International Journal of Microbiology Research
ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 9, Issue 12, 2017, pp.-976-980.
Available online at http://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000234

Research Article

METHICILLIN-RESISTANCE OF *Staphylococcus* species IN SOUTHERN BENIN: RESISTANCE GENE, VIRULENCE FACTOR ASSOCIATED AND STAPHYLOCOCCAL CHROMOSOMAL CASSETTE DISTRIBUTION

KOUDOKPON HORNEL[1], DOUGNON TAMÈGNON VICTORIEN*[1], KISSIRA SETONDJI ISLAMIATH[1], ANIAMBOSSOU ALIDAH[1], FANOU V. BRICE ARMAND[1], LOKO FRÉDÉRIC[1], BANKOLE HONORÉ SOUROU[1], DIENE M. SEYDINA[2] AND ROLAIN JEAN-MARC[2]

[1]Research Unit in Applied Microbiology and Pharmacology of natural substances, Research Laboratory in Applied Biology, Polytechnic School of Abomey-Calavi, University of Abomey-Calavi, 01 PO Box 2009 Cotonou, Benin
[2]URMITE, Aix-Marseille University, 27 Bd Jean Moulin, 13385 Marseille Cedex 5, France.
*Corresponding Author: Email-victorien88@hotmail.com

Received: November 21, 2017; Revised: December 11, 2017; Accepted: December 12, 2017; Published: December 28, 2017

Abstract- Methicillin resistance of staphylococcal strains remains a public health concern. The present study aims to detect the carriage of the mecA gene by staphylococcal strains and the associated virulence factors. Seventy-eight staphylococcal strains collected from three hospitals in southern Benin were identified in Maldi T of MS. Antibiotic sensitivity was determined by antibiotic disk diffusion on Mueller Hinton-2 medium. The search for the mecA gene, the production of Panton-Valentine PVL, Leucocidin, Toxic shock syndrome toxin TSST and detection of Staphylococcal chromosomal cassette were performed by Real Time - Polymerase Chain Reaction. The results obtained show that *Staphylococcus saprophyticus* was the most isolated species in the urine (38.4%) followed by *Staphylococcus* aureus (21.8%), *Staphylococcus sciuri* (21.8%), *Staphylococcus conhii* (6, 4%), *Staphylococcus heamolyticus* (1.3%), *Staphylococcus hominis* (1.3%) and *Staphylococcus xylosus* (1.3%). In the cervico-vaginal secretion specimens, *Staphylococcus aureus* was the most isolated with a proportion of 5.1%. From the study of antibiotic sensitivity, there is a strong resistance of strains to beta-lactams but no resistance to glycopeptides. 24.4% of the staphylococcal strains harbored the mecA gene. Between them, 52.4% of the *Staphylococcus aureus* strains were carriers of the mecA gene and 41.2% of the *Staphylococcus sciuri* strains also carried them. 33.3% of strains of *Staphylococcus aureus* produced PVL and 14.3% of the TSST. Two types of cassettes were identified in *Staphylococcus aureus* ccrB IV (n = 3) and ccrC (n = 4). Only one type of cassette was found in the strains of *Staphylococcus sciuri* bearing the mecA gene, this is ccrB II (n = 1). It is therefore important to initiate the search for these genes routinely for the proper taking care of patients.

Keywords- Public Health, Antimicrobial Resistance, *Staphylococcus spp*, Molecular diagnosis, Bacteriology.

Citation: Koudokpon Hornel, et al., (2017) Methicillin-Resistance of *Staphylococcus* Species in Southern Benin: Resistance Gene, Virulence Factor Associated and Staphylococcal Chromosomal Cassette Distribution. International Journal of Microbiology Research, ISSN: 0975-5276 & E-ISSN: 0975-9174, Volume 9, Issue 12, pp.-976-980.

Academic Editor / Reviewer: Gopalakrishnan Reshmi, Garima Kapoor, Shivanna Vijaya

Introduction

Staphylococci are a large family of ubiquitous pathogens that are very common in community and nosocomial infections [1]. *Staphylococcus aureus* is the leader of this family due to its involvement in severe suppurative, localized or systemic pathologies in humans [2,3]. Nonetheless, other staphylococcal species can cause a lot of damage to their hosts. This is the case, for example, with *Staphylococcus saprophyticus*, which is the second bacterium responsible for urinary tract infection after *Escherichia coli*. What makes staphylococci species even more redoubtable is their ability to quickly acquire resistance to a wide range of antibiotics [4,2]. Methicillin resistance in these staphylococcal strains has been and still a major health concern worldwide [5]. A special feature has been put in recent years on methicillin-resistant *Staphylococcus aureus* strains. Indeed, an estimated 20% mortality associated with systemic infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) [6], because it is a pathogen difficult to treat because of its selectivity to many antibiotics [3]. In addition, cases of resistance to glycopeptides, treatment of choice against MRSA have already been revealed [7]. Negative Coagulase Staphylococci are also concerned by this resistance even though it is less cited [8-10]. The acquisition of the mecA gene contained in the staphylococcal cassette chromosome (SCC) is responsible for the production of PLP 2a causing loss of affinity to penicillin is at the cause of this resistance [11,3].The majority of MRSA acquired by hospitals (HA-MRSA) carry *SCCmec* types I, II and III, whereas MRSA (CA-MRSA) acquired by the community usually carries SCCmec types IV and V [12,13],

A new PBP2a counterpart has recently been described as encoded by mec C who shares 70% identity with mec A [14]. This new gene is also involved in resistance to cefoxitin and oxacillin in strains of *Staphylococcus aureus* [15] especially in animals, or there is growing fear of transmission to humans [16]. Added to this is the production of toxins such as Panton-Valentin leucocidin (PVL) and Staphylococcal toxic shock toxin STST associated with the resistance of these strains [17,18]. In Benin, the first epidemiological studies on resistance to methicillin began in 2006 [19]. Since then, numerous studies have been carried out both on clinical strains of infection and on animal strains [20-23]. But the vast majority of these studies focused on *Staphylococcus aureus*. The present study aims to take stock of the methicillin resistance of staphylococcal species in South Benin and the production of toxin by these strains.

Material and Methods

Bacteriological identification

The present study focused on 508 urine and cervico-vaginal secretions collected in three years in three hospitals in southern Benin: Menontin District Hospital, Bethesda Hospital and the Departmental University Hospital Ouémé Plateau. A

total of 286 samples were positive with 315 strains isolated. Of these strains, 78 strains of staphylococci have been identified (Gram-positive Cocci, Catalase +). Once collected, these strains were then sent to the Laboratory of Research in Applied Biology for control and conservation (BTS + Glycerol at -80 ° C). The identification was carried out by Maldi Tof-type Mass Spectrometry at UMRITE, IHU-Marseille [24].

Antibiogram

A panel of 16 antibiotics were used to evaluate the sensitivity of staphylococcal strains to the antibiotic, namely Penicillin (P10µg), Oxacillin (OX5µg), Cefoxitin (FOX30µg), Vancomycin (VA30µg), Teicoplanin (TEC30µg), Clindamycin (DA15), Erythromycin (E15µg), Pristinamycin (PT15µg), Gentamycin (CN 15µg), Ciprofloxacin (CIP5µg), Linezoid (LNZ30µg), Fosfomycin (FF50µg), Doxycycline (OD 30UI), Fusidic Acid (10µg), Rifampicin (RA30µg)), trimethoprim-sulphamethoxazol (SXT25µg). A 0.5 Mc Farland bacterial suspension was seeded by swabbing on Commercial Square Muller Hinton medium and the deposited antibiotic discs. The agar plates were then incubated at 37 ° C for 24 hours [25]. Diameters of inhibition were then measured and compared to the diameter proposed by the CASFM 2013 in order to determine the resistance profile with regard to antibiotics [25].

Table-I *Sequences of the primers and probes used*

Gene		Sequences	References
mecA	Primers F	GTTAGATTGGGATCATAGCGTCATT	(Bittar *et al.*, 2009)
	Primers R	TGCCTAATCTCATATGTGTTCCTGTAT	
	Probe	TTCCAGGAATGCAGAAAGACCAAAGC AT	
PVL	Primers F	AAAATGCCAGTGTTATCCAGAGGTA	(Francois *et al.*, 2004)
	Primers R	TTTGCAGCGTTTTGTTTTCG	
	Probe	CTTCAATCCAGAATTTATTGGTGT	
TSST-1	Primers F	TCATCAGCTAACTCAAATACATGGATT	(Schlebusch *et al.*, 2009)
	Primers R	TGTGGATCCGTCATTCATTGTT	
	Probe	TCCAATAACCACCCGTTTTATCGCTTG AA	
ccrB-I	Primers F	TTTGGCACGTAATACTTCCGATT	(Francois *et al.*, 2004; Ito *et al.*, 2003)
	Primers R	AAAATTCAACATTTTGGCGATGA	
	Probe	ACTTACAATAGTCGAAGAAC	
ccrB-II	Primers F	AACGAGACGTGCCCAAGAAG	(Francois *et al.*, 2004; Ito *et al.*, 2003)
	Primers R	CATCAGTTCATGTTTACTATTAGGTAT TTTGTC	
	Probe	ATTTGCCGCTGGGCT	
ccrB-III	Primers F	ACAATCCACAGTCATTACAT	(Francois *et al.*, 2004; Ito *et al.*, 2003)
	Primers R	AGTTACGACTTTCTGTTTCA	
	Probe	CATCAGTTCATGTTTACTATTAGGTAT TTTGTC	
ccrB-IV	Primers F	GAACAGACCTGAGCTCCAACGT	(Francois *et al.*, 2004; Ito *et al.*, 2003)
	Primers R	TCGGTTTGTTTTGTAGATCATAACACA	
	Probe	ATGCAAAAGAAGGCAATAT	
sccV	Primers F	TCTGGGAGTTCTGCCTGTCA	(Bittar *et al.*, 2009)
	Primers R	TCACATTTGACGCAATCTGCT	
	Sonde	TGCTGAAGTCGTCGAACGCGTAATCA	

Research of mecA gene, production of PVL and TSST toxins and detection of Staphylococcal chromosomal cassette

A pure colony of each identified strain was used for DNA extraction by the EZ-1 technique. The search for the mecA resistance gene, and the PVL, TSST virulence genes were performed by real-time PCR. The primers and probes used are presented in [Table-I]. The reaction medium consists of the following sample: Quantitec 10µl, F 1µl primers, R 1µl primers, DNase-free water 2µl, 1µl probe and 5µl of DNA. RT PCR was performed with CFX96 (Bio Rad, France) [5]. The positive control used is the *Staphylococcus aureus* strain 7204948 for the Mec A gene and the PVL gene and the *Staphylococcus aureus* strain 5268501 for the STST gene.

Results

Between the 78 staphylococcal strains collected, of which 70 came from the urine and 8 from the cervico-vaginal secretion samples, *Staphylococcus saprophyticus*, *Staphylococcus aureus* and *Staphylococcus sciuri* were the most isolated with proportions of 39.8%; 26.9%; 21.8% as shown in [Table-II]. *Staphylococcus saprophyticus* was the most isolated in urine while *Staphylococcus aureus* was the most isolated in cervico-vaginal secretion specimens.

[Table-III] shows the susceptibility of the strains to the antibiotics tested. Large proportions of resistance have generally been observed with beta-lactams including Penicillin at 10 µg, Oxacillin at 5 µg and Cefoxitin at 30 µg. However, we did not observe any resistance to the tested glycopeptides Vancomycin at 30 µg and Teicoplanin at 30 µg. From RT-PCR results, the mecA gene was found in 19 staphylococcal strains. Strains of *Staphylococcus aureus* are the most carriers of the gene with 11 cases out of 19. As for toxins, 7 cases of PVL production and 3 cases of STST production have been identified in *Staphylococcus aureus*. No production of both toxins was observed in other Staphylococcus species [Table-IV]. Three cases of PVL production and 3 cases of STST production were observed in *Staphylococcus aureus* strains carrying the mecA gene as shown in [Table-V]. The study of the beta-lactam resistance phenotype shows that 7 strains of *Staphylococcus aureus* out of the 8 carrying the mecA gene all have the P-OX-FOX phenotype. Only one case of this phenotype was found in strains of *Staphylococcus sciuri* carrying the mecA gene [Table-VI].

Table-II *distribution of strains according to the origin of the sample*

	Urines	Vaginal swab	Total
Staphylococcus saprophyticcus	30 (38,4%)	1 (1,3%)	31 (39,8%)
Staphylococcus aureus	17(21,8%)	4 (5,1%)	21 (26,9%)
Staphylococcus sciuri	17(21,8%)	0 (0%)	17(21,8%)
Staphylococcus conhii	5 (6,4%)	0 (0%)	5 (6,4%)
Staphylococcus heamolyticus	1 (1,3%)	1 (1,3%)	2 (2,6%)
Staphylococcus xylosus	1 (1,3%)	0 (0%)	1 (1,3%)
Staphylococcus hominis	1 (1,3%)	0 (0%)	1 (1,3%)
Total	72 (92,3%)	6 (7,7%)	78 (100%)

Table-III *Resistance profile of staphylococci strains to antibiotics*

	P	OX	FOX	VA	TEC	DA	E	PT	GEN	CIP	LNZ	FF	DO	FA	SXT	RA
S. saprophyticcus	9/31 (29%)	24/31 (77%)	9/31 (29%)	0/31 (0%)	0/31 (0%)	0/31 (0%)	4/31 (13%)	0/31 (0%)	5/31 (16%)	0/31 (0%)	0/31 (0%)	0/31 (0%)	0/31 (0%)	14/31 (45%)	4/31 (13%)	0/31 (0%)
S. aureus	17/21 (81%)	18/21 (86%)	15/21 (71%)	0/21 (0%)	0/21 (0%)	0/21 (0%)	4/21 (19%)	0/21 (0%)	6/21 (29%)	7/21 (33%)	0/21 (0%)	0/21 (0%)	0/21 (0%)	9/21 (43%)	6/21 (29%)	0/21 (0%)
S. sciuri	8/17 (47%)	17/17 (100%)	1/17 (6%)	0/17 (0%)	0/17 (0%)	6/17 (35%)	6/17 (35%)	0/17 (0%)	1/17 (6%)	0/17 (0%)	0/17 (0%)	0/17 (0%)	0/17 (0%)	17/17 (100%)	0/17 (0%)	0/17 (0%)
S. conhii	2/5 (40%)	2/5 (40%)	1/5 (20%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	2/5 (40%)	0/5 (0%)	1/5 (20%)	1/5 (20%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	2/5 (40%)	1/5 (20%)	0/5 (0%)
S. hemolyticus	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	1/2 (50%)	0/2 (0%)	0/2 (0%)	1/2 (50%)	0/2 (0%)	1/2 (50%)	0/2 (0%)
S. xylosus	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	0/1 (0%)
S. hominis	1/1 (100%)	1/1 (100%)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
Total	38/78 (49%)	63/78 (81%)	28/78 (36%)	0/78 (0%)	0/78 (0%)	6/78 (8%)	17/78 (22%)	0/78 (0%)	14/78 (18%)	9/78 (12%)	0/78 (0%)	9/78 (12%	0/78 (0%)	43/78 (55%)	12/78 (15%)	0/78 (0%)

Table-IV *Portage of the mecA gene and PVL and STST toxins in staphylococcal strains.*

		mecA	PVL	TSST
S. saprophyticcus	*urines*	0/30	0/30	0/30
	Vaginal swab	0/1	0/1	0/1
S. aureus	*urines*	10/17	6/17	3/17
	Vaginal swab	1/4	1/4	0/4
S. sciuri	*urines*	6/17	0/17	0/17
	Vaginal swab	0	0	0
S. conhii	*urines*	0/5	0/5	0/5
	Vaginal swab	0	0	0
S. hemolyticus	*urines*	1/1	0/1	0/1
	Vaginal swab	0/1	0/1	0/1
S. xylosus	*urines*	0/1	1/1	0/1
	Vaginal swab	0	0	0
S. hominis	*urines*	1/1	1/1	0/1
	Vaginal swab	0	0	0
Total	*urines*	15/72	6/72	3/72
	Vaginal swab	1/6	1/6	0/6
	Total	19/78 (24,4%)	7/78 (9%)	3/78 (2,5%)

Table-V *Relation betweenmecA gene, PVL and STST toxins.*

N°	mecA	PVL	STST	Antibiotic Profile		
				P 10	OXA5	FOX30
1	-	+	-	*S*	*R*	*R*
2	+	-	-	*R*	*R*	*R*
3	-	+	-	*R*	*R*	*R*
4	+	+	-	*R*	*R*	*R*
5	+	+	-	*R*	*R*	*R*
6	+	-	-	*R*	*R*	*R*
7	+	-	+	*R*	*R*	*R*
8	-	+	-	*R*	*R*	*S*
9	+	-	-	*S*	*R*	*S*
10	+	-	+	*R*	*R*	*R*
11	+	-	+	*R*	*R*	*R*
12	+	+	-	*R*	*R*	*R*
13	-	+	-	*R*	*R*	*S*
14	+	-	-	*R*	*R*	*S*
15	+	-	-	*R*	*S*	*S*

Table-VI *Beta-lactam resistance profile of strains carrying the mecA gene*

Strains		Resistance Profile	
S. aureus	*mecA +*	*P-OXA-FOX*	8
		P-OXA	1
		OXA	1
		P	1
	mecA -	*P-OXA-FOX*	3
		OXA-FOX	2
		P-FOX	2
		P-OXA	3
S. sciuri	*mecA +*	*P-OXA-FOX*	1
		P-OXA	4
		OXA	1
	mecA -	*P-OXA*	2
		OXA	7
		P	2
S. hemoliticus	*mecA +*	*P-OXA-FOX*	1
S. hominis	*mecA +*	*P-OXA-FOX*	1

We therefore wanted to know the cassettes which the mecA-isolated gene belongs in Benin. Therefore, we carried out an Real Time-PCR of the genes ccrB I to IV and the gene ccrV or ccrC. The primers and probes used are presented in [Table-I]. Two types of cassette were identified in *Staphylococcus aureus* ccrB IV (n = 3) and ccrC (n = 4). Only one type of cassette was found in the strains of *Staphylococcus sciuri* bearing the mecA gene, this is ccrB II (n = 1).

All strains producing PVL and bearing the mecA gene also bear the ccrB IV gene and all strains producing TSST- 1 bear the SCC V [Table-VII].

Discussion

Methicillin resistance in staphylococci remains a hot topic in developing countries.

The present study took into account 78 strains of staphylococci isolated from urine samples and cervico-vaginal secretion. *Staphylococcus saprophyticus* was the most isolated germ of urine (38.4%). This result confirms many other studies that have shown that this germ was the second leading cause of urinary tract infection after *Escherichia coli* [26-28]. In Benin, the studies carried out are mostly limited to the search for *Staphylococcus aureus* [29,30] in cases of infection because of its redoubtable character and simplicity of presumption while the identification of *Staphylococcus saprophyticus* among routine Negative Staphylococci Coagulase is more difficult. These studies are therefore limited to the identification of Negative Coagulase Staphylococci [31]. These findings once again show the importance of new technique such as Maldi Tof, MS in routine diagnosis [24]. It was noted a predominance of *Staphylococcus aureus*. The presence of *Staphylococcus aureus* in cervico-vaginal secretion specimens could reflect very serious conditions such as toxin production of toxic shock syndrome involved in infertility cases [32], sepsis and vaginal injury during menstruation [33].

Table-VII *Distribution of Staphylococcal chromosomal cassette*

N° Ech		Origine	ccr types	Resistance to Beta lactamine			Others genes
				P10	OXA5	Fox30	
1	*S. aureus*	*Urine*	*C(V)*	*R*	*R*	*R*	-
2	*S. aureus*	*Urine*	*B(IV)*	*R*	*R*	*R*	*PVL*
3	*S. aureus*	*Urine*	*B(IV)*	*R*	*R*	*R*	*PVL*
4	*S. aureus*	*Urine*	*C(V)*	*R*	*R*	*R*	-
5	*S. aureus*	*Urine*	*C(V)*	*R*	*R*	*R*	*TSST*
6	*S. aureus*	*Urine*	*NT*	*R*	*R*	*R*	-
7	*S. aureus*	*Urine*	*C(V)*	*R*	*R*	*R*	*TSST*
8	*S. aureus*	*Urine*	*C(V)*	*R*	*R*	*R*	*TSST*
9	*S. aureus*	*Urine*	*B(IV)*	*R*	*R*	*R*	*PVL*
10	*S. aureus*	*Urine*	*NT*	*R*	*R*	*R*	-
11	*S. aureus*	*Urine*	*NT*	*R*	*R*	*R*	-
12	*S. Sciuri*	*Urine*	*B(II)*	*R*	*R*	*R*	-
13	*S. Sciuri*	*Urine*	*NT*	*R*	*R*	*R*	-
14	*S. Sciuri*	*Urine*	*NT*	*R*	*R*	*R*	-
15	*S. Sciuri*	*Urine*	*NT*	*R*	*R*	*R*	-
16	*S. Sciuri*	*Urine*	*NT*	*S*	*S*	*S*	-
17	*S. Sciuri*	*Urines*	*NT*	*R*	*R*	*R*	-
18	*S. hominis*	*Urines*	*NT*	*S*	*S*	*S*	-
19	*S. heamolyticus*	*Urines*	*NT*	*R*	*R*	*R*	-

The susceptibility to antibiotics of its strains showed a strong resistance of strains to beta-lactams. However, we have not observed cases of resistance to glycopeptides which is the remedy in case of resistance to methicillin. This study is therefore in addition to recent studies that have shown the absence of glycopeptide resistance in clinical strains of staphylococci in Benin [21,30,22]. There have been cases of resistance to glycopeptides associated with nosocomial infections [34], and in cattle carcasses [23]. It is therefore important to carry out a surveillance policy in order to avoid the transmission of this resistance to community strains.

24.4% of the staphylococci strains overall had the mecA gene. 11 of the 21 *staphylococcus aureus* strains were carriers of the mecA gene, with a prevalence of 52.4% of SAMR. This prevalence is above that found by Affolabi *et al.*, 2014 who had a prevalence of 24.3%. 35.2% of strains of *Staphylococcus sciuri* carried the mecA gene. Many authors have shown that this resistance of *Staphylococcus sciuri* originated from the acquisition by the latter of a homologous gene of the mecA gene including mecA-like [35,36]. Toxin production by *Staphylococcus aureus* strains was 33.33% for PVL and 14.3% for STST. Prevalence of 60.70% [37], 70% [21]. Sina *et al.*, 2013, 14,8% [22]. Affolabi *et al.*, 2014b, have been noted for PV production by *Staphylococcus aureus* strains in Benin. As for the production of TSST by *Staphylococcus aureus* strains in Benin, studies [38], showed a prevalence of about 30% in urogenital and pus samples [21], noted a low proportion of TSST production in boils (1%). 14.28% of the strains of *Staphylococcus aureus* produced PVL were associated with the carriage of the gene mec A and 9.5 of TSST. No strain associated the two toxins with both the mecA gene. According to some authors, the combination of its virulence factors

constitutes a major complication in the treatment of these infections [32], [39].
The ccrV were the most identified in the present study in *Staphylococcus aureus* strains followed by ccr IV [Table-II]. These results are in agreement with [40,3] instead found a predominance of type I associated with resistance to mupirocin. A type II presence of ccrB was noted in *Staphylococcus Sciuri* mecA. Of the many cases of untyped strain was also reported by [41], for *Staphylococcus hominis* strains of *Staphylococcus heamolyticus* and by [42], for *Staphylococcus sciuri* strains. All strains producing PVL and bearing the mecA gene also bear the ccrB IV gene. This confirms the studies of [43,13] which found a strong relationship between the production of PVL and ccrB [44] did not observe any relationship between the portage of ccrB I and the production of PVL and TSST.
The distribution of resistance phenotypes between SAMR and SASM shows us that the only consideration of cefoxitin disks for assuring resistance to methicillin remains erroneous. It is therefore necessary to combine several beta-lactams for a better interpretation of the resistance phenotype. In *Staphylococcus sciuri* strains, we observed only one case of resistance to cefoxitin in strains carrying the mecA gene or not. All this shows the importance of mecA gene research by molecular techniques which remain the best track for identifying methicillin resistance in Staphylococci strains.

Conclusion

The present study has shown that resistance to methicillin in staphylococci strains remains valid in Benin. Although the present study did not record cases of glycopeptide resistance, a permanent watch strategy is required to ensure that cases detected in cattle carcasses and hospital strains do not spread in the population. Toxin production by staphylococcus aureus strains remains a complication of staphylococcal infections. The production of PVL and TSST by strains isolated in this study should be a concern for proper management of its infections.

Application of research

This study could lead to the implementation of a permanent surveillance strategy to ensure that cases of methicillin-resistant staphylococci detected in cattle carcasses and hospital strains do not spread in the population. The production of toxins by *Staphylococcus aureus* strains remains a complication of staphylococcal infections. The production of PVL and TSST by strains isolated in this study should be a concern for the correct management of its infections.

Topic of research

Methicillin-resistance of Staphylococcus species clinical strain in South Benin: Resistance gene, virulence factor associated and Staphylococcal chromosomal cassette distribution.

Acknowledgement/Funding: This study has been done with the support of the Ministry of Higher Education and the Scientific Research in Benin.

Author Contributions: All the authors equally contributed to the manuscript.

Author statement: All authors read, agree and approved the final manuscript.

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

Abréviations

SAMR: Methicillin-Resistant *Staphylococcus aureus*
SCC: Staphylococcal Cassette Cphromosome
PVL: Panton-Valentin Leucocidin
RT PCR: Retro Transcryptase Polymerase Chain Reaction
TSST: Toxic shock syndrome toxinPVL
Conflict of Interest: None to declare
STST: Staphylococcal Toxic Shock Toxin

References

[1] Boukadida J., AbdallahH B. & Boukadida N. (2003) *Bull Soc Pathol Exot,* 96, 283–285.
[2] Boukhatem N., Ferhat M., Hadj Mohamed R. & Lalaoui N. (2015) *J Fundam Appl Sci., 7*(2), 260–270.
[3] Joshi P. R., Acharya M., Aryal R., Thapa K., Kakshapati T., Seng R., Singh A. & Sitthisak S. (2017) *Asian Pac J Trop Biomed*.
[4] Ito T., Okuma K., Ma X. X., Yuzawa H., Hiramatsu K., Fluit A., Coombs G., Pearman J., Tenover F. & other authors (2003) *Drug Resist Updat,* 6, 41–52.
[5] Kim J.-U., Cha C.-H., An H.-K., Lee H.-J. & Kim M.-N. (2013) *J Clin Microbiol,* 51(3), 1008–1013.
[6] Callejo-Torre F., Maria J., Bouza E., Astigarraga P. O., Coma M. J., Corral D., Palomar Martínez M., Alvarez-Lerma F. & Jesús López-Pueyo M. (2016) *Le Infez Med.,* 3, 201–209.
[7] Panesso D., Planet P. J., Diaz L., Hugonnet J.-E., Tran T.T., Narechania A., Munita J. M., Rincon S., Carvajal L. P. & other authors (2015) *Emerg Infect Dis.,* 21(10), 1844–1848.
[8] Chah K. F., Gómez-Sanz E., Nwanta J. A., Asadu B., Agbo I. C., Lozano C., Zarazaga M. & Torres C. (2014) *Brazilian J Microbiol.,* 45, 215–20. Elsevier.
[9] Ugwu C.C., Gomez-Sanz E., Agbo I.C., Torres C. & Chah K. F. (2015) *Brazilian J Microbiol.,* 46, 885–892.
[10] Fariña N., Samudio M., Carpinelli L., Nentwich M. M. & de Kaspar H. M. (2016) *Int Ophthalmol* 1–7.
[11] Ito T., Hiramatsu K., Oliveira D. C., De Lencastre H., Zhang K., Westh H., O'Brien F., Giffard P. M., Coleman D. & other authors (2009) *Antimicrob Agents Chemother*.
[12] Stefani S., Chung D. R., Lindsay J. A., Friedrich A. W., Kearns A. M., Westh H. & MacKenzie F. M. (2012) *Int J Antimicrob Agents,* 39, 273–282.
[13] Valsesia G., Rossi M., Bertschy S. & Pfyffer G. E. (2010) *J Clin Microbiol.,* 48, 720–7.
[14] Szczuka, E., Krajewska, M., Lijewska, D., Bosacka, K. & Kaznowski, A. (2016). Diversity of staphylococcal cassette chromosome mec elements in nosocomial multiresistant Staphylococcus haemolyticus isolates. *J Appl Genet*57, 543–547. Springer.
[15] Ballhausen, B., Kriegeskorte, A., Schleimer, N., Peters, G. & Becker, K.(2014). The mecA homolog mecC confers resistance against β-lactams in Staphylococcus aureus irrespective of the genetic strain background. *Antimicrob Agents Chemother*58, 3791–8. American Society for Microbiology (ASM).
[16] Espinosa-Gongora, C., Harrison, E. M., Moodley, A., Guardabassi, L. & Holmes, M. A. (2015). MRSA carrying mecC in captive mara. *J Antimicrob Chemother*70, 1622–1624.
[17] Durand G. (2009) *Thèse Dr Univ Claude Bernard LYON I,* 219p.
[18] Ouedraogo, A.-S., Dunyach-Remy, C., Kissou, A., Sanou, S., Poda, A., Kyelem, C. G., Solassol, J., Bañuls, A.-L., Van De Perre, P. & et al. (2016) *Front Cell Infect Microbiol* 7, 1406-1407.
[19] Ahoyo A.-T., Baba-Moussa L., Makoutode M., Gbohoun A., Bossou R., Dramane K., Sanni A. & Prévost G. (2006) *Arch Pédiatrie.,* 13, 1391–1396.
[20] Baba-Moussa L., Sina H., Scheftel J. M., Moreau B., Sainte-Marie D., Kotchoni S. O., Prévost G. & Couppié P. (2011) *PLoS One,* 6.
[21] Sina H., Ahoyo T. A., Moussaoui W., Keller D., Bankolé H. S., Barogui Y., Stienstra Y., Kotchoni S. O., Prévost G. & Baba-Moussa L. (2013) *BMC Microbiol.,* 13, 188.
[22] Affolabi D., Odoun M., Faihun F., Damala R., Ahovegbe L., Wachinou Prudence, Kehinde A., Bekou W., Massougbodji A. & Anagonou S. (2014b) *Int J Curr Res.,* 6, 4616–4620.

[23] Ahouandjinou H., Baba-Moussa F., Sina H., Moussé W., Zull-Kifoul A., Akim S., Bonou J., Toukourou F. & Baba-Moussa L. (2016) *Int J Sci Environement,* 5(5), 3275–3288.
[24] Seng P., Abat C., Rolain J. M., Colson P., Lagier J. C., Gouriet F., Fournier P.E., Drancourt M., Scola B. La & Raoult D.(2013) *J Clin Microbiol.,* 51, 2182–2194.
[25] Nizami Duran, Burcin Ozer, Gulay Gulbol Duran, Yusuf Onlen & Cemil Demir (2012) *Indian J Med Res. Mar,* 135(3), 389–396.
[26] Ipe D. S. & Ulett G. C.(2016) *J Microbiol Methods,* 127, 164–171.
[27] Kline K. A. & Lewis A. L. (2016) *Microbiol Spectr,* 4.
[28] Odolini S., Parola P., Gkrania-Klotsas E., Caumes E., Schlagenhauf P., López-Vélez R., Burchard G.-D., Santos-O'Connor F., Weld L. & other authors (2012) *Clin Microbiol Infect,* 18, 468–474.
[29] Baba-Moussa L., Anani L., Scheftel J. M., Couturier M., Riegel P., Haïkou N., Hounsou F., Monteil H., Sanni A. & Prévost G. (2008) *J Hosp Infect.,* 68, 32–38.
[30] Sina H., Baba Moussa F., Ahoyo T. A., Mousse W., Anagonou S., Gbenou J. D., Preacute vost G., Kotchoni S. O. & Baba Moussa L. (2011) *African J Microbiol Res.,* 5, 2797–2803.
[31] Bankole H. S., Dougnon T. V., Koudokpon H., Gnonlonfoun Shadrac, Hounmanou G., de Souza M. & Baba-moussa Lamine Soumanou M. (2015) *Rev Ivoir Sci Technol.,* 26, 212–226.
[32] Akhi M. T., Esmailkhani A., Sadeghi J., Niknafs B., Farzadi L., Akhi A. & Nasab E. N. (2017) *Int J Fertil Steril.,* 11, 28–32.
[33] Sada R., Fukuda S. & Ishimaru H. (2017) *IDCases,* 8, 77–80.
[34] Ahoyo T., Bankolé H., Adéoti F., Gbohoun A., Assavèdo S., Amoussou-Guénou M., Kindé-Gazard D. & Pittet D. (2014) *Antimicrob Resist Infect Control,* 3, 17.
[35] Rahman M. T., Kobayashi N., Alam M. M. & Ishino M. (2005) *Microb Drug Resist.,* 11, 205–214.
[36] Wu S. W., Lencastre H. De & Tomasz A. (2005) *Microb Drug Resist.,* 11, 215–224.
[37] Ahoyo T. A., Martin-Odoom A., Bankole H., Baba-Moussa L., Zonon N., Loko F., Prevost Gi., Sanni A. & Dramane K. (2012) *Curr Clin Top Infect Dis.,* 9, 234–240.
[38] Attien P., Sina H., Moussaoui W., Zimmermann-Meisse G., Dadié T., Keller D., Riegel P., Edoh V., Kotchoni S. O. & other authors (2014) *Biomed Res Int.,* 2014.
[39] Correa-Jiménez O., Pinzón-Redondo H. & Reyes N. (2016) *J Infect Public Health.,* 9, 415–420.
[40] Hannan A., Javed F., Saleem S., Tahira K., Jahan S. & Hannan A. (2015) *Open J Med Microbiol.,* 5, 69–75.
[41] Machado A. B. M. P., Reiter K. C., Paiva R. M. & Barth A. L. (2007) *J Med Microbiol.,* 56, 1328–1333.
[42] Nemeghaire S., Vanderhaeghen W., Argudín M. A., Haesebrouck F. & Butaye P. (2014) *J Antimicrob Chemother.,* 69, 2928–2934.
[43] Francois P., Renzi G., Pittet D., Bento M., Lew D., Harbarth S., Vaudaux P. & Schrenzel J. (2004) *J Clin Microbiol*.
[44] Budimir A., Deurenberg R. H., Plecko V., Vink C., Kalenic S. & Stobberingh E. E. (2006) *J Antimicrob Chemother.,* 57, 331–334.