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Full Length Research Paper

Toxins and adhesion factors associated with *Staphylococcus aureus* strains isolated from diarrhoeal patients in Benin

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Staphylococcus aureus **is a causative agent of acute and infectious diarrhoea. In Africa, there is no sufficient information on the virulence and the degree of factors produced by its diarrhoea-isolated strains. Clinical features and virulence factors produced by *S. aureus* isolated from diarrhoeal-patients admitted at the Hospital Hubert Koutoukou Maga (HKM) in Cotonou was investigated. The virulence factors were screened by radial immunoprecipitation and multiplex polymerase chain reaction (PCR). Fifteen antibiotics were tested. Among independent 115 patients examined for diarrhoea, 32 had faeces positive for *S. aureus* isolated as pure culture. Most of these patients were hospitalized (21/32) and developed aqueous, bloody and painful diarrhoea, after antimicrobial therapy. About 62% were resistant to oxacillin. Genes encoding for clumping factor B and for laminin binding protein were detected in 62% of *S. aureus* isolates. About 94% of LukE-LukD producing strains have been isolated from patients developing post-antibiotic associated diarrhoea (PAAD). The Panton-Valentine Leucocidin (PVL) was produced by 19% of isolates, all from PAAD. This study points out new data concerning virulence factors and adhesion factor produced by *S. aureus* strains isolated from diarrhoea in Benin. The culture of the faeces will not always allow the diagnosis. It is important to update a technique, which enables researchers to carry out the virulence factors produced by these bacteria.**

Key words: Adhesion factors, diarrhoea, enterotoxins, *Staphylococcus aureus*, leucotoxins, Benin, Africa, PCR.

INTRODUCTION

Acute infectious diarrhoea is a major public health concern in the world, particularly in developing countries where such diseases remain endemic and the resulting dehydration complicates prognosis (Scopetti et al., 1983).

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Antibiotic-associated diarrhoea (AAD) represents a clinical entity leading to prolonged hospital stays and resulting in additional costs. Early diagnosis and appropriate therapy can reduce the morbidity of such infections. *Staphylococcus aureus* is the causative agent of various infections and the most frequently isolated bacterium in hospital settings (Gravet et al., 1999). The genetic content allows *S. aureus* to infect a wide range of host systems, being responsible for about 5 - 20% average

of nosocomial infections (Emori and Gaynes, 1993), but it often exists in a commensally state (Emori and Gaynes, 1993). About 30-50% of healthy individuals may be *S. aureus* occasional carriers (Emori and Gaynes, 1993). In 1982, *S. aureus* have been reported to be responsible for 10 cases of intestinal infections (McDonald et al., 1982). Nine (9) cases have been also reported in 1983 (Scopetti et al., 1983). The bacteria have been associated with antibiotic associated diarrhea (Scopetti et al., 1983; Gravet et al., 1999) where most of the isolates were methicillin resistant (Dagnra et al., 2001; Baba-Moussa et al., 1999). In addition, other antibiotics leading to therapeutic failures, sepsis and morbidity inducing extra costs in hospitalization have been reported (Edelsberg et al., 2008). While many strains can produce one or more enterotoxins (Baladan and Rasooly, 2001), others strains have been recognized to be responsible for gastrointestinal symptoms during food intoxication (Baladan and Rasooly, 2001). Risk factors in Europe and USA included antibiotic treatment, age, and recent intestinal surgery (Scopetti et al., 1983; Edelsberg et al., 2008). Fluoroquinolones given alone have been under-lined as favoring emergence of such diarrhoea (Gravet et al., 1999). The prevalence of methicillin-resistant *S. aureus* (MRSA) strains is increasing in Africa (Dagnra et al., 2001; Baba-Moussa et al., 1999). In most developing countries including Benin, patients suffering from diarrhoea often receive antibiotics without final diagnosis, which may be performed only when the diarrhea persists. Data on virulence factors produced by strains of *S. aureus* in Benin remain scarce (Voss and Doebbeling, 1995; Baba-Moussa et al., 2008). Despite an established involvement in diarrhea, *S. aureus* may not always be screened from stool samples at hospital and the risk of consecutive bacteraemia was pointed out (Gravet et al., 1999). Therefore, it is important to check its presence in diarrhoea. As for many *S. aureus* infections, virulence is determined by the interplay of adhesion, opsonization and cell targeting. It is also important to consider a wide panel of virulence determinants that may be involved with diseases.

The aim of this work was to determine toxins and adhesion factors produced by *S. aureus* isolated from patients suffering from diarrhoea and admitted at the Hospital Hubert Koutoucou Maga in Cotonou, Benin (CHU-HKM), for the examination and testing of their antibiotics resistance.

MATERIALS AND METHODS

Patients and isolates

A total of 115 patients suffering from diarrhea and admitted into the medical units of CHU-HKM of Cotonou (Benin) from October to December 2007, were investigated in this study. Diarrhoea was defined as at least three liquid or semi-liquid motions per day. Resolution of diarrhoea was defined as normal stool frequency and normal consistency for at least, 3 consecutive days. One liquid or semi-liquid motion per day per patient was collected during 48 h. Samples collection was carried out for a three month period. An individual questionnaire was filled for each patient, giving personal and clinical features including age, sex, clinical admission, beginning of an antibiotherapy to relevant diseases and associated symptoms, as well as the duration of the diarrhoea.

Identification of isolates

Bacterial samples (230) were collected at the bacteriology laboratory of CHU-HKM from the investigated patients as recommended (Freney et al., 2000). *S. aureus* strains have been primarily investtigated for morphological and biochemical characteristics including Gram staining, aerobic, anaerobic optional, catalase, acetoïne and coagulase producing. Other enteropathogenic bacteria have also been checked. Infection due to the parasites which were recognized as common diarrheic pathogens (*Amoebae*, *Giardia*, *Cryptosporidia*, *Strongyloides*) justified exclusion of patients. Only samples in which *S. aureus* were at pure/major culture of aerobic flora (≥ 90% UFC) have been considered for further investigation.

Antibiograms

Antimicrobial susceptibility was tested by agar diffusion method on Mueller Hinton agar (Bio-Rad-Diagnostic Pasteur, Marnes la Coquette, France) as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (NCCLS, 2002). Interpretation of antimicrobial susceptibility followed the recommendations of the Antibiogram Committee of the French Microbiology Society (CA-SFM) (Soussy, 2005). Evaluation of methicillin resistance was performed by plating the strains on buffered Mueller-Hinton (Bio-Rad-Diagnostic Pasteur, Marnes la Coquette, France) with NaCl 2% (wt/vol) at 37°C for 24 h. Fifteen antibiotics were tested (Table 1)

Phenotypic detection of the toxins

Bicomponent leucotoxins and epidermolysins

The different leucotoxins (Panton-Valentine leucocidin [PVL], leucotoxins LukE-LukD and epidermolysins A (ETA) and B (ETB) were evidenced from culture supernatants after 18 h of growth in yeast-Casamino acids-pyruvate (YCP) medium by radial gel immunodiffusion as previously described (Gravet et al., 1998).

Enterotoxins A, B, C and D and TSST-1

Five superantigens (enterotoxins A, B, C, D, E, TSST-1) were detected using the semi-quantitative reversed passive latex agglutination detection kits SET-RPLA^{TM} and TST-RPLA^{TM} (Oxoid, Basingstoke, England) as previously described (Brett, 1998; Gravet et al., 1999).

Genotypic detection of toxins and adhesion factors

The genes encoding for toxins and adhesion factor for which the

Table 1. Antibiotic susceptibility of S. *aureus* strains isolated from diarrhea in Benin.

Antibiotics	PAAD (%) (n = 21)	NAAD (%) (n = 11)	TOTAL PAAD+NAAD (n = 32)
Oxa	95	0	20/32 (62%)
PeG	100	100	32/32 (100%)
VA	0	0	0/32 (0)
TEC	0	0	0/32 (0)
FA	6	0	8/32 (25%)
K	29	18	8/32 (25%)
T	29	18	8/32 (25%)
Cl	22	0	7/32 (22%)
GM	6	0	2/32 (6%)
RA	6	0	2/32 (6%)
SXT	19	20	6/32 (19%)
OXF	0	0	0/32 (0)
PT	2	0	2/32 (6%)
LZ	0	0	0/32 (0)
E	57	45	17/32 (53%)

PAAD = Post-antibiotic associated diarrhoea; NAAD = non-antibiotic associated diarrhoea.

antibodies were not found have been checked by Multiplex PCR. Presence of genes encoding enterotoxins E (*see*), G (*seg*), H (*seh*), K (*sek*), L (*sel*), T (*set*), epidermolysin D (*etd*), Epidermal differenttiation Factors EDIN A, (*edinA*) and genes encoding 7 adhesion factors: Fibronectin Binding Proteins A and B (*fnbA and fnbB*), Bone Sialoprotein Binding Protein (*bbp*), Clumping Factor B (*clfb*), Fibrinogen Binding Protein (*fib*), Elastin Binding Protein (*ebp*) and Laminin Binding Protein (*lbp*) were detected by Multiplex PCR as previously described (Baba-Moussa et al., 2008). The sequences of primers used are shown in Table 2.

RESULTS

Clinical results

Among the 230 stool samples examined, 32 isolates, that is, 13.91% were identified as *S. aureus* while 18.7% (43/230) were identified as *Salmonella* sp., 13.5% (31/230) as *Vibrio cholerae*, 4.3%), (10/230) as *Shigella* sp., 35.21 % (81/230) as *E. coli*, 8.7% (20/230) were *Clostridium difficile* and 5.6% (13/230) for the other Gram negative bacteria. All the 32 strains of *S. aureus* were from 32 different patients. Among these patients, 17 were females (sex ratio = 1.13). Age of patients with *S. aureus* ranged from 1 year to 70 year-old (y.o.) for females, and from 8 months to 60 years for males. Mean age ± standard deviations were 31.8 ± 20.3 (1 - 70 y.o.) and 28.9 ± 23.1 (8 m – 60 y.o.) for females and males, respectively. Two patients were hospitalized in pediatric units, 10 in the parasitological unit, 5 had a surgical operation and 15 were admitted in medical units for different affections such as urinary tract infections (2), cirrhosis (5) and pancreatitis (2). The occurrence of malaria was not critical in this series. Among the 15 patients admitted in the medical units, 6 had an infection from unknown origin. Among the patients which had a surgical operation, 4 had appendicitis and 1 had an intestinal occlusion. Eleven (11) of these 32 patients did not receive an antibacterial treatment prior to the diarrhoea, but were concurrently admitted for malaria. Five of these eleven patients developed diarrhoea before admission to hospital. In fact, these five patients took a traditional anti-malarial treatment with medicinal plants at home. The other 21 patients with predominant *S. aureus* isolates in stools received an antimicrobial treatment for more than 2 days before the beginning of diarrhoea and more than 3 days after admission, which exclusively occurs at hospital after more than 3 days of antimicrobial treatment. These antibiotic treatments were consecutive to fever developed after surgery and mainly because of bacterial infection. Seven patients received an ampicillin regimen, 10 received amoxicillin, 3 had a gentamycin-based therapy and the last received cefuroxim. Another systematic sampling control was carried out four days after antibiotic treatment. The diarrhea mostly disappeared when an appropriate anti-staphylococcal treatment was administered, such as oral vancomycin® (14 cases) or stop of antibacterial treatment (18 cases). The surgical operation in most (5) of the cases concerned appendicitis (3) and the recovery was gained within one week to a 10 day period after treatment. Twenty of the 32 patients presented an elevation of C-reactive protein (CRP) and were febrile (38 - 40°C). Four patients consecutively developed a septicemia after diarrhea during their hospitalization and one died among them. A 4 year old child received a

Table 2. Primers used for PCR detection of genes encoding virulence factors in this study.

Oligonucleotides sequences	size pb/PCR set	accession n°
edinA 1: 5'-TCATAGAAGTATCTAATACTTCTTTAGCA-3'	604 – 2	M63917
edinA 2 : 5'-TCCAACACGGTATTCTGTGCCTCTAGGTA-3'		
etd 1: 5'-AATACATATGAAGAATCTGAAATTTTA3'	800 – 4	AB057421
etd 2: 5'-AAGTTATTCCATAATGATTAGAATGA-3'		
see 1: 5'-CTTACCGCCAAAGCTGTCG-3'	159 – 3	M21319
see 2': 5'-GTCCACTTGTAAATGGTAGCGAGAA-3'		
seg 1: 5'-AATTATGTGAATGCTCAACCCGAT-3'	408 – 3	AF064773
seg 2: 5'-CTTTAGTGAGCCAGTGTCTTGCTTTG-3'		
seh 1: 5'-CATCTACCCAAACATTAGCACC-3'	222 - 5	U11702
seh 2: 5-TAGAAATCAAGGTGATAGTGGCAA-3''		
sek 1: 5'-TGATACTCCTATAGCTAATCAACTACA-3'	300 – 3	U93688
sek 2: 5-ACATCAATCTCTTGAGCGGTAACA-3'		
sel 1: 5'-ACCAGAATCACACCGCTTAGAATAC-3'	422 – 2	AF217235
sel 2: 5'-TGGAATACTACACTCCCCTTATCAAAAG-3'		
set1-1: 5'-GAAGGTCTACAAGGCCAAAATGTCT-3'	363 – 6	BX571856
set1-2: 5'-TCAACACATCGCCCATGCGCTCGA-3'		
bbp 1: 5'-CGGCTAGTGATAATAAAGAAGTAGTG-3'	550 – 1	BA000018
bbp 2: 5'-CTTGTTGGAGCTGTAGCAACTGGTTT-3'		
clfB 1: 5'-ATTAGTGCAAACACAAACAGTGCG-3'	305 – 4	AJ224764
clfB 2: 5'-AGTTCCTTGCGCATTGGAAATCGT-3'		
ebp 1: 5'-AGACCAATCAGAATTAGAACATCA-3'	378 – 4	U48826
ebp2: 5'-TCAGAAACTGTTGAATGCTCAGTGT-3		
fib 1: 5'-AGCGGCAATAGGTATTACTACAACT3'	220 – 2	X72013
fib 2: 5'-CGAATGTACCATCGTTAAATTCAT-3'		
fnbA 1: 5'-TTAACTTGGGATAATGGTTTAGTTT-3'	273 – 7	AJ629521
fnbA 2: 5'-GCTGATGAATCCGTTTCTTCTATTG-3'		
fnbB 1: 5'-TGGAAGAAACTAAAGCGACAGGTAC-3'	317 – 1	AJ629502
fnbB 2: 5'-TTCTTTAAACGTATATCTAACTTTTC-3'		
lbp 1: 5'-TGGTGTATATGACTACAGTAAGTT-3'	410 – 5	AF065394
lbp 2: 5'-CGTTTGTAGCAACAGCAATATCAGC-3'		

digestive surgery (appendicitis).

Antibiotic resistance

Table 1 shows the susceptibilities to 15 antibiotics of the 32 isolates *S. aureus* issued from stools. All strains harbored resistance against penicillin G. Twenty-five percent (25%) of the isolates was simultaneously resistant to kanamycin, tobramycin and chloramphenicol, whereas 53% were resistant to erythromycin. Amongst all isolates, 62% harbored resistance against oxacillin/methicillin. All these 20 MRSA isolates were issued from the group of patients concerned with antibiotic-associated diarrhoea (AAD) (20/21). No tolerance was noticed to vancomycin, teicoplanin and linezolid.

Presence of genes encoding for adhesion factors

Genes encoding for clumping factor B (*ClfB*) and Laminin Binding Protein (*LBP*) were always simultaneously found in 62.5% of the *S. aureus* isolates associated with AAD. Similarly, 53% of strains harbored gene encoding for elastin binding protein (*EBP*) and 71.4% (15/21) of them were issued from AAD isolate (Table 3). Fibronectin binding protein A was present in all isolates, whereas the serotype B (FnbB) was found in 5 out of the 32 isolates, that is, 16%. Conversely, genes encoding for collagen (*Cbp*), bone sialo-binding protein (BBP) and fibrinogen

Table 3. Genes encoding adhesion factors from *S. aureus* isolates issued from diarrhea in Benin.

Genes	PAAD (%) (n = 21)	NAAD (%) (n = 11)	TOTAL PAAD+NAAD (%) (n = 32)
FnbB	14	18	16
fnbA	100	100	100
bbp	0	0	0
clfb	95	0	62.5
fib	0	0	0
ebp	71	18	53
lbp	95	0	62.5

PAAD = Post-antibiotic associated diarrhoea; NAAD = non-antibiotic associated diarrhoea.

Table 4. Toxins produced by *S. aureus* isolates issued from diarrhea in Benin (%).

Toxins	PAAD (%) (n = 21)	NAAD (%) (n = 11)	TOTAL PAAD+NAAD (%) (n = 32)
PVL	28.5	0	19
LukE-LukD	81	9	56
EtA	5	9	19
EtB	0	0	0
SEA	62	0	40
SEB	38	0	25
SEC	0	0	0
SED	0	0	0
TSST	29	9	22
etd	0	0	0
see	0	0	0
seg	14	0	5
seh	0	0	0
sek	0	0	0
sel	0	0	0
set	0	0	0
edinA	14	0	9

1=Post-antibiotic associated diarrhoea 2=Non-antibiotic associated diarrhoea

binding protein (Fib), were never detected in any isolates.

Toxin production

The panton and valentine leucocidin (PVL) was produced by 6/32 (19%) isolates (Table 4). All the PVL-producing strains were from patients with AAD. The leucotoxin LukE-LukD was produced in 56% of strains. Eighty percent (80%) of the isolates from the group of 21 patients with AAD produced antigens fully related to LukE-LukD. Enterotoxins A (SEA) and B (SEB) were produced in 40% (13/32) and 25% (8/32) of the 32 isolates, respectively, but 12.5% (4/32) produced both SEA and SEB simultaneously. All the enterotoxin A- and/or B-producing isolates are from the group of patients concerned by AAD and represent 81% (17/21) of these patients. All the strains that produced enterotoxins A also harbored leucotoxin LukE/LukD (13/32) (40%). A total of 78% (25/32) of the isolates produced or harbored the gene encoding at least one enterotoxin. Six isolates produced simultaneously the PVL and LukE-LukD and are among the isolates corresponding to AAD. The TSST was produced by 7/32 of these isolates and ETA was produced by 2/32. Epidermal differentiation inhibitory factor A (EDINA) was present in 3 out of 32 (9%) isolates.

DISCUSSION

Few data were available on the resistance of *S. aureus* strains to antibiotics in Africa (Voss and Doebbeling,

1995), particularly in Benin. The results obtained from this study showed that most of *S. aureus* isolates were resistant to methicillin (62%). *S. aureus* isolates involved in diarrhoea harbor specific resistance to antibiotics. 95% of the 32 isolates resistant to methicillin corresponded to post-antibiotic associated diarrhoea and concerned hospitalized patients. It has been reported that 5 MRSA out of 72 strains, that is, only 7%, were isolated from sporadic diarrhoea in children less than 5 years old in Nigeria (Efuntoye and Adetosoye, 2003). This difference could be due to the high number of patients, including adults and old people which have been investigated in this study. Nevertheless, our results are similar to those obtained in a study carried out at the CHU of Tokoin in Lomé, Togo which showed 67% of resistance to methicillin (Dagnra et al., 2001). However, in this study most of the resistant strains to methicillin have been isolated from patients under antibiotherapy. MRSA were assumed to be the cause of 10 cases of antibiotic-associated diarrhoea observed over a 12-month period at the Australian Royal Melbourne Hospital (McDonald et al., 1982). Moreover, it has been reported that MRSA enterocolitis can occur in patients with antibiotic-related diarrhoea (Fujita et al., 2004). The diarrhoeas often followed antibiotic treatment and practically disappeared when the treatment stopped or when vancomycin was administrated. Diarrhoea was observed in 30% of cases during antibiotic treatment (http://www.medix.free.fr/sim/diarrhee-infectieuse-urgence.php; 2003; http://www.medix.free.fr/sim/diarrhee-infectieuse-urgence.php, 2009). Generally, they are benign, but under some circumstances (e.g., ecological perturbation of intestinal flora induced by the antibiotherapy) they allowed emergence of infectious and pathogenic agents such as, *S. aureus*. In France where medical care is better than in African countries, more than 20% of nosocomial infections were related to the MRSA. Although no data were available for African countries, it could be anticipated that the frequency of such infections might be that higher. *S. aureus* resistance to methicillin became a worldwide problem (Wanne et al., 2005). Nowadays treatment focused on vancomycin. As shown in Table 1, many of the collected *S. aureus* isolates in Benin resisted to that antibiotic.

Table 4 showed that 65% of strains produced enterotoxins A or B. Enterotoxin A was produced by 40%, whereas the B type was produced by 25% of the strains. It is well known that enterotoxin A was frequently associated with staphylococcal food-poisoning (SFP) (John et al., 2005). The type B might be responsible for 20% of these kinds of infections. The two types of enterotoxin are responsible for 80% of SFP in France (John et al., 2005). This study showed that enterotoxin-producing isolates of MRSA may cause nosocomial antibiotic-associated diarrhoea (John et al., 2005). The prevalence of these enterotoxins observed in Benin for *S. aureus* strain isolated from diarrhoea motion was similar to that observed in France (Gravet et al., 1999). A study made in Germany showed that among the 198 *S. aureus* investigated from stools, a total of 114 *S. aureus* strains produced the following enterotoxins *in vitro*: SEA, 36; SEB, 20; SEC, 19 and SED, 68 (Flemming and Ackerman, 2007). Enterotoxins are excreted and activate intestinal wall receptors, which stimuli reached the vomiting centre through the vague nerve (Ghia et al., 2006; Borison and Wang, 1950). *S. aureus* may produce other non-identified enterotoxins using serologic methods. Genes encoding for enterotoxins G (9%) and E (6%) were identified in this study by multiplex PCR.

The panton and valentine leucocidin (PVL) was produced by 6 out of 32 strains, that is, 19%, whereas 56% of strain produced leucotoxin lukE-LukD. Strains producing PVL also harbor LukE/LukD. All the PVL-producing strains were MRSA and concerned hospitalized patients. The results obtained for PVL production using strains collected in Benin were in contrast with those obtained in a previous study where any strain produce PVL (Gravet et al., 1999). This difference could be explained by the fact that PVL is produced at a level of 30% by African strain when the strain was isolated from all kind of infection (Baba-Moussa et al., 1999), the percentage being only 2.5% in Europe. Nineteen percent (19%) of the strains produced epidermolysin A (Table 4). Interestingly, all the strains that produced enterotoxin A also harbored leucotoxin LukE-LukD. These results were similar to those obtained in France using *S. aureus* isolated from post antibiotic diarrhoea (Gravet et al., 1999). This study showed that *S. aureus* strains isolated from post antibiotic diarrhea often produced LukE-LukD and SEA. Results from a thesis at CHU of Lille and Amiens CHU in France, reported the pathogenic role of *S. aureus* in post-antibiotic associated with diarrhea (Gravet, 2001). *S. aureus* strains isolated from pure cultures have been studied at the Bacteriologic Institute at the Medicine Faculty of “Université Louis Pasteur” in Strasbourg (France) and the study showed that all these strains produced enterotoxin A and Leucotoxin LukE-LukD (Gravet et al., 1999). In contrast, this study reports that 6 out of 21 strains from post-antibiotic associated with diarrhoea produced the toxic shock syndrome toxin (TSST). One case of pseudomembranous enterocolitis caused by methicillin-resistant *S. aureus* in Japan was founded despite vancomycin administration. The patient died from a septic shock (Fujita et al., 2004). Consequently, TSST may play a role in post-antibiotic diarrhoea associated to *S. aureus*.

S. aureus virulence requires a colonizing step involving fixation on target organs. Adhesion factors that have been identified in this study play an important role in this fixation. Table 3 shows that the factors frequently

encountered in strains were *ClfB* (62.5%), *EBP* (53%) and *LBP* (62.5%). All isolates carrying *ClfB* and LBP were from patient with AAD. Although these data were specific to *S. aureus* isolates issued from diarrhea, no evidence suggested a possible close relation with other infectious strains. It has been reported that these three adhesion factors were present with similar occurrences in *S. aureus* strains isolated from nasal, osteomyelitis and cardiac puncture (Tristan et al., 2003). The frequency of these adhesion factors in isolates from diarrhoea motions may be due to the fact that *S. aureus* is an entero-pathogenic bacteria, which can colonize small mucous intestinal without overrunning it, using attachment factors (Hance et al., 1998). *S. aureus* settles in cells through proteins surface, adhesion factors, which are anchored in the peptidoglycan. *S. aureus* strains that specifically express receptors for fibrinogen and fibronectin are associated with infective endocarditis, while strains that produce receptors for bone sialoprotein, collagen and fibronectin are associated with osteomyelitis and arthritis (Tristan et al., 2003). A markedly high frequency of some adhesion factors was observed with these diarrheas: *FnbA*, *clf, lbp,* but they do not seem to be associated with a given group of isolates

Conclusion

Antibiotic treatment is not always required in the presence of a pathogenic agent and even when required, pathogenic agent may be useful. The purpose is to avoid complications in case of bacteriemia. Bacterial food contamination may induce gastro-intestinal symptoms or other ailments. These ailments are derived from an earlier excreted toxin when foods are ingested. Therefore, the culture of the motion will not generally allow the diagnosis. It is important to update a technique, which enables carrying out the virulence factors produced by these bacteria. Patients with staphylococcal diarrhoea present a significant risk of cross infection. Early diagnosis, treatment and isolation are recommended. Greater recognition of this disease should result in more rapid appropriate treatment of affected patients. MRSA enterocolitis can occur in patients with antibiotic-related diarrhoea and physicians should be aware of its rapid clinical course and possible lethal outcome.

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Abbreviations:

Antibiotics: Ox, Oxacillin; **PeG,** Penicillin G; **VA,** Vancomycin; **TEC,** Teicoplanin; **RA,** Rifampicin; **FA,** Fusidic acid; **GM,** Gentamicin; **K,** Kanamycin; **T,** Tobramycin; **CI,** Chloramphenicol; **SXT,** Trimetroprim sulfamethozolin; **OXF,** Ofloxacin; **PT,** Pristinamycin; **LZ,** Linezolid; **E,** Erythromicin. **Enterotoxins:** E (*see*), G (*seg*), H (*seh*), K (*sek*), L (*sel*), T (*set*), ***etd*****,** epidermolysin D; **adhesion factors:** ***fnbA and fnbB*****,** Fibronectin Binding Proteins A and B**;** ***bbp*****,** Bone Sialoprotein Binding Protein; ***clfb*****,** Clumping Factor B; ***fib*****,** Elastin Binding Protein; ***ebp,*** Fibrinogen Binding Protein; ***lbp*****,** Laminin Binding Protein; **ETA,** Epidermolysins A; **ETB,** Epidermolysins B.

REFERENCES

Baba-Moussa L, Sanni A, Dagnra AY, Anagonou S, Prince-David P, Edoh V, Befort JJ, Prévost G, Monteil H (1999). Approche épidémiologique de l'antibiorésistance et de la production de leucotoxines par les souches de *Staphylococcus aureus* isolées en Afrique de l'Ouest. Méd. Mal. Infect. 29: 689-696.

Baba-Moussa L, Anani L, Scheftel JM, Couturier M, Haïkou N, Hounsou F, Monteil H, Sanni A, Prévost G (2008). Virulence factors produced by strains of Staphylococcus aureus isolated from urinary tract infections, J. Hosp. Infect. 68: 32-38.

Baladan N, Rasooly A (2001). Analytical chromatography for recovery of small amounts of staphylococcal enterotoxins from food. Int. J. Food Microbiol. 64: 33-40.

Borison HL, Wang SC (1950). Further Studies on the Vomiting Center Federation. Proceedings, 9: 14-15.

Brett M (1998). Kits for the detection of some bacterial food poisoning toxins: problems, pitfalls and benefits. J. Appl. Microbiol. 84: S110-S118

Dagnra AY, Hounkpatin A, Prince-David M (2001). Fort pourcentage de souches de *Staphylococcus aureus* résistantes à la méthicilline au CHU de Lomé (Togo). Med. Mal. Infect. 31: 14-15.

Edelsberg J, Berger A, Schell S, Mallick R, Kuznik A, Oster G (2008). Economic consequences of failure of initial antibiotic therapy in hospitalized adults with complicated intra-abdominal infections. Surgl. Infect. 9: 335-347.

Efuntoye MO, Adetosoye AI (2003). Enterotoxigenicity and drug sensitivity bof Staphylococci from children aged five years and below with sporadic diarrhoea. E. Afr. Med. J. 12: 656-659.

Emori TG, Gaynes RP (1993). An overview of nosocomial infections, including the role of microbiology laboratory. Clin. Microbiol. Rev. 6: 428-442.

Flemming K, Ackermann G (2007). Prevalence of enterotoxin producing *Staphylococcus aureus* in stools of patients with nosocomial diarrhea. Infection, 35: 356-358.

Freney J, Renaud F, Hansen W, Bollet C (2000). *Précis de Bactériologie Clinique.* Eds ESKA Paris.

Fujita K, Honda Y, Kurihara T, Ohba H, Okimoto N (2004). A case of pseudomembranous enterocolitis caused by methicillin-resistant *Staphylococcus aureus*. *Kansenshogaku Zasshi.* 78: 905-909.

Ghia JE, Blennerhassett P, Kumar-Ondiveeran H, Verdu EF, Collins SM (2006). The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. Gastroenterology. 131: 1122-1130.

Gravet A, Colin D, Keller D, Girardot R, Monteil H, Prevost G (1998). Characterization of a novel structural member, LukE-LukD, of the bi-component staphylococcal leucotoxins family. FEBS Lett. 436: 202-208.

Gravet A, Rondeau M, Harf- Monteil C, Grunenberger F, Monteil H, Scheftel JM, Prevost G (1999). Predominant *Staphylococcus aureus* isolated from antibiotic-associated diarrhea is clinically relevant and

produces enterotoxin A and the bicomponent toxin LukE-LukD. J. Clin. Microbiol. 37: 4012-4019.
Gravet A (2001). PhD Thesis, Université Louis Pasteur, Strasbourg, France.
Hance P, Teyssou R, Nicand E, Buisson Y (1998). Sources alimentaires des diarrhées bactériennes. Toxi-infections alimentaires collectives. *Médecine thérapeutique/Pédiatrie*. 1 : 25-30.
John M, Boyce MD, Nancy L, Havill MT (2005). Nosocomial antibiotic-associated diarrhea associated with enterotoxins-producing strains of methicillin-resistant *Staphylococcus aureus*. Am. J. Gastroenterol. 100: 1828-1834.
McDonald M, Ward P, Harvey K (1982) Antibiotic-associated diarrhea and meticillin-resistant *Staphylococcus aureus*. Med. J. Aust. 1: 462-464.
NCCLS (2002). Performance standards for antimicrobials susceptibility testing. NCCLS, Wayne, Pa. Supplement M100-S12 .
Scopetti F, Orefici G, Biondi F, Benini F (1983). Staphylococcus aureus resistant to methicillin and gentamicin as a cause of outbreak of epidemic enteritis in a hospital. *Boll. Ist. Sieroter Milan*. 62: 406-411.
Soussy CJ (2005). Société Française de Microbiologie, communiqué Coordonnateur; http://www.sfm.asso.fr/ Tristan A, Ying L, Bes M, Etienne J, Vandenesch F, Lina G (2003). Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. J. Clin. Microbiol. 41: 4465-4467.
Voss A, Doebbeling BN (1995). The worldwide prevalence of methicillin-resistant *Staphylococcus aureus*. Int. J. Antimicrob. Agents, 5: 101-106.
Wanne WJ, Spalburg E, Heck ME, Pluister GN, Tiemersma E, Willems RJ, Huijsdens XW, de Neeling AJ, Etienne J (2005) Emergence of virulent methicillin-resistant *S. aureus* strains carrying Pantone Valentine leucocidin genes in the Netherlands. J. Clin. Microbiol. 43: 3341-3345
www.medix.free.fr/sim/diarrhee-infectieuse-urgence.php (2003). Diarrhées aiguës infectieuses. Cours de réanimation-urgences. Medix cours de médecine.
www.medix.free.fr/sim/diarrhee-infectieuse-urgence.php (2009). Diarrhées aiguës infectieuses. Cours de réanimation-urgences. Medix cours de médecine.