Environmental heterogeneity of *Staphylococcus* species from alkaline fermented foods and associated toxins and antimicrobial resistance genetic elements

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Abstract

Different samples of three products including Bikalga and Soumbala from Burkina Faso (West Africa) and Ntoba Mbodi from Congo-Brazzaville (Central Africa) were evaluated. The bacteria (400) were phenotyped and genotypically characterized by Rep-PCR, PFGE, 16S rRNA and *rpoB* gene sequencing and *spa* typing. Their PFGE profiles were compared with those of 12,000 isolates in the Center for Disease Control (CDC, USA) database. They were screened for the production of enterotoxins, susceptibility to 19 antimicrobials, presence of 12 staphylococcal toxin and 38 AMR genes and the ability to transfer erythromycin and tetracycline resistance genes to *Enterococcus faecalis* JH2-2.

Fifteen coagulase negative (CoNS) and positive (CoPS) species characterised by 25 Rep-PCR/PFGE clusters were identified: *Staphylococcus arlettae*, *S. aureus*, *S. cohnii*, *S. epidermidis*, *S. gallinarum*, *S. haemolyticus*, *S. hominis*, *S. pasteuri*, *S. condimenti*, *S. piscifermentans*, *S. saprophyticus*, *S. sciuri, S. simulans*, *S. warneri* and *Macrococcus caseolyticus*. Five species were specific to Soumbala, four to Bikalga and four to Ntoba Mbodi. Two clusters of *S. gallinarum* and three of *S. sciuri* were particular to Burkina Faso. The *S. aureus* isolates exhibited a *spa* type t355 and their PFGE profiles did not match any in the CDC database. Bacteria from the same cluster displayed similar AMR and toxin phenotypes and genotypes, whereas clusters peculiar to a product or a location generated distinct profiles. The toxin genes screened were not detected and the bacteria did not produce the staphylococcal enterotoxins A, B, C and D. AMR genes including *blaz*A, *cat*501, *dfr*(A), *dfr*(G), *mec*A, *mecA1*, *msr(*A) and *tet*(K) were identified in CoNS and CoPS. Conjugation experiments produced JH2-2 isolates that acquired resistance to erythromycin and tetracycline, but no gene transfer was revealed by PCR.

The investigation of the heterogeneity of *Staphylococcus* species from alkaline fermented foods, their relationship with clinical and environmental isolates and their safety in relation to antimicrobial resistance (AMR) and toxin production is anticipated to contribute to determining the importance of staphylococci in alkaline fermented foods, especially in relation to the safety of the consumers.

Key words: Alkaline fermented foods; *Staphylococcus* spp.; diversity; distribution; safety; toxins; AMR

1. Introduction

Alkaline fermented foods are products that are generally rich in proteins (30-50%) and their production involves a fermentation process associated with an increase of pH to values as high as 10.0 (Parkouda et al., 2009; Vouidibio Mbozo et al., 2017). They are mainly known in Africa and Asia, where they constitute low cost sources of protein foods and play an important part in the diet of people (Parkouda et al., 2009). It is generally recognized that the pH rise is the result of accrued degradation of raw material proteins that leads to the release of peptides, essential amino acids as well as alkalinizing substances such as ammonia (Kiers et al., 2000). In some cases, alkali-treatments during the production are responsible for the pH augmentation (Parkouda et al., 2008).

Bikalga, Soumbala, and Ntoba Mbodi are alkaline fermented foods which are produced through the traditional fermentation of roselle seeds (*Hibiscus sabdariffa*), African locust bean seeds (*Parkia biglobosa*) and cassava leaves (*Manihot esculenta* Crantz) respectively. Production of Bikalga and Soumbala comprises several processing stages of the seeds: cleaning, boiling (about 95°C for 24-40 h), dehulling (Soumbala), fermentation (48-72 h), steaming (Bikalga) and drying (Ouoba et al., 2008a; Parkouda et al., 2008; Parkouda et al., 2009). They are the most popular traditional fermented foods used as condiments in Burkina Faso (West Africa) and are also produced in other African countries. Ntoba Mbodi, which is popular in the Republic of the Congo (Central Africa), is made of cassava leaves and the production process does not involve heating steps. After being cleaned and cut, the leaves are distributed into small portions in larger leaves, wrapped and left to ferment for 2-4 days (Vouidibio Mbozo et al., 2017). The fermented cassava leaves are then used to prepare sauces, which are eaten with starch-based foods. In Bikalga, Soumbala and Ntoba Mbodi, pH

values up to 8.5, 9 and 10 have been respectively reported (Ouoba, 2015; Ouoba, 2017; Parkouda et al., 2009; Vouidibio Mbozo et al., 2017).

The key microorganisms involved in alkaline fermentations are *Bacillus* species such as *B. subtilis*, *B. licheniformis*, *B. amyloliquefaciens*, *B. pumulis*, and *B. xylanilyticus* that can degrade proteins, lipids, and carbohydrates of the raw materials and bring about the nutritional quality and safety of the product (Parkouda et al., 2009). However, secondary microorganisms such as lactic acid bacteria, *Staphylococcus* spp. and various Gram-positive and Gram-negative bacteria are often isolated with no clear indication of their role in the fermentation. The production techniques of most alkaline fermented foods are traditional, leading to variability of microbial population, nutritional quality, product stability and potential safety issues. It is believed that some of the secondary microorganisms are in fact contaminants from the producers, processing materials and environments that have poor hygienic quality in most traditional fermentation settings. Some secondary microorganisms are part of the original microbiota of the raw materials that are suppressed by products released during the fermentation. For example, those that are alkali sensitive will be eliminated or reduced during the fermentation.

Staphylococci are Gram-positive non-motile cocci ubiquitously spread in nature, and are commonly isolated from humans, animals, food and environmental sources. More than 70 species and subspecies of the *Staphylococcus* genus have been characterized so far (LPSN, 2018) with *Staphylococcus aureus* being one of the most studied species due to its clinical importance. *Staphylococcus* species are usually divided into two groups i.e. coagulase-positive (CoPS) and coagulase-negative (CoNS) according to their ability to coagulate rabbit plasma. Various *Staphylococcus* species have been recurrently isolated in many alkaline and non-

alkaline vegetable based fermented foods, but their role in such fermentation is unknown. However, their importance in the fermentation of meat-based products has been well documented. They occur in the production of various types of sausages where they constitute a part of the autochthonous microbiota or are added as starter cultures in combination with lactic acid bacteria (Fonseca et al., 2013; Milicevic et al., 2014). *Staphylococcus equorum*, *S. saprophyticus S. xylosus* and *S. carnosus* are the most common species found in fermented sausages, but other species such as *S. warneri*, *S. vitulinus*, *S. pasteuri*, *S. epidermidis*, *S. lentus*, *S. haemolyticus*, *S. intermedius*, and *S. hominis* can also be occasionally isolated (Milicevic et al., 2014). They have been reported to be involved in biochemical activities during meat fermentation such as the degradation of proteins, lipids and peroxide as well as the reduction of nitrite (Casaburi et al., 2008; Milicevic et al., 2014). These activities generate compounds such as peptides, amino acids, carbonyls and volatile compounds that contribute to the nutritional quality, specific organoleptic properties and safety of the fermented meat products (Milicevic et al., 2014).

Although beneficial properties of *Staphylococcus* species in food have been documented, many species are most known worldwide for causing food poising and other types of clinical infections in humans and animals including e.g. bacteraemia, wound infections, pyogenic lesions, and mastitis. Moreover, they present detrimental antimicrobial resistance (AMR) profiles that make some of their infections hard to treat. With regard to toxin production in food, staphylococcal food poisoning is one of the most common food-borne diseases. It results from the ingestion of heat stable staphylococcal enterotoxins (SEs) produced in food by enterotoxigenic strains of mainly *S. aureus* (Hennekinne et al., 2012). Symptoms related to ingestion of such toxins are fast-acting, occurring generally within 30 min to 8 h and lasting for about 24

hours. Individuals generally experience vomiting, nausea, stomach cramps, and diarrhoea. Severe illnesses related to staphylococcal food poisoning are rare but occasionally, it can be deadly, especially in children, elderly and patients weakened by a long-term illness. *Staphylococcus aureus* is considered one of the world's leading causes of disease outbreaks associated with food consumption. Other CoPS such as *S. intermedius* and CoNS such as *S. epidermidis*, *S. sciuri*, *S. simulans*, *S. xylosus*, *S. equorum*, *S. lentus* and *S. capitis* have been reported to harbour genes coding for SEs and to produce some SEs (Casaes Nunes et al., 2015; Crass and Bergdoll, 1986). However, information on their real implication in staphylococcal food poising outbreaks is scarce (Breckinridge and Bergdoll, 1971; Podkowik et al., 2013). Significantly, the incidence of AMR has continually increased in staphylococci, as well as in other types of bacteria. This is attributed to factors such as the frequent antibiotic administration to livestock, or misuse and overuse of antimicrobials. Therefore, the food chain constitutes one possible route of AMR transfer (Angulo et al., 2004). Both CoPS and CoNS from food origin exhibit a wide range of AMR genetic elements coding for resistance to different antimicrobials such as methicillin, tetracycline, penicillin, and vancomycin (Gundogan et al., 2005; Simeoni et al., 2008). The most frequently encountered resistant staphylococcus is methicillin-resistant *Staphylococcus aureus* (MRSA), which is resistant to multiple antimicrobials and is responsible for hard-to-treat infections. Thus, it constitutes a serious public health concern, as it causes thousands of deaths per year worldwide and constitutes a global economic burden.

The study aimed to screen the environmental heterogeneity of *Staphylococcus* species from alkaline fermented foods, their relationship with clinical isolates worldwide and their safety with regards to genes encoding antimicrobial resistance

(AMR) and toxin production. This is anticipated to elucidate their distribution and importance in such foods, especially in relation to the safety of the consumers.

2. Materials and Methods

2.1 Identification of the bacteria

2.1.1 Phenotypic characterisation

The bacteria (400) were isolated from different samples of Bikalga (9), Ntoba Mbodi (8) and Soumbala (10) collected at different markets and production sites in Burkina Faso (Bikalga and Soumbala) and the Republic of the Congo (Ntoba Mbodi), from areas constituting major consumers of these food products. Each sample (10 g) was homogenised for 1 min in 90 ml of maximum recovery diluent (MRD, Oxoid) and ten-fold dilutions (10^{-2} - 10^{10}) prepared. The dilutions (0.1 ml) were spread on nutrient agar (NA; Oxoid CM0003, Basingstoke, UK) for the enumeration of total aerobic mesophilic bacteria. The plates were incubated aerobically at 37°C and the count recorded after 2 days. For enumeration and isolation of the presumptive *Staphylococcus* population, the dilutions were spread on Baird Parker (BPA; Oxoid) and Mannitol Salt (MSA; Oxoid) agars and the plates incubated aerobically at 37°C for 2 days. Bacteria isolated from the two the latter media were purified by repeated streaking on NA, maintained in nutrient broth (NB; Oxoid CM001) containing 20% (v/v) glycerol (Sigma G8773, Gillingham, UK) as well as on cryobeads and stored at -20°C for further analyses.

For the phenotypic characterization, the isolates were streaked on NA and examined for colony and cell morphology, as well as tested for Gram, catalase, oxidase and coagulase reactions. The Gram reaction was evaluated using the KOH method (Gregersen, 1978) and the oxidase test using *Pseudomas fragi* as positive control and *Brochothrix thermosphacta* as negative control. The coagulase test was

performed using the Staphylase test kit (Oxoid DR0595) and following the manufacturer's instructions. The cell morphology was determined by light microscopy (Nikon Model Eclipse, E400, Japan). The phenotypic screening resulted in the elimination from the study of 310 non-staphylococcal isolates which were able to grow on BPA and MSA and whose colony morphologies initially suggested they were potential *Staphylococcus* spp. Thus, 90 isolates were retained for further characterization.

2.1.2 Molecular typing

2.1.2.1 DNA extraction

The bacteria were grown on Brain Heart Infusion Agar (BHI-A) prepared by mixing BHI (Oxoid CM1135) and technical agar (Oxoid LP0013, 15g/L). After incubation at 37°C for 48 h, the DNA was extracted from purified cultures following the manufacturer's instructions using InstaGene Matrix (Bio-Rad 732-6030, Hemel Hempstead, UK).

2.1.2.2 Repetitive element palindromic-polymerase chain reaction (rep-PCR)

The isolates were characterized by rep-PCR using the GTG5 primer as described by Ouoba et al. (2008a). Bacteria showing the same DNA profile were clustered in the same group by visual screening and cluster analysis using the Bionumerics system (2.50, Dice's Coefficient of similarity, Unweighted pair group method with arithmetic: UPGMA; Applied Maths, Saint-Martens-Latem, Belgium).

2.1.2.3 Pulse Field Gel Electrophoresis (PFGE)

In order to assess the worldwide distribution of the types of *S. aureus* found in the fermented foods, PFGE of SmaI-digested genomic DNA was performed according to the method proposed by the Center for Disease Control and Prevention/Pulse Net (CDC, USA) as described by Van Balen et al. (2013). The Salmonella serotype

Branderup strain H9812 digested with XbaI was used as a molecular size marker. The PFGE profiles of the *Staphylococcus* isolates were compared with those in a CDC database that contains worldwide profiles of about 12,000 *S. aureus* strains recovered from outbreaks, surveillance, reference and the environment, and submitted to the database.

2.1.2.4 Sequencing of the 16S rRNA and rpoB genes

The bacteria were identified by the amplification and sequencing of the 16S rRNA and *rpoB* genes. For the 16S rRNA gene amplification, the method and the pA and pE primers described by Ouoba et al. (2008a) were used. For the amplification of the *rpoB* gene, primers 2491F and 3554R described by Drancourt and Raoult (2002) were used with the following PCR mixture: 5 µl of 10 X PCR buffer containing 15 mM of $MgCl_2$ (Applied Biosystems N8080160), 0.5 µl of dNTP (1.25 mM), 0.5 µl of each primer (21 pmol/µl), 0.2 µl of AmpliTaq polymerase (5 U; Applied Biosystems N808-0160) and 41.3 µl of sterile high purity water. The PCR conditions were as follows: initial denaturation at 94°C for 2 min, 35 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 1 min and a final extension step at 72°C for 5 min.

After the amplification of both genes, positive amplicons were checked by electrophoresis and purified using the QIAquick PCR purification kit (Qiagen, Crawley, UK). The 16S rRNA and *rpoB* genes were sequenced (SourceBioscience, Cambridge, UK) using primers pD (3.2 pmol/µl) and 2491F/ 3554R (3.2 pmol/µl) respectively. The 16S rRNA gene sequences were analysed in the ezbiocloud server (Yoon et al., 2017) whereas those of the *rpoB* gene were screened in the GenBank/EMBL/DDBJ database (National Center for Biotechnology, MD, USA).

2.1.2.4 *Spa* typing

A multiplex PCR method and primers recommended by the European Reference Laboratory for antimicrobial resistance (EURL-AR, 2012), and that allows at the same time the detection of the *spa* gene and the *S. aureus* methicillin resistance genes *mec*A, *mec*C was used. The method includes the use of a PCR mixture containing 2.5 µl of 2xGreen PCR Master Mix (Thermo Scientific K1081), 2 µl (10 µM) of a mixture of the forward primers, 2 µl (10 µM) of a mixture of the reverse primers and 6.5 µl of high purity water. The PCR conditions described by Stegger et al. (2012) were applied.

2.2 Screening haemolysis and toxin production potential of the bacteria

2.2.1 Haemolytic activity on blood agar

The *Staphylococcus* isolates were screened for their haemolytic activity on blood agar as follows: Columbia agar base (Oxoid CM003) was autoclaved at 121 °C for 15 min and sheep blood (5%; Oxoid SR0051B) added after cooling to 50 °C, before distribution into Petri dishes. The isolates were then streaked on the agar and incubated at 37 °C for 48 h. Haemolysis was identified by the appearance of a zone of clearing around the colonies.

2.2.2 Production of staphylococcal enterotoxins A, B, C and D

The ability of the bacteria to produce the staphylococcal enterotoxin A, B, C and D was investigated using the staphylococcal enterotoxin reversed passive latex agglutination (SET-RPLA) toxin detection kit (Oxoid TD0900). Each isolate was grown at 37°C for 24h in 10 ml of Tryptone Soya broth (TSB; Oxoid CM0129) and 1ml of the culture centrifuged using a benchtop centrifuge at 3000 rpm for 20 min. The presence of toxins was screened in the supernatant according to the manufacturer's instructions.

2.2.3 Detection of staphylococcal enterotoxin, exfoliative and toxic shock syndrome toxin genes

253 The isolates were tested for the presence of the staphylococcal enterotoxin
254 genes *sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, exfoliative toxin genes *eta* and *etb*,
255 and toxic shock syndrome toxin gene tsst-1. Primers described by Johnson et al.
256 (1991), Omoe et al. (2002) and Nashev et al. (2004) were used. The PCR mixture was
257 that used by Ouoba et al. (2008b). The PCR conditions were as follows: 94°C for 2
258 min followed by 35 cycles of denaturation at 94°C for 1 min, annealing at the
259 corresponding temperature for 1 min and elongation at 72°C for 1 min. The PCR ended
260 with a final extension at 72°C for 5 min.

261 2.3 Screening the AMR phenotypic and genotypic profiles of the bacteria

262 2.3.1 Inoculum preparation

263 The isolates were grown on BHI-A and pure colonies re-suspended in 1 ml of
264 Maximum Recovery Diluent (MRD; Oxoid CM0733). A quantity of the latter suspension
265 was transferred to 10 ml of MRD to obtain a turbidity of 0.5 (10^7-10^8 CFU/ml) on the
266 McFarland scale using a nephelometer (TREK Diagnostic systems, West Sussex,
267 UK).

268 2.3.2 Determination of the susceptibility of the bacteria to antimicrobials

269 The Minimal Inhibitory Concentration (MIC) for 19 antimicrobials (Table 1) was
270 determined using a 96 well sensititre^{TM} plate (EUST, TREK Diagnostic Systems Ltd,
271 East Grinstead, UK) containing varying amounts of each antimicrobial. The method
272 recommended by EURL-AR (DTU, Denmark) was applied (EURL-AR, 2009). Briefly,
273 50 µl of the inoculum was mixed with 10 ml Mueller Hinton broth (MHB; Oxoid
274 CM0405) and 50 µl of this mixture were dispensed in each well. Incubation was carried
275 out aerobically at 37°C for 24 h and visible growth recorded. The susceptibility of the
276 isolates to each antimicrobial was established using the cut-off values recorded in
277 Table 1.

278 2.3.3 Detection of AMR genes

279 The presence of 38 genes encoding resistance to chloramphenicol,
280 erythromycin, gentamycin, methicillin, penicillin, quinupristin/dalfopristin, tetracycline,
281 trimethoprim, streptomycin and vancomycin were investigated (Table 1).

282 For the detection of the genes, the PCR mixtures described by Naghizadeh
283 Raeisi et al. (2018) were used. However, for the detection of the *S. aureus* methicillin
284 resistance genes *mec*A and *mec*C, the multiplex PCR (described in 2.1.2.4) and
285 primers recommended by EURL-AR (2012) were used. For the *mecA1* gene pertaining
286 to *S. sciuri*, the same mixture (except the primers) as that used for the detection of
287 erythromycin (Naghizadeh Raeisi et al., 2018) was applied. The references of the
288 primers are depicted in Table 1. Positive control isolates (EURL-AR) for most genes
289 screened were included. Moreover, all positive amplicons were sequenced to confirm
290 the identity of the genes.

291 All PCR amplifications were performed in a thermocycler (GeneAmp PCR 2700
292 system) using the following program: initial denaturation at 94°C for 3 min, followed by
293 25 - 35 cycles of 94°C for 1 min, 45-68°C for 1 min depending on the annealing
294 temperature for the individual set of primers, 72 °C for 1 min and a final extension at
295 72 °C for 10 min. After purification, the presumptive positive amplicons were
296 sequenced using the amplification primers at a concentration of 3.2 pmol/µl and the
297 similarity of the sequence tested against published sequences in GenBank.

298 2.3.4 Screening the transferability of AMR

299 The ability of *S. warneri* 105 to transfer the *msr*(A) gene to *Enterococcus*
300 *faecalis* JH2-2 and *S. saprophyticus* A17M105 to transfer the *tet*(K) gene to the same
301 recipient was investigated by conjugation experiments according to Ouoba *et al.*
302 (2008c) and Naghizadeh Raeisi et al. (2018). The donors are resistant to erythromycin

(*S. warneri*) and tetracycline (*S. saprophyticus*), but sensitive to rifampicin and fusidic acid. The recipient *E. faecalis* JH2-2 is resistant to rifampicin and fusidic acid and sensitive erythromycin and tetracycline.

Inocula were prepared as described above and 10 ml of the donor suspension and 1 ml of that of the recipient mixed and filtered through a sterile membrane filter (0.45 µm) (Whatman Laboratory Division, Maidstone, UK) using a vacuum pump (Welch Thomas 2522C-02, Skokie, Illinois, USA). The filters were incubated aerobically on BHI-A at 37 °C for 48h. The growth was washed off the filter with 3 ml of MRD, diluted (up to 10^{-5}) and 100 µl of each dilution spread on agar plates containing different antimicrobials. For the *msr*(A) gene, the inoculation was made on BHI-RFE [rifampicin (25µg/ml), fusidic acid (25 µg/ml) and erythromycin (16 µg/ml)], BHI-RF [rifampicin (25 µg/ml) and fusidic acid (25 µg/ ml)], BHI-E [Erythromycin (16 µg/ml)] and BHI agar plates. For the tet(K) gene transfer, the mating mixtures were spread on BHI-RFT [rifampicin (25µg/ml), fusidic acid (25 µg/ml) and tetracycline (10 µg/ml)], BHI-RF [rifampicin (25 µg/ml) and fusidic acid (25 µg/ ml)], BHI-T [(tetracycline (10 µg/ml)] and BHI agar plates. The plates were incubated at 37 °C for 7-21 days. Cells of the donors and recipient that had not undergone a conjugation procedure were all spread on the same type of media as those used to inoculate the mating mixtures.

Only recipients that had acquired resistance to erythromycin and tetracycline are able to grow on BHI-RFE and BHI-RFT agar plates, respectively. Potential *E. faecalis* JH2-2 transconjugant (PET) DNA fingerprints were compared with those of the recipients and donors by rep-PCR using the GTG5 primer (Ouoba et al., 2008a). In addition, their MICs for erythromycin and tetracycline were determined.

2.3.5 Determination of the genetic background of acquired erythromycin and tetracycline resistance in the PETs

2.3.5.1 Extraction of DNA

Total DNA was extracted as described above. Plasmid DNA was extracted as follows: the donors, recipient and PETs were cultured for 48 h on BHI-A. A pure colony was then transferred into 10 ml BHI and incubated in a shaking water bath at 37°C for 12 h. Plasmid DNA was extracted using the QIAGEN Plasmid miniprep Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions.

2.3.5.2 Amplification of the AMR genes in the PETs

PCR using total and plasmid DNA were performed to detect the presence of the *msr*(A) and *tet*(K) genes in the PETs. Since the *S. warneri* isolate contained also the *cat*501, *blaz*A and *dfr*(A) genes, these were also screened in the PETs. The use of plasmid DNA aimed to determine the location of genes in the bacteria and to increase the chance to detect a potential transfer in the PETs.

3. Results

3.1 Identification of the bacteria

The pH values of the products were 5-6 in the unfermented samples and 8-9 in the fermented products. The bacterial numbers recovered (CFU/g) for each product were as follows: Soumbala: 10^6-10^{13} CFU/g on NA, 10^3-10^{10} on MSA, 10^1-10^8 on BP; Bikalga: 10^8-10^{11} on NA, 10^7-10^8 on MSA, 10^4-10^8 on BP; Ntoba Mbodi: 10^9-10^{11} on NA, 10^8-10^{10} on MSA, 10^5-10^8 on BP. Out of 27 samples analysed, *Staphylococcus* species were recovered from 18 samples. The products where *Staphylococcus* isolates were not detected included mainly samples from the production steps of Bikalga and Soumbala that had undergone heat treatment, but also one sample of each of the three foods obtained from different markets. As mentioned earlier, many bacteria recovered on MSA and BP did not possess typical staphylococcal characteristics and were not included in the advanced screening. Most of those

353 bacteria were Gram-positive (many of which were spore-forming) and Gram-negative
354 rods that were able to grow on the selective media (BPA and MSA). The 90 isolates
355 that were further screened showed variable colony and cell morphology. All were
356 cocci, arranged differently (single, pairs, clusters) according to the isolate, catalase
357 and Gram positive and oxidase negative. Two isolates from Bikalga were coagulase
358 positive and the remaining 88 bacteria were coagulase negative.

359 The microbial profile was variable according to the fermented product, the
360 production site and country (Table 2, Table 3). Genotyping by rep-PCR and PFGE
361 both generated 25 clusters representing 15 species (Table 2, Fig 1). The comparison
362 of the PFGE profiles of the *S. aureus* isolates did not generate a similarity with any of
363 the 12,000 profiles in the CDC database. By sequencing the 16S rRNA and *rpo*B
364 genes, the bacteria of eight rep-PCR/PFGE clusters were identified as *S. sciuri* (45.6
365 % of the total isolates), three as *S. gallinarum* (7.8 %), two as *S. cohnii* (4.4%), two as
366 *S. warneri* (5.6%) and one cluster each as *S. arlettae* (2.2%), *S. aureus* (2.2%), *S.*
367 *epidermidis* (1.1%), *S. haemolyticus* (1.1%), *S. hominis* (1.1%), *S. pasteuri* (1.1%), *S.*
368 *condiment* (1.1%), *S. piscifermentans* (1.1%), *S. saprophyticus* (1.1%), *S. simulans*
369 (18.9%), and *Macrococcus caseolyticus* (previously designated as *S. caseolyticus*,
370 5.5%). In general, *S. sciuri* was the most frequently isolated species and was dominant
371 in the samples of Bikalga (42.9%) and particularly Ntoba Mbodi (71.1%) where it
372 occurred from the beginning of the fermentation and persisted throughout the process.
373 In Soumbala, *S. simulans* (54.8%) was the dominant species. The isolate of *S.*
374 *haemolyticus* was clearly identified by 16S rRNA gene sequencing/EZtaxon analysis
375 with 100 % similarity, but the *rpo*B gene sequencing generated a 96.5% (30 base pairs
376 difference) similarity with *S. hominis* and 93.5% (30 base pairs difference) with *S.*
377 *haemolyticus*. The spa type of the *S. aureus* isolate was identified as t355.

The species of *S. sciuri* and *S. gallinarum* were common to the two countries. One cluster (5) of *S. sciuri* was detected in all three types of food although this particular cluster was not present in all samples (Table 3). Clusters 4 and 6 of the same species were detected in samples from both countries but not in all three products. *Staphylococcus gallinarum* was also recovered from both countries but not all products, with one cluster (15) observed in Soumbala and Ntoba Mbodi. Nine species were specific to Burkina Faso including five isolated solely from Soumbala (*S. epidermidis*, *S. pasteuri*, *S. condimenti*, *S. piscifermentans*, *S. simulans*) and four from Bikalga (*S. aureus*, *S. cohnii*, *S. haemolyticus, M. caseolyticus*). On the other hand, four species were peculiar to Congo/ Ntoba Mbodi (*S. arlettae*, *S. hominis, S. saprophyticus, S. warneri*). Moreover, two clusters of *S. gallinarum* (1, 23) and two of *S. sciuri* (2, 7) were peculiar to Soumbala while one cluster of *S. sciuri* was particular to Bikalga.

3.2 Haemolysis on blood agar and toxin production

Haemolytic activity of the isolates on sheep blood agar was variable as seen in Table 4. Positive haemolysis was observed mainly in isolates of *S. aureus* and *S. simulans*. *Staphylococcus epidermidis*, *S. haemolyticus*, *S. pasteuri* and S. *warneri* exhibited partial haemolysis, whereas the rest of the species did not show haemolytic activity within the 48 h of incubation. The different species clusters showed similar haemolysis potential.

None of the bacteria screened produced the staphylococcal enterotoxins A, B, C and D. Furthermore, the genes coding for the pre-cited enterotoxins as well as the other eight genes were not detected in the isolates.

3.3 Antimicrobial resistance

All bacteria were susceptible to gentamycin, kanamycin, streptomycin, and vancomycin. For the rest of the antimicrobials, susceptibility was variable according to the species, the cluster, and the antimicrobial screened (Table 4). Most of the staphylococci were resistant to trimethoprim with 87.8 % of the isolates, representing 11 species, exhibiting phenotypic resistance to that antimicrobial. Both *S. cohnii* clusters exhibited the highest phenotypic resistance profiles involving 11 antimicrobials. The bacteria with the least phenotypic resistance were *S. condimenti* and *S. pasteuri,* which showed reduced susceptibility toward sulfamethoxazole only. Within the same species, the different clusters exhibited different resistance patterns, but all isolates from the same cluster showed a similar pattern irrespective of the origin. For example, isolates of cluster 1 of *S. gallinarum* were resistant to cefoxitin, clindamycin, quinupristin/dalfopristin and tiamulin, whereas those of cluster 15 were resistant to clindamycin, fusidate, penicillin, quinupristin/dalfopristin, tiamulin, sulfamethoxazole, and trimethoprim, resulting in phenotypic resistance profiles which differ by five antimicrobials (Table 4).

None of the 38 AMR genes screened was detected in the isolates of *S. condimenti*, *S. pasteuri*, S. *piscifermentans*, *S. simulans*, and *M. caseolyticus* (Table 4). For the other species, different genes encoding resistance to multiple antimicrobials including penicillin: *blaz*A, chloramphenicol: *cat*501, trimethoprim: dfr(A), dfr(G), erythromycin: *msr(*A), methicillin: *mec*A, *mecA*1 and tetracyline: *tet* (K) were detected, with a degree of AMR genotypic profile variability observed between some species and clusters of the same species (Table 4). The *mecA1* gene was detected in 45.6% of the isolates, *mec*A in 20%, *tet* (K) in 11.1 %, *blaz*A in 10 %, *dfr*(A) in 10%, *msr*(A) in 6.7%, *cat*501 in 1.1%, and *dfr*(G) in 1.1 %. Isolates of all six clusters of *S. sciuri* showed *mec*A1, but bacteria in only three clusters displayed *mec*A. None

427 of the *S. aureus* isolates contained *mec*A, *mec*A1 or *mec*C. The isolates of *S.*
428 *epidermidis* and *S. warneri* showed the highest numbers (4) of AMR genes, including
429 *blaz*A, *dfr*(A), *dfr*(G) and *tet*(K) for *S. epidermidis* and *blaz*A, *Cat*501, *dfr*(A) and *msr*(A)
430 for cluster 13 of *S. warneri*. The *blaz*A, *mec*A, *mec*A1 and *tet* (K) genes were found in
431 isolates from all products and all countries, *cat*501 in Ntoba Mbodi /Congo, *dfr*(A) in
432 Ntoba Mbodi/Congo and Soumbala/Burkina Faso, *dfr*(G) in Soumbala/Burkina Faso,
433 and *msr*(A) in Ntoba Mbodi /Congo and Bikalga/Burkina Faso

434 Conjugation experiments produced *E. faecalis* JH2-2 PETs with increased
435 resistance to erythromycin and tetracycline. The MIC of some PETs increased up to 6
436 and 4 fold for erythromycin and tetracycline respectively (Table 5). All PETs showed
437 the same rep-PCR profiles as that of the recipient *E. faecalis* JH2-2 (results not
438 shown). No bacteria were recovered on the antimicrobial selective agar plates
439 inoculated with cells which did not undergo a conjugation process. Positive amplicons
440 were obtained for the genes screened for transfer using both total and plasmid DNA
441 samples from the donors. However, no positive amplicon was observed with DNA
442 samples from the recipient and the PETs under the experimental conditions used.

443 **4. Discussion**

444 The specificity in the bacterial profile of the products can be attributed to
445 differences in the raw materials and production processes. Also, environmental factors
446 can affect bacterial populations in a given geographical area and its associated plants
447 and thus can affect the bacterial population of the products derived from such plants.
448 However, since a similar type of fermentation occurs during production of the
449 fermented foods, similarities can be anticipated. High bacterial counts as observed in
450 our study have been reported in other studies describing the microbial composition of
451 alkaline fermented products (Ahaotu et al., 2013; Azokpota et al., 2006; Dakwa et al.,

2005; Ouoba et al., 2010). Furthermore, the increase of pH constitutes a selection of analogous alkali resistant microorganisms in the foods. The presence of the same clusters of *S. sciuri* and *S. gallinarum* in both countries suggests a wide distribution of those strains in the environment. A peculiarity of the *S. sciuri* isolates recovered from the three products is that they were all oxidase negative. This seems unusual because the *S. sciuri* group of species including *S. sciuri*, *S. fleuritti*, *S. lentus*, *S. stepanovicii*, and *S. vitulinus* are known as the only oxidase positive species of the *Staphylococcus* genus (Becker et al., 2014). The discrepancy could be related to the origin specificity and to a lesser extent, the screening methods. The fact that the *S. aureus* PFGE profile did not match that of any clinical and environmental isolates in the CDC database suggests that the cluster is uncommon and not globally distributed.

The species of *S. sciuri*, *S. gallinarum*, *S. epidermidis*, *S. aureus* and *M. caseolyticus* have been reported in other African alkaline fermented products such maari, dawadawa, and soydawadawa (Parkouda et al., 2009). However, most of the other species, as well as the high species and strain diversity in the *Staphylococcus* profiles observed in the current study, seem to have not been reported before. The reason could be that *Staphylococcus* populations in such foods are usually screened as part of the general microbial population and are not addressed specifically and thoroughly using both phenotypic and genotypic methods. The species of *S. condimenti* and *S. piscifermentans* have been reported to be typically associated with fermented foods such as fermented fish, sausages and their starter cultures (Becker et al., 2014). It is believed that *Staphylococcus* spp. from fermented foods are contaminants from the raw materials, equipment, environment and possibly the producers. To produce Ntoba Mbodi, no heating step is included in the process, thus the original *Staphylococcus* isolates, derived from the cassava and wrapping leaves,

that can withstand alkaline conditions probably constitute the major part of the staphylococcal population. However, production of Soumbala and Bikalga includes long cooking steps (24-48h), so it can be assumed that there is a recontamination of the products from the processing and storage materials, the producers and the environment, especially during the drying process. The limited recovery of *Staphylococcus* isolates (90/400 presumptive isolates) supports their status as a minor component of the microflora of the foods. They have often been isolated from alkaline fermented foods as secondary microorganisms, but a clear indication of their role in the fermentation is not established. The main microorganisms responsible for alkaline fermentation, and which bring about desired changes to the foods, have been demonstrated to be *Bacillus* species (Odunfa and Adewuyi, 1985; Ouoba et al., 2017). Fermentation trials conducted by Odunfa and Adewuyi (1985) showed that *S. saprophyticus* was unable to ferment African locust bean seeds to generate a product with the typical organoleptic and nutritional properties. Nevertheless, it can be suggested that the presence of *Staphylococcus* species contributes to some biochemical activities such as those related to the degradation of proteins and lipids that some species are able to perform during meat fermentation to produce sausages (Casaburi et al., 2008; Milicevic et al., 2014).

Staphylococcus spp., especially CoPS, are known to cause various illnesses including food intoxication due to their capacity to produce heat resistant enterotoxins. Interestingly, none of the isolates investigated, including the two CoP *S. aureus,* exhibited a potential toxin-producing phenotype and genotype. It cannot be ruled out that they may contain other toxin genes that were not screened, but since the most common genes were not detected, it can be assumed that these bacteria do not represent a serious threat for consumers. In Burkina Faso and the Republic of the

Congo, there are no official reports of foodborne illnesses resulting from the consumption of alkaline traditional fermented foods, although this may be related to non-availability of data and lack of statistics. If unrecorded cases had occurred, these would probably have been attributed to toxins elaborated by the fermenting *B. cereus* strains that commonly occur in the products (Ouoba et al., 2008 b; Vouidibio-Mbozo et al., 2017). In the study by Fowoyo and Ogunbanwo (2017a), the *sea*, *seb*, *sec* and *sed*, and *tsst-1* genes were detected in CoNS, including *S. epidermidis*, *S. simulans*, *S. xylosus*, *S. kloosii* and *S. capra*e, isolated from six Nigerian traditional fermented foods including iru, an alkaline fermented African locust bean product that is similar to Soumbala. The positive amplicons were not sequenced to confirm the identity of the gene, but the ability of some isolates to produce the staphylococcal toxins A, B, C, and D suggest that these bacteria may pose a potential threat to consumers.

Antimicrobial resistances that are not associated with the presence of a gene are of concern, but those involving a gene represent an enhanced threat, due to the potential for transfer to other bacteria by mechanisms such as horizontal transfer, and therefore can be spread. The *tet* (K), *blaz*A, *dfr*(A), *msr*(A), *cat*501, and *dfr*(G) genes detected can be associated with transferable mobile genetic elements such as plasmids and transposons, and can therefore potentially be transferred to other bacteria including pathogenic microorganisms. In the current research, *tet* (K), *blaz*A, *msr*(A), *cat*501 and *dfr*(A) genes were amplified using plasmid DNA, suggesting that they are located on plasmids, at least in the isolates screened. The AMR threat is greater with bacteria with multi-drug genotypic profiles, such as one of the isolates of *S. warneri* which exhibited AMR genes for four antimicrobials: erythromycin, chloramphenicol, penicillin and trimethoprim, and those of *S*. *epidermidis* and *S.*

526 *hominis* which showed respectively four and three genes encoding resistance to
527 penicillin, tetracycline and chloramphenicol.

528 Methicillin resistance is an important factor when screening AMR in
529 *Staphylococcus*. This is because MRSA is responsible for a serious and persistent
530 problem in treatment of infections caused by such bacteria. The most virulent MRSA
531 are also resistant to other beta-lactam antimicrobials. Methicillin resistance is usually
532 caused by the presence of the *mecA* gene coding for a penicillin-binding protein
533 (PBP2a) associated with a significantly lower affinity for beta-lactams. Thus, cell-wall
534 biosynthesis by the bacteria continues, even when a typically inhibitory concentration
535 of antibiotic is administered (Peacok et al., 2015). Variants of *mec*A include genes
536 such as *mec*A and *mec*C ($mecA_{LGA251}$) originally found in *S. aureus* isolates, *mec*A1,
537 *mec*A2 and *mec*B (*mec*Am) initially detected in the *S. sciuri* group, *S. vitulinus* and *M.*
538 *caseolyticus* respectively (Ito et al., 2012). Isolates of three clusters of *S. sciuri* from
539 both countries exhibited both *mec*A1 and *mec*A, whereas the isolates of the three
540 other clusters also found in both countries exhibited only *mec*A1. The presence of the
541 *mec*A1 gene in *S. sciuri* is a common trait of this species and does not generally imply
542 methicillin resistance in the bacteria (Couto et al., 2000). However, the presence of
543 *mec*A in the isolates implicates methicillin resistance and represents a threat, although
544 ~~a~~ phenotypic resistance was not observed for cefoxitin. Couto et al. (2000) showed
545 that only *S. sciuri* isolates that possess a copy of *mec*A in addition to *mec*A1 exhibit
546 significant levels of resistance to beta-lactams. In our study, such isolates displayed
547 phenotypic resistance to penicillin. Interestingly, the *S. aureus* did not display any of
548 the methicillin resistance genes screened and were susceptible to cefoxitin. However,
549 the strains were resistant to penicillin and contained the *blaZ* gene.

550 The prevalence of AMR genes in various CoNS and CoPS from processed
551 and unprocessed foods such as meat, fish and fermented dairy and meat products
552 from different origins has been widely documented (Argudín et al., 2011; Kürekci,
553 2016; Perreten et al.,1998; Zdolec et al., 2012). However, such information on CoNS
554 and CoPS from alkaline fermented products and especially those from Africa are, to
555 the best of our knowledge, non-existent. This can be due to the limited importance
556 attributed to these bacteria in alkaline fermentation. The study by Fowoyo and
557 Ogunbanwo (2017b) on CoNS from different Nigerian fermented foods, reported
558 phenotypic resistance to antimicrobials such as trimethoprim-sulfamethoxazole,
559 ciprofloxacin, erythromycin, gentamicin, and cefotaxime, but no AMR gene was
560 reported, probably because only *mec*A was screened in the isolates with no positive
561 results.

562 The significant increase of the MIC above the cut-off values in the PETs for
563 erythromycin and tetracycline indicates that they have acquired resistance to the
564 antimicrobials. Since the genes screened were not detected in the PETs, it can be
565 assumed that they were not transferred, but that the isolates acquired resistance
566 through other mechanisms, such as mutations. The occurrence of resistances was
567 triggered by the presence of the *Staphylococcus* donors because PETs were not
568 recovered from recipient cells which did not undergo a conjugation process. Mutations
569 can occur as a result of a mechanism by which the *Staphylococcus* spp. stimulate an
570 evolution of *E. faecalis* JH2-2 by increasing, for instance, the expression of error-prone
571 DNA repair mechanisms (Naghizadeh Raeisi, 2018). Alternatively, the growth of the
572 recipient could be stimulated by the donor leading to a larger recipient population in
573 which mutations can arise (Naghizadeh Raeisi, 2018). However, it is worth noting that
574 when a low number of plasmid copies which carry a gene is transferred, it may not be

575 detected using conventional PCR. Moreover, other genes coding for resistance to the
576 antimicrobials and which were not screened, may be present in the donors and
577 transferred to the recipients. Transfer potential varies according to different factors
578 related to the environment, type of donor and recipient, and the location of the AMR
579 genes. Thus, the genes screened may have not been transferred into *E. faecalis* JH2-
580 2 and therefore the threat with regards to the potential of the donors to spread AMR is
581 limited. However, the possibility of gene transfer to other types of bacteria cannot be
582 ruled out since the genes are located on plasmids which are mobile genetic elements
583 that can be transferred in appropriate conditions.

584 The current study demonstrates ecological similarities and differences in the
585 *Staphylococcus* population of different types of alkaline fermented foods. The main
586 microorganisms identified are primarily CoNS with a very limited number of CoPS, as
587 would be expected in non-clinical samples. The bacteria did not suggest a safety issue
588 with regards to the potential to produce toxins responsible for foodborne diseases.
589 Due to the presence of multiple AMR genes in some isolates, they are reservoirs of
590 AMR determinants which may be spread under particular conditions. However, in
591 general, the results of the study herein reported suggest little safety importance of the
592 staphylococcus isolates in the fermented products screened.

593

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788

Figure caption

Figure 1: Cluster analysis (Bionumerics, Dice's Coefficient of similarity, Unweighted pair group method with arithmetic: UPGMA) of the different rep-PCR and PFGE fingerprints of *Staphylococcus species* isolated from Bikalga, Soumbala and Ntoba Mbodi

Table 1: Antimicrobials studied and their effect on the *Staphylococcus* isolates screened

Antimicrobials	Concentration Range screened (mg/L)	Cut off values(mg/L)	Genes screened [e]	*Staphylococcus* isolates		
				[f]MIC Range (mg/L)	Resistant (%)	Susceptible (%)
Cefoxitin	0.5 - 16	4 [a,b]	*mec*A, *mec*A1 and *mec*C for methicillin resistance	1 - 8	8.9	91.1
Chloramphenicol	4 - 64	8 [c]	*cat*501, *cat*A1, *cml*A	<4 - 64	52.2	47.8
Ciprofloxacin	0.25 - 8	1 [a]		<0.25 - 2	1.1	98.9
Clindamycin	0.12 - 4	0.25 [a]		<0.12 - 4	41.1	58.9
Erythromycin	0.25 - 8	1 [a]	*erm*(A), *erm*(B), *erm*(C), erA, erB, *msr*A, *mph*A, *mef*A/*mef*E	<0.25 - >8	11.1	88.9
Fusidate	0.5 - 4	1 [c]		<0.5 - >4	43.3	56.7
Gentamicin	1 - 16	2 [a]	aac(6′)aph(2″), aac(3″)II, aac(3″)IV, ant(2″)-I	<1	0	100
Kanamycin	4 - 64	16 [d]		<4	0	100
Linezolid	1 - 8	4 [a]		<1 - 8R	1.1	98.9
Mupirocin,	0.5 - 256	1 [a]		<0.5 - > 256	1.1	98.9
Penicillin	0.12 - 2	0.125 [b]	*BlaZ*	<0.12 - >2	43.3	56.7
Quinupristin-dalfopristin	0.5 - 4	1 [a]	*Vat*D, *Vat*E	<0.5 - 4	64.4	35.6
Rifampicin	0.016 - 0.5	0.5 [c]		<0.016 - >0.5	4.4	95.6
Streptomycin	4 - 32	16 [b]	*Str*A, *Str*B, *aad*A, *aad*E	<4 - 16	0	100
Sulfamethoxazole	64 - 512	128 [a]		<64 - >512	61.1	38.9
Tetracycline	0.5 - 16	1 [a]	*tet*(M), *tet*(L), *tet*(S), *tet*(Q), *tet*(K), *tet*(O), *tet*(W)	<0.5 - >16	6.7	93.76
Tiamulin	0.5 - 4	2 [a]		<0.5 - > 4	48.9	51.1
Trimethoprim	2 - 32	2 [a]	*dfr*(A), *dfr*(G), *dfr*(K)	<2 - >32	87.8	12.2
Vancomycin	1 - 16	2 [a]	*Van* A, *Van*B, *Van*X	<1	0	100

Cut-off values according to a) EFSA (2012), b) *EURL-AR (2017), c)* EUCAST (2018) and d) Virdis et al. (2010)

e) Most primer sequences are from EURL-AR (2013) and Ouoba et al. (2008), except for *mec*A1 (Tsubakishita et al., 2010), *erA*, *erB*, *msr*A, *mph*A, *mef*A/*mef*E (Sutcliffe et al.,1996), *dfr(*G), *dfr*(K (Argudín et al., 2011)

f) MIC: Minimal Inhibitory Concentration

Table 2: Identity and origin of the *Staphylococcus* species recovered from Soumbala, Bikalga, and Ntoba Mbodi

Samples/Origin	Fermentation time/ sampling site[a]	Isolates	[b]Rep-PCR/ PFGE clusters	Identification by 16S RNA and *rpo*B gene sequencing
Soumbala				
Burkina Faso				
S3	Site 1/ 0h	S35M (47)	14	*Staphylococcus epidermidis*
S5	Site 1/ 48h	S51B (33)	23	*Staphylococcus gallinarum*
		S54a M (34)	23	*Staphylococcus gallinarum*
		S54b M (35)	23	*Staphylococcus gallinarum*
S6	Site 1/ Dried	S63aM (41)	3	*Staphylococcus simulans*
SF	Site 2/ ≥72h	SF1M (1)	1	*Staphylococcus gallinarum*
		SF3M (2)	3	*Staphylococcus simulans*
		SF2M (3)	5	*Staphylococcus sciuri*
		SF2bB (6)	3	*Staphylococcus simulans*
		SF2aB (7)	3	*Staphylococcus simulans*
		SF1B (8)	3	*Staphylococcus simulans*
		SF8M (21)	2	*Staphylococcus sciuri*
		SF7M (22)	3	*Staphylococcus simulans*
		SF6M (23)	7	*Staphylococcus sciuri*
SP	Site 3/ ≥72h	SP6M (26)	10	*Staphylococcus piscifermentans*
		SP5M (27)	3	*Staphylococcus simulans*
		SP3M (28)	3	*Staphylococcus simulans*
		SP1M (29)	11	*Staphylococcus pasteuri*
		SP4B (30)	3	*Staphylococcus simulans*
		SP2B (31)	3	*Staphylococcus simulans*
		SP1B (32)	3	*Staphylococcus simulans*
SZ	Site 4/ ≥72h	SZ1B (10)	3	*Staphylococcus simulans*
		SZ2B (11)	3	*Staphylococcus simulans*
		SZ6B (13)	3	*Staphylococcus simulans*
		SZ1M (14)	5	*Staphylococcus sciuri*
		SZ3M (15)	3	*Staphylococcus simulans*
		SZ4M (16)	5	*Staphylococcus sciuri*
		SZ5M (17)	9	*Staphylococcus condimenti*
		SZ6M (18)	15	*Staphylococcus gallinarum*
		SZ7M (24)	3	*Staphylococcus simulans*
		SZ9M (25)	3	*Staphylococcus simulans*
Bikalga				
Burkina Faso				
B2	Site 5/ 48h	B21M (50)	6	*Staphylococcus sciuri*
		B22M (51)	4	*Staphylococcus sciuri*
		B27aM (53)	16	*Staphylococcus cohnii*
		B27bM (54)	16	*Staphylococcus cohnii*
		B25M (56)	16	*Staphylococcus cohnii*
		B25B (57)	8	*Staphylococcus sciuri*
		B24B (58)	24	*Staphylococcus cohnii*
B3	Site 5/ 72h	B35M (60)	5	*Staphylococcus sciuri*
		B36B (61)	5	*Staphylococcus sciuri*
		B34M (62)	5	*Staphylococcus sciuri*
		B32B (64)	5	*Staphylococcus sciuri*
		B31M (65)	5	*Staphylococcus sciuri*
		B31B (66)	8	*Staphylococcus sciuri*

[a]Site 1: Ouagadougou (Samandin production site), Site 2: Fada market, Site 3: Ouagadougou (Patte d'Oie market), Site 4: Ouagadougou (Zogona market), Site 5: Ouagadougou (Zone Pilote production

828 site) [b] Rep-PCR : Repetitive element palindromic-polymerase chain reaction ; PFGE : Pulse Field Gel
829 Electorphoresis

Table 2 *continued*: Identity of the *Staphylococcus* species recovered from Soumbala, Bikalga, and Ntoba Mbodi

Samples/Origin	Fermentation time/ sampling site[a]	Isolates	[b]Rep-PCR/ PFGE clusters	Identification 16S RNA and *rpoB* gene sequencing
B5	Site 5/ Dried	B57B (69)	18	*Staphylococcus aureus*
		B51B (70)	18	*Staphylococcus aureus*
BP	Site 6/ Dried	BP2B (73)	19	*Macrococcus caseolyticus*
		BP2M (74)	19	*Macrococcus caseolyticus*
		BP3B (75)	19	*Macrococcus caseolyticus*
		BP6B (76)	19	*Macrococcus caseolyticus*
		BP5B (77)	19	*Macrococcus caseolyticus*
BB	Site 7/ Dried	BB3B (84)	20	*S. haemolyticus*
Ntoba Mbodi				
Congo				
AU0	Site 8/ 0h	A04M (93)	25	*Staphylococcus sciuri*
		A05M (94)	12	*Staphylococcus warneri*
		A08M (95)	12	*Staphylococcus warneri*
		A07M (185)	12	*Staphylococcus warneri*
		A06B (193)	12	*Staphylococcus warneri*
AU1	Site 8/ 24h	A12B (96)	6	*Staphylococcus sciuri*
		A15M (104)	25	*Staphylococcus sciuri*
		A17M (105)	13	*Staphylococcus warneri*
		A18M (106)	17	*Staphylococcus arlettae*
		A15M (176)	4	*Staphylococcus sciuri*
		A16M (177)	6	*Staphylococcus sciuri*
		A111B (186)	5	*Staphylococcus sciuri*
		A110B (194)	5	*Staphylococcus sciuri*
AU2	Site 8/ 48h	A25B (111)	17	*Staphylococcus arlettae*
		A215B (113)	15	*Staphylococcus gallinarum*
		A21M (115)	4	*Staphylococcus sciuri*
		A27B (178)	5	*Staphylococcus sciuri*
		A29B (179)	6	*Staphylococcus sciuri*
		A26M (180)	6	*Staphylococcus sciuri*
		A23M (191)	4	*Staphylococcus sciuri*
		A24M (192)	4	*Staphylococcus sciuri*
AU3	Site 8/ 72h	A37bB (123)	6	*Staphylococcus sciuri*
		A39B (124)	21	*Staphylococcus hominis*
		A34M (128)	6	*Staphylococcus sciuri*
		A31M (188)	6	*Staphylococcus sciuri*
		A33M (187)	6	*Staphylococcus sciuri*
JO0	Site 9/ 0h	J03B (131)	6	*Staphylococcus sciuri*
		J04B (132)	6	*Staphylococcus sciuri*
		J01B (189)	4	*Staphylococcus sciuri*
		J02B (190)	6	*Staphylococcus sciuri*
JO3	Site 9/ 72h	J03M (138)	22	*Staphylococcus saprophyticus*
		J311B (148)	15	*Staphylococcus gallinarum*
		J33M (150)	6	*Staphylococcus sciuri*
		J34M (151)	6	*Staphylococcus sciuri*
		J31M (181)	6	*Staphylococcus sciuri*
		J32M (182)	6	*Staphylococcus sciuri*
MB	Site 10/ 72-96h	MB1M (156)	5	*Staphylococcus sciuri*
		MB11M (184)	5	*Staphylococcus sciuri*

[a]Site 5: Ouagadougou (Zone Pilote production site), Site 6: Ouagadougou (Patte d'Oie market), Site 7: Bobo Dioulasso (market), Site 8: Brazzaville (Production site A), Site 9: Brazzaville (Production site B), Site 10: Brazzaville (market)

[b] Rep-PCR : Repetitive element palindromic-polymerase chain reaction ; PFGE : Pulse Field Gel Electorphoresis

857 Table 3 Geographical distribution of the *Staphylococcus* species and associated clusters
858

Species	Rep-PCR/ PFGE Clusters	Burkina Faso/ Soumbala				Burkina Faso/ Bikalga			Congo/ Ntoba Mbodi		
		Site 1[b]	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8	Site 9	Site 10
S. arlettae	17	-[c]	-	-	-	-	-	-	+	-	-
S. aureus	18	-	-	-	-	+	-	-	-	-	-
S. cohnii	16	-	-	-	-	-	+	-	-	-	-
	24	-	-	-	-	-	+	-	-	-	-
S. epidermidis	14	+	-	-	-	-	-	-	-	-	-
S. gallinarum	1	+	-	-	-	-	-	-	-	-	-
	15	-	-	-	+	-	-	-	+	+	-
	23	+	-	-	-	-	-	-	-	-	-
S. haemolyticus	20	-	-	-	-	-	-	+	-	-	-
S. hominis	21	-	-	-	-	-	-	-	+	-	-
S. pasteuri	11	-	-	+	-	-	-	-	-	-	-
S. condimenti	9	-	-	-	+	-	-	-	-	-	-
S. piscifermentans	10	-	+	-	-	-	-	-	-	-	-
S. saprophyticus	22	-	-	-	-	-	-	-	-	+	-
S. sciuri	2	-	+	-	-	-	-	-	-	-	-
	4	-	-	-	-	+	-	-	+	+	-
	5	-	+	-	+	+	-	-	-	-	+
	6	-	-	-	-	+	-	-	+	+	-
	7	-	+	-	-	-	-	-	-	-	-
	8	-	-	-	-	+	-	-	-	-	-
	25	-	-	-	-	-	-	-	+	-	-
S. simulans	*3*	+	+	+	+	-	-	-	-	-	-
S. warneri	12	-	-	-	-	-	-	-	+	-	-
	13	-	-	-	-	-	-	-	+	-	-
M. caseolyticus	19	-	-	-	-	-	+	-	-	-	-

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860 [a] Rep-PCR : Repetitive element palindromic-polymerase chain reaction ; PFGE : Pulse Field Gel Electrophoresis

861 [b]Site 1: Ouagadougou (Samandin production site), Site 2: Fada market, Site 3: Ouagadougou (Patte d'Oie market), Site 4: Ouagadougou (Zogona market),
862 Site 5: Ouagadougou (Zone Pilote production site), Site 6: Ouagadougou (Patte d'Oie market), Site 7: Bobo Dioulasso (market), Site 8: Brazzaville
863 (Production site A), Site 9: Brazzaville (Production site B), Site 10: Brazzaville (market)

864 [c] - : not detected, + : detected

865 Table 4 Profiles of antimicrobial resistance (AMR) and toxin production potential of the species and clusters of *Staphylococcus* from
866 Bikalga, Soumbala, and Ntoba Mbodi

Origin [a]	Rep-PCR/ PFGE Cluster	Species	Number of Isolates	AMR phenotype [b]	AMR genes	Toxin genes/ Toxin A, B, C, D	Haemolysis on blood agar
Ntoba Mbodi (Co)	17	*S. arlettae*	2	CHL, CLI, FUS, SYN, SMX, TIA, TMP	dfr(A)	- [c]	-
Bikalga (BF)	18	*S. aureus*	2	CHL, ERY, PEN, SYN, SUL	blazA	-	+
Bikalga (BF)	16	*S. cohnii*	3	FOX, CHL, CLI, ERY, FUS, PEN, RIF, SMX, SYN, TIA, TMP	*msr*(A)	-	-
Bikalga (BF)	24	*S. cohnii*	1	FOX, CLI, ERY, FUS, PEN, RIF, SMX., SYN, TET, TIA, TMP	*msr*(A)	-	-
Soumbala (BF)	14	*S. epidermidis*	1	CHL, PEN, SMX, TET, TMP	blazA, dfr(A), dfr(G) tet(K)	-	±
Soumbala (BF)	1	*S. gallinarum*	1	FOX, CLI, SYN, TIA	*tet*(K)	-	-
Soumbala, Ntoba Mbodi (BF, Co)	15	*S. gallinarum*	3	CLI, FUS, PEN, SYN, TIA, SMX, TMP	*tet*(K)	-	-
Soumbala (BF)	23	*S. gallinarum*	3	FOX, CLI, FUS, PEN, SYN, TIA, TMP	*tet*(K)	-	-
Bikalga (BF)	20	*S. haemolyticus*	1	CLI, ERY, PEN, SYN, TIA, TMP	tet(K), *msr*(A)	-	±
Ntoba Mbodi (Co)	21	*S. hominis*	1	CHL, PEN, SMX, TET, TMP	blazA, *tet*(K), dfr(A)	-	-
Soumbala (BF)	11	*S. pasteuri*	1	SMX		-	±
Soumbala (BF)	10	*S. piscifermentans*	1	CHL, ERY,SMX, TIA		-	-
Soumbala (BF)	9	*S. condimenti*	1	SMX		-	-
Ntoba Mbodi (Co)	22	*S. saprophyticus*	1	CHL, PEN, TET, TMP	*tet*(K)	-	-
Soumbala (BF)	2	*S. sciuri*	1	CLI, FUS, SMX, TIA, TMP	*mec*A, *mec*A1	-	
Bikalga, Ntoba Mbodi (BF, Co)	4	*S. sciuri*	6	CLI, FUS, TIA, TMP	*mec*A1	-	-
Bikalga, Soumbala, Ntoba Mbodi (BF, Co)	5	*S. sciuri*	13	CLI, FUS, TMP	*mec*A1	-	-
Bikalga, Ntoba Mbodi (BF, Co)	6	*S. sciuri*	16	CHL, PEN, SYN, SMX, TIA, TMP	*mec*A, *mec*A1	-	-
Soumbala (BF)	7	*S. sciuri*	1	CLI, ERY, FUS, SYN, TIA, TMP	*mec*A, *mec*A1	-	-
Bikalga (BF)	8	*S. sciuri*	2	CHL, PEN, SYN, TET, TIA, TMP	*mec*A1	-	-
Ntoba Mbodi (Co)	25	*S. sciuri*	2	CLI, FUS, SMX, SYN, TIA, TMP	*mec*A1		
Soumbala (BF)	3	*S. simulans*	17	CHL, LZD, SYN, SMX, TMP		-	+
Ntoba Mbodi (Co)	12	*S. warneri*	4	FUS, PEN, SYN, SMX, TMP	blazA, dfr(A)	-	±
Ntoba Mbodi (Co)	13	*S. warneri*	1	CHL, ERY, PEN, SMX, TMP	blazA, Cat501 dfr(A), *msr*(A)	-	-
Bikalga (BF)	19	*M. caseolyticus*	5	CIP, MUP		-	-

867 [a] BF: Burkina Faso, Co: Congo

868 [b] The profile represents the antimicrobials to which the isolates were resistant to; Cefoxitin: FOX, Chloramphenicol: CHL, Ciprofloxacin: CIP,
869 Clindamycin: CLI, Erythromycin: ERY, Fusidate: FUS, Mupirocin: MUP, Quinupristin/Dalfopristin (Synercid): SYN, Linezolid: LZD, Penicillin:
870 PEN, Rifampicin: RIF, Sulfamethoxazole: SMX, Tiamulin: TIA, Trimethoprim: TMP [c] -: negative, ±: partial haemolysis, +: positive haemolysis

871 Table 5: Minimal Inhibitory Concentration (MIC) for donors, recipient and potential *E. faecalis* JH2-2 transconjugants (PET)

MIC (mg/L): Erythromycin		MIC (mg/L): Tetracycline	
Donor *S. warneri*	>32	Donor S. *saprophyticus*	64
Recipient *E. faecalis*	<1	Recipient *E. faecalis*	1
PET-E1	16	PET-T1	8
PET-E2	<1	PET-T2	8
PET-E3	>32	PET-T3	16
PET-E4	<1	PET-T4	8
PET-E5	<1	PET-T5	8
PET-E6	4	PET-T6	16
PET-E7	<1	PET-T7	16
PET-E8	<1	PET-T8	4
PET-E9	>32	PET-T9	16
PET-E10	<1	PET-T10	4
PET-E11	32		
PET-E12	<1		
PET-E13	>32		
PET-E14	<1		

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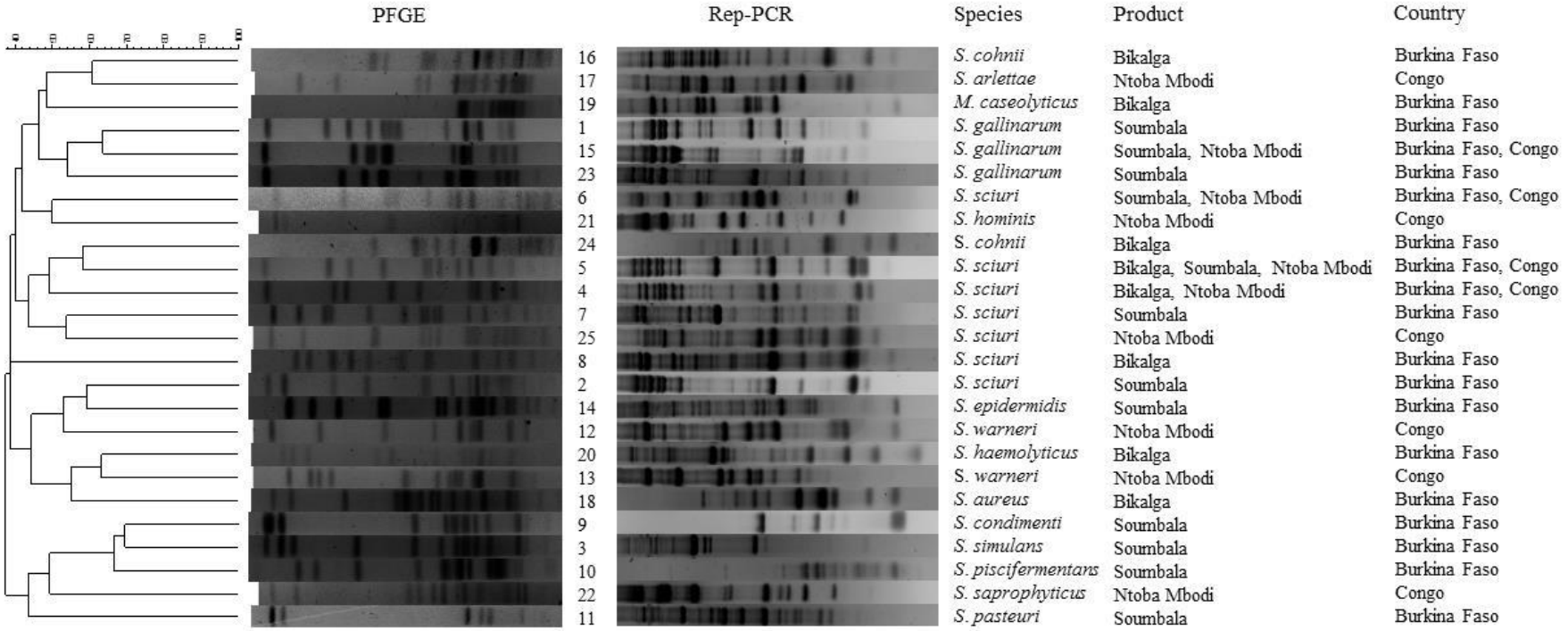

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875 Figure 1