 01, Cote d'Ivoire.
[2]Department of Microbiology, University of Cocody, Unit of Formation and Research of Medical Sciences, Abidjan, Cote d'Ivoire.
[3]University Hospital Center of Cocody, Abidjan, Cote d'Ivoire.

Accepted 24 June, 2018

The worldwide spread of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) skin infections is becoming an emerging problem. These bacteria can produce virulence factors. The objectives of this study were to determine the rate of MRSA and frequency of *mecA* gene by PCR and detect genes *eta* and *etb* encoding exfoliatin A and B. This is a prospective study focused on school children of 5 to 15 years with a cutaneous infection from October 2007 to June 2008. The skin samples taken were allowed to isolate strains of *S. aureus* by standard bacteriological methods. Susceptibility testing was performed to detect strains of MRSA with cefoxitin disk. Detection of *mecA*, *eta* and *etb* genes was performed using PCR. The rate of MRSA was 14.8%. The *mecA* gene was present in 2 strains of *S. aureus* (3.7%). The genes encoding the exfoliatin were detected in 5.6%.

Key words: Community-acquired skin infection, MRSA, exfoliatin A and B.

INTRODUCTION

Staphylococcus aureus, though a commensal bacteria of the skin might be responsible for cutaneous infections. Strains of *S. aureus* resistant to methicillin (MRSA) are often responsible for nosocomial infections, but more recently their involvement has been reported in community-acquired infections (Lowy, 1998; Huletsky et al., 2004; Chambers, 2001). MRSA is no longer an exclusively nosocomial pathogen. Its acquisition in the community by children without risk factors has emerged in many countries in recent years (Hussain et al., 2000; Zetola et al., 2005). Their pathogenicity is linked to the expression of virulence factors (Rojo et al., 2010). Delaporte (2006) in a study on the emergence of multi-drug resistant bacteria reported that over 60% of staphylococcal skin infections are community caused by methicillin-resistant strains. The emergence of community-acquired MRSA is associated to the misuse and inappropriate use of antibiotics (Trystam et al., 2004). Rapid identification of MRSA and appropriate treatment is essential. Conventional methods are often limited by the heterogeneous expression of resistance (Kohner et al., 1999; Geha et al., 1994). Among the approaches to improve the detection of methicillin resistance, PCR is considered to be the gold standard method (Mohanasoundaram and Lalitha, 2008; Sakoulas et al., 2001). In Africa, there is a paucity of data on community-acquired MRSA skin infections. The aims of this study were to determine the rate of MRSA and frequency of *mecA* gene and detect genes *eta* and *etb* encoding exfoliatin A and B by PCR.

MATERIALS AND METHODS

The cross-sectional study was conducted from October 2007 to

*Corresponding author. E-mail: knadele@yahoo.fr. Tel: (225) 04 03 86 82.

Table 1. Sequences of primers used for multiplex PCR.

Product gene	Primer name	5' ---------- 3'	Size (pb)	Reference
Coa	COAG1	ATACTCAACCGACGA CAC CG	440 - 915	Ahrens and Andresens, 2004
	COAG4	GATTTTGGATGAAGC GGATT		
	COAG2	CGAGACCAAGATTCAACAAG		
	COAG3	AAAGAAAACCACTCACATCA		
mecA	*Mec*A1	GTAGAAATGACTGAACGTCCGATA	310	Pérez-Roth et al., 2001
	*Mec*A2	CCAATTCCACATTGTTTCGGTCTAA		
eta	ETA-3	CTAGTGCATTTGTTATTCAAGACG	119	Becker et al., 1998
	ETA-4	TGCATTGACACCATAGTACTTATTC		
etb	ETB-3	ACGGCTATATACATTCAATTCAATG	262	Becker et al., 1998
	ETB-4	AAAGTTATTCATTTAATGCACTGTCTC		

June 2008 in a school in the community of Abobo. The school had 1520 students. This study included children of 5 to 15 years attending such institution and who had a skin infection, suppurative skin infection regardless of sex. The type of skin infection have been described on the advice of a dermatologist and included impetigo (5), skin abscess with necrotic infection (147). For each child, a swab of purulent skin lesions was collected. Culture was performed on blood agar sheep (Columbia base agar containing 5% sheep blood) and mannitol salt agar (selective media for staphylococcus). This study was conducted at the Department of Bacteriology-Virology, Pasteur Institute of Côte d'Ivoire.

Identification of *S. aureus*

Species identification of staphylococci was carried out by standard microbiology methods. It focused on the morphology of bacteria to the gram stain, the presence of catalase, deoxyribonuclease (DNase), the API STAPH ® (REF 20500 bioMerieux SA) and an agglutination test Slidex Staph Plus (BioMerieux ®) which detects clumping factor simultaneously, protein A and capsular antigens of *S. aureus*.

Antibiotic sensitivity test

Susceptibility testing was performed for using the diffusion method of Kirby Bauer agar as recommended by the French Society for Microbiology antibiogram committee (FSM-AC 2008). Antibiotics tested were: Cefoxitin 30 µg; Erythromycin 15 UI; Lincomycin 15 µg; Pristinamycin 15 µg, gentamicin 10 UI, Ciprofloxacin 5 µg, rifampicin 30 µg and vancomycin 30 µg. Cefoxitin responds to methicillin. Quality control was performed with the reference strain *S. aureus* ATCC 29213.

Detection of *coa*, *mecA*, *eta* and *etb* genes

Preparation of DNA extracts

The strains of *S. aureus* were inoculated onto blood agar (5% sheep blood) for 24 h. Bacterial DNA was extracted by thermal shock. Briefly, a loop of typical colonies was suspended in 400 µl of sterile distilled water. The suspension was frozen at -20°C for 20 min and then the frozen suspension was incubated for 20 min in a heating block preheated to 100°C after centrifugation at 13,000 rpm for 10 min, the supernatant containing bacterial DNA was recovered and stored at -20°C for gene amplification.

Gene amplification (PCR)

Gene amplifications were performed to detect 4 genes that are the *coa* gene encoding staphylocoagulase by nested-PCR (Ahrens and Andresen, 2004), the *mecA* gene that encodes resistance to methicillin includes modification of PBP2a (Pérez-Roth et al., 2001) and both *eta* and *etb* genes that encode exfoliatin A and B by multiplex PCR (Becker et al., 1998). Sequences of the primers used in this study are compiled in Table 1. Different genes have been detected in a reaction carried out in a final volume of 50 mu.l containing: 10 mM Tris-HCl (pH 8.9), 50 mM KCl, 3 mM MgCl2, 200 microM dNTPs, 0.6 mM of each primer, 1 U Taq DNA polymerase (Promega, Madison, USA) and 5 mu.l of DNA extract.

For the *coa* gene, the amplification conditions, performed with the primers COAG1/COAG4 are as follows: initial denaturation of DNA at 94°C for 5 min was followed by another 40 cycles at 94°C about 30 s, hybridization pause for 30 s at 55°C and elongation for 2 min 30 s at 72°C. The amplification is completed by a final elongation phase for 10 min at 72°C. The second amplification performed with primers COAG2/COAG3 is under the same conditions as previously using 1 µl of the first amplification reaction.

For the *mecA* gene, the amplification conditions are as follows: after incubating for 5 minutes at 94 ° C, two cyclical phases are carried out as follows: an initial cycle repeated 10 times consisted of a denaturation step at 94°C for 30 s, a primer binding step at 64°C for 30 s and an elongation step at 72°C for 45 s. The second phase of cyclical repeats 25 times consists of a denaturation step at 94°C for 45 s, a primer binding step at 50°C for 45 s and an elongation step at 72°C for 1 min. The amplification is completed by a final elongation phase at 72°C for 10 min.

For the simultaneous detection of eta and etb genes, the amplification conditions are identical to that of the coa gene, the only difference is that the number of repeated cycles is 30 and the incubation time for the elongation cycle is 30 s.

The size of PCR products (amplicons) was verified by agarose gel electrophoresis (1.5% w/v) containing ethidium bromide (EtBr)

Table 2. Methicillin-résistance and exfoliatin genes of *Staphylococcus* sp.

Product gene	*S. aureus* (N = 54) (%)	Coagulase negative *Staphylococcus* spp. (N = 7) (%)
Meti-R *mecA*(+)	2 (3.7)	1 (14.3)
Meti-R *mecA*(-)	6 (11.1)	0
mecA (+) *eta-etb* (+)	1(1.9)	0
eta-etb (+)	1(1.9)	0
mecA (+)*etb* (+)	1(1.9)	0

of 0.5 µg/ml and revealed on an ultraviolet table (UV).

RESULTS

One hundred and fifty-two (152) children of mean age 9 years (range: 5 to 15 years) were included. The age group most represented was that of children aged 9 to 11 years with 45.4%. One hundred and five (105) children (69. 1%) were male and 47 (30.9%) were female giving a sex ratio of 2.23. Suppurative skin lesions on the lower limbs (80.9%), upper limbs (12.8%), head (4.2%) or on the trunk (2.1%). out of the 152 samples analyzed, 61 strains of staphylococci were isolated (40.1%). Among these isolates, 54 *S. aureus* (88.5%) and 7 coagulase negative *Staphylococcus* species (CNS) with 3 species: *Staphylococcus lentus* (3), *Staphylococcus sciuri* (3) and *Staphylococcus cohnii urealyticum* (1). The rate of methicillin-resistant *S. aureus* (MRSA) was 14.8%. One strain of *S. lentus* was methicillin-resistant. Resistance to erythromycin was 3.7% for *S. aureus* and 14.3% for SNC; these strains were also MLSB inducible. All strains of *Staphylococcus* isolated were sensitive to gentamicin, ciprofloxacin, rifampicin and vancomycin. The *mecA* gene was detected in 3 phenotypically methicillin-resistant strains (4.9%) and 2 were *S. aureus* (3.7%). The MRSA phenotype observed in 6 strains lacked the *mecA* gene. The genes encoding the exfoliatin were detected in 3 strains of *S. aureus* (5.6%). Two (2) strains of *S. aureus* (3.7%) were simultaneously eta and etb genes that encode, respectively for exfoliatin A and B, a strain carrying the *mecA* gene. Only one strain was *etb* and *mecA* gene positive (Table 2).

DISCUSSION

Staphylococcus skin infections are common in children. The *S. aureus* prevalence was relatively high, 40.1%. Bernard et al. (2004) reported a rate of 53% of *S. aureus* in primary cutaneous infections and 47% in secondary skin infection. Could the lack of hygiene be incriminated as a factor favoring the frequent occurrence of skin infection of the child (Maguire et al., 1998)? The level of CA-MRSA skin infections was 14.8%. This rate is higher than those reported in France, 5.8% and 8.2% (Bernard et al., 2008; Hubiche et al., 2008). In the U.S., study on pediatric dermatology infections revealed that 32% of strains were MRSA (Ortega-Loayza et al., 2010). In India, Thind et al. (2010) showed a low rate of CA-MRSA in pyodermas (9.6%). Nagaraju et al. (2004) reported in an isolation rate of MRSA as 10.9% from community acquired pyoderma. In Iraq the level of resistance was very high with 90% of CA-MRSA. These data suggests that most MRSA were community acquired with limited nosocomial spread (Co et al., 2011). In China, 54.1% isolates were MRSA in skin and soft tissue infections (Yao et al., 2010). In previous study in China in 2008, 10.4% of isolates were MRSA (Ho et al., 2008). The emergence of MRSA in the community is a growing concern, although strains of MRSA hospital can be transmitted in the community, some authors argue that the increasing prevalence of MRSA acquisition in the community can not be explained by the spread of hospital strains. Strains acquired in the community are often different from clinical strains and it appears to have risen outside the health care environment because of the selective pressure of antibiotics (Buckingham et al., 2004; Fridkin et al., 2005). However, lower rates of MRSA observed in Europe could be explained due to a better standard of hygiene reduces the movement of bacteria in the communities. They could also result in good policies initiated in the use of antibiotics in these countries (Trystam et al., 2004). All strains of MRSA in this study were not associated with another antibiotic resistance. The erythromycin resistance of 3.7% was also isolated; it was an inducible MLSB phenotype. Resistance in France was reported by 32% and the rate of multidrug resistance was 10.7% in skin infections (Bernard et al., 2008). The *mecA* gene was present in 3.7% of *S. aureus*, out of the 9 strains of MRSA only 3 carried the *mecA* gene. In Nigeria, the absence of *mecA* gene among strains of MRSA has been reported (Olayinka et al., 2009). This difference shows that the phenotypic characteristics alone do not explain all the mechanisms of resistance observed, therefore it is important to look into genotypic characters. If the main mechanism of methicillin resistance is the modification of the target (PBP2a) encoded

by the *mecA* gene, other mechanisms are also involved. This is the case of hyper-production of beta-lactamase, hyper-production of PBP4 and production of methicillinase (Nour et al., 2005). The gold standard for MRSA detection is the amplification of *mecA* gene by PCR (Berger-Bach and Roher, 2002). Furthermore, the *mecA* gene was detected in a strain of coagulase negative *Staphylococcus* (CNS). These strains were often regarded as the reservoir of genes encoding resistance to antibiotics. These resistance genes were transferred to *S. aureus* and other bacteria "gram-positive." However, neither the mechanism of transfer of *mecA* nor the organizations involved in this transfer are known to date. However, it was suggested that the circulation of *mecA* would be in the direction from CNS to *S. aureus* (Ito et al., 2001). Piemont et al. (1984) reported that about 5% of strains of *S. aureus* isolated from clinical specimens produced exfoliatin either A or B or both. In Nigeria, several authors argued that exfoliatin A would be the most frequent (Adesiyun et al., 1991; Ladhani, 2001). The association of MRSA and exfoliatin genes was not common about 1.4% in Tunisia (Ben et al., 2006), and it is very important for understanding gene expression and regulation in the endeavor to determine the mechanisms of CA-MRSA virulence, and the development of anti-infective strategies to combat this emerging pathogen (Loughman et al., 2009).

In conclusion, the circulation of MRSA in the community is significant and must be used to establish appropriate infection control programmes and health intervention strategies. More studies are needed to investigate the clonal evolution of MRSA over time and the emergence of CA-MRSA. The association of MRSA and exfoliatin genes leads to search for other virulence factors such as Panton-Valentine leukocidin.

REFERENCES

Adesiyun AA, Lenz W, Schaal KP (1991). Exfoliative toxin production by *Staphylococcus aureus* strains isolated from animals and human beings in Nigeria. Microbiologica, 14: 357-362.

Ahrens P, Andresens LO (2004). Cloning and sequence analysis of genes encoding *Staphylococcus hyicus* exfoliative toxin types A, B, C and D. J. Bacteriol., 186(6): 1833-1837.

Becker K, Roth R, Peters G (1998). Rapid and specific detection of toxigenic *Staphylococcus aureus*: use of two multiplex PCR enzyme immunoassays for amplification and hybridization of staphylococcal enterotoxin genes, exfoliative toxin genes, and toxic shock syndrome toxin 1 gene. J. Clin. Microbiol., 36(9): 2548-2553.

Ben NM, Mastouri M, Frih S, Sakly N, Ben Salem Y, Nour M (2006). Molecular characterization of methicillin-resistant *Staphylococcus aureus* isolated in Tunisia. Diagn. Microbiol. Infect. Dis., 55(1): 21-26.

Berger-Bächi B, Rohrer S (2002). Factors influencing methicillin-resistance in staphylococci. Arch. Microbiol., 178: 165-171.

Bernard P, Jarlier V, Santerre-Henriksen A (2008). Antibiotic susceptibility of *Staphylococcus aureus* strains responsible for community-acquired skin infections. Ann. Dermatol. Venereol., 135(1): 13-19.

Buckingham SC, McDougal LK, Cathey LD Comeaux K, Craig AS, Fridkin SK, Tenover FC (2004). Emergence of community-associated methicillin - resistant *Staphylococcus aureus* at a Memphis, Tennessee Children's Hospital. Pediatr. Infect. Dis. J., 23: 619-624.

Chambers HF (2001). The changing epidemiology of *Staphylococcus aureus*? Emerg. Infect. Dis., 7: 178–182.

Co EM, Keen EF, Aldous WK (2011). Prevalence of methicillin-resistant *Staphylococcus aureus* in a combat support hospital in Iraq. Mil. Med., 176(1): 89-93.

French Society for Microbiology Antibiogram Committee. Recommendations (2008). Reference guide for antibiotic susceptibility, p. 50.

Delaporte E (2006). Emergence de bactéries multirésistantes: «the growing menace». Ann. Dermatol. Venereol., 133: 7-8.

Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabenti K, Jernigan JA, Harriman K, Harrison LH, Lynfield R, Farley MM (2005). Active Bacterial Core Surveillance Program of the Emerging Infections Program Network. Methicillin-resistant *Staphylococcus aureus* disease in three communities. N. Engl. J. Med., 352: 1436-1444.

Geha DJ, Uhl JR, Gustaferro CA, Persing DH (1994). Multiplex PCR for identification of methicillin-resistant staphylococci in the clinical laboratory. J. Clin. Microbiol., 32: 1768-1772.

Hubiche T, Duchemin D, Lehours P, Boralevi F, Taieb A, et Léauté-Labrèze C (2008). Incidence of methicillin-resistant *Staphylococcus aureus* in community-onset paeditric skin infections: A retrospective study 2000-2005. Ann. Dermatol. Venereol., 135(5): 361-365.

Huletsky A, Giroux R, Rorsbach V, Gagnon M, Vaillancourt M , Bernier M Gagnon F, Truchon K, Bastien M, Picard FJ, Van Belkum A, Ouellette M, Roy PH, Bergeron MG (2004). New real time PCR assay for rapid detection of methicillin resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. J. Clin. Microbiol., 42 (5): 1875-1884.

Hussain FM, Boyle-Vavra S, Bethel CD, Daum RS (2000). Current trends in community-acquired methicillin-resistant *Staphylococcus aureus* at a tertiary care pediatric facility. Pediatr. Infect. Dis. J., 19: 1163-1166.

Ho PL, Chuang SK, Choi YF, Lee RA, Lit AC, Ng TK, Que TL, Shek KC, Tong HK, Tse CW, Tun WK, Yung RW (2008). Hong Kong CA-MRSA surveillance network. (2008). Community-associated méthicilline-resistant and méthicilline-sensitive *Staphylococcus aureus*: skin and soft tissue infections in Hong Kong. Diagn. Microbiol. Infect. Dis., 61(3): 245-250.

Ito T, Katayama Y, Asada K, Mori N, Tsutsumimoto K, Tiensasitorn C, Hiramarsu K (2001). Structural comparison of three types of staphylococcal cassette chromosome mec integrated in the chromosome in methicillin-resistant *Staphylococcus aureus*. Antimicrob. Agents Chemother., 45: 1323–1336.

Kohner P, Uhl J, Kolbert C, Persing D, Cockerill F (1999). Comparison of susceptibility testing methods with Mec A gene analysis for determining methicillin resistance in clinical isolates of *Staphylococcus aureus* and coagulase negative *Staphylococcus* spp. J. Clin. Microbiol., 37: 2952-2961.

Ladhani S (2001). Recent developments in staphylococcal scalded skin syndrome. Clin. Microbiol. Infect., 7: 301-307.

Loughman JA, Fritz SA, Storch GA, Hunstad DA (2009). Virulence gene expression in human community-acquired *Staphylococcus aureus* infections. J. Infect. Dis., 199(3): 294-301.

Lowy FD (1998). *Staphylococcus* infections. N. Engl. J. Med., 339: 520-532.

Maguire GP, Arthur AD, Boustead PJ, Dwyer B, Currie BJ (1998). Clinical experience and outcomes of community-acquired and nosocomial methicillin-resistant *Staphylococcus aureus* in a northern Australian hospital. J. Hosp. Infect., 3: 273-281.

Mohanasoundaram KM, Lalitha MK (2008). Comparison of phenotypic versus genotypic methods in the detection of methicillin resistance in *Staphylococcus aureus* Indian J. Med. Res., 127: 78-84.

Nagaraju U, Bhat G, Kuruvila M, Pai GS, Javalakshmi, Babu RP (2004). Methicillin-resistant *Staphylococcus aureus* in community-acquired pyoderma. Int. J. Dermatol., 43(6): 412-414.

Olayinka BO, Olayinka AT, Obajuluwa AF, Onaolapo JA, Olurinola PF (2009). Absence of *mecA* gene in methicillin-resistant *Staphylococcus aureus* isolates. Afr. J. Infect. Dis., 3(2): 49-56.

Nour M, Mastouri M, Ben Nejma M (2005). Methicillin resistance in

Staphylococcus aureus; emergence and molecular basis. Pathol. Biol. (Paris) 53(6): 334-40

Ortega-Loayza AG, Diamantis SA, Gilligan P, Morrell DS (2010). Characterization of *Staphylococcus aureus* cutaneous infections in a pediatric dermatology tertiary health care outpatient facility. J. Am. Acad. Dermatol., 62(5): 804-811.

Pérez-Roth E, Claverie-Martín F, Villar J, Méndez-Álvare S (2001). Multiplex PCR for simultaneous identification of *Staphylococcus aureus* and detection of methicillin and mupirocin resistance. J. Clin. Microbiol., 39: 4037-4041.

Piemont Y, Rasoamananjara D, Fouace JM, Bruce T (1984). Epidemiological investigation of exfoliative toxin-producing *Staphylococcus aureus* strains in hospitalized patients. J. Clin. Microbiol., 19: 417-420.

Rojo P, Barrios M, Palacios A, Gomez C, Chaves F (2010). Community-associated *Staphylococcus aureus* infections in children. Expert Rev. Anti- Infect. Ther., 8(5): 541-554.

Sakoulas G, Gold HS, Vankataraman L, Degirolami PC, Eliopoulos GM, Qian Q (2001). Methicillin- resistant *Staphylococcus aureus*: comparison of susceptibility testing methods and analysis of *mecA* positive susceptible strains. J. Clin. Microbiol., 39: 3946-3951.

Thind P, Prakash SK, Wadhwa A, Garg VK, Pati B (2010). Bacteriological profile of community-acquired pyodermas with special reference to méthicilline resistant *Staphylococcus aureus*. Indian J. Dermatol. Venerol. Leprol., 76: 572-574.

Trystam D, Varon E, Pean Y, Grundmann H, Gutmann L, Jarlier V, Aubry-Damon H (2004). European Antimicrobial Resistance Surveillance System (EARSS): reference data of 2002, France. BEH. 32-33: 142-144.

Yao D, Yu FY, Qin ZQ, Chen C, He SS, Chen ZQ, Zhang XQ, Wang LX (2010). Molecular characterization of *Staphylococcus aureus* isolates causing skin and soft tissue infections (SSTIs). BMC Infect. Dis., 10: 133.

Zetola N, Francis SF, Nuermberger EL, Bishai WR (2005). Community-acquired methicillin-resistant *Staphylococcus aureus: a*n emerging threat. Lancet Infect. Dis., 5: 275-286.