An assessment on DNA microarray and sequence-based methods for the characterization of methicillin-susceptible Staphylococcus aureus from Nigeria

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Staphylococcus aureus is an important human pathogen causing nosocomial and community-acquired infections worldwide. In the characterization of this opportunistic pathogen, DNA microarray hybridization technique is used as an alternative to sequence based genotyping to obtain a comprehensive assessment on the virulence, resistance determinants, and population structure. The objective of this study was to characterize a defined collection of S. aureus isolates from Nigeria using the microarray technique, and to assess the extent that it correlates with sequence-based genotyping methods. The clonal diversity and genomic content of 52 methicillin-susceptible Staphylococcus aureus (MSSA) were investigated by spa typing, MLST and DNA microarray hybridization. More than half (55.8%) of these isolates were associated with clonal complexes (CCs) typically associated with methicillin-resistant S. aureus (MRSA) clones i.e., CC1, CC5, CC8, CC30, and CC45. Certain genes linked with virulence (hlgA and clfA) and adherence (ebpS, fnbA, sspA, sspB, and sspP) were detected in all isolates. A number of genes or gene clusters were associated with distinct clonal types. The enterotoxin gene cluster (egc) was linked with CC5, CC25, CC30, CC45, and CC121, enterotoxin H gene (seh) with CC1, exfoliative toxin D gene (etd) with CC25 and CC80, and the epidermal cell differentiation inhibitor B gene (edinB) with CC25, CC80, and CC152. The excellent agreement between data from DNA microarray and MLST in the delineation of Nigerian MSSA isolates indicates that the microarray technique is a useful tool to provide information on antibiotic resistance, clonal diversity and virulence factors associated with infection and disease.

Keywords: Staphylococcus aureus, microarray, MLST, genotyping, Nigeria

Abbreviations: Agr, accessory gene regulator; CC, Clonal complex; CLSI, Clinical Laboratory Standards Institute; MSSA, Methicillin susceptible Staphylococcus aureus; MLST, Multilocus sequence typing; PVL, Panton-Valentine Leukocidin; S.aureus, Staphylococcus aureus; SCCmec, Staphylococcal chromosome cassette mec; spa, Staphylococcus aureus protein A; ST, Sequence Type.
INTRODUCTION

*Staphylococcus aureus* is implicated in a variety of human infections with high rates of morbidity and mortality (Lowy, 1998; Corey, 2009). In infection, *S. aureus* exhibits a coordinated and regulated expression of a wide variety of cell and surface-associated virulence factors (Foster and Höök, 1998; Novick, 2006). These factors mediate adherence to host cells and damaged tissue, facilitate tissue destruction and spreading, promote iron uptake and evasion of host immune system, as well as tissue damage (Skaar and Schneewind, 2004; Grumann et al., 2014). Recent studies in Cameroon (Kihla et al., 2014), Egypt (Ahmed et al., 2014), Gabon (Alabi et al., 2013), Nigeria (Jido and Garba, 2012; Oladeinde et al., 2013), South Africa (Groome et al., 2012; Naaidoo et al., 2013), and Tanzania (Kayange et al., 2010; Mhada et al., 2012) have identified *S. aureus* as the main etiological agent for various infections in Africa. Moreover, this species has been recognized as one main cause of community-acquired neonatal sepsis in Africa (Waters et al., 2011). These studies clearly establish the important role of this major human pathogen in tropical Africa.

In many health care institutions in sub-Saharan Africa, the lack of skilled laboratory manpower and resources is a major constraint in the identification of bacterial pathogens from clinical samples. If such analysis can be provided at all, identification of *S. aureus* typically relies on phenotypic methods precluding in-depth strain characterization. Molecular analysis of clonal attribution and presence of single genes contained in *S. aureus* isolates have emerged in pilot studies from select African centers, areas and populations (Ateba Ngoa et al., 2012; Shittu et al., 2012; Seni et al., 2013; Aiken et al., 2014; Eggyir et al., 2014; Oosthuysen et al., 2014; Conceição et al., 2015; De Boeck et al., 2015; Kraef et al., 2015; Schaumburg et al., 2015). Nevertheless, in view of the impact of *S. aureus* disease in sub-Saharan Africa, the clonal characterization in concert with a comprehensive analysis of the hitherto ill-described virulence factor armamentarium of *S. aureus* isolates from this region is urgently warranted. Such analyses should target a broad spectrum of variable staphylococcal factors such as genes or gene clusters conferring antibiotic resistance, toxins, virulence, adhesion or immune evasion factors. These analyses have not been performed on a collection of *S. aureus* isolates in Nigeria, and reports from African countries are limited and only addressed a limited and select analytical spectrum (Raji et al., 2013; Aiken et al., 2014; Rovira et al., 2015).

The DNA microarray used for this analysis is a unique and comprehensive genotyping technique based on the analysis of 334 target sequences corresponding to approximately 170 distinct genes and their allelic variants. It enables the simultaneous identification of various gene classes including species markers, genes encoding resistance and virulence properties, exotoxin and adhesion factors, accessory gene regulator (*agr*), capsule, and SCCmec types (Monecke et al., 2011). Based on the observation of a high level of genetic diversity from previous investigations on methicillin-susceptible *S. aureus* (MSSA) in Nigeria (Shittu et al., 2011, 2012; Kolawole et al., 2013), we studied MSSA isolates obtained from various clinical sources in Nigeria using this comprehensive, array-based approach to provide an insight on the major factors associated with infection and disease.

MATERIALS AND METHODS

Identification and Antibiotic Susceptibility Testing of *S. aureus* Isolates

The isolates (*n* = 52) were obtained from samples processed as part of surveillance activities in the microbiology laboratories of six health care institutions located in Ado-Ekiti, Ille-Ife, Osogbo, Lagos, and Ibadan in South-West Nigeria, and Maiduguri in North-East Nigeria. The duration of collection of isolates was from March 2009 to April, 2010. Only the isolates were analyzed in this study. Preliminary verification as *S. aureus* was based on colony characteristics on blood agar, positive results for catalase, coagulase and DNase tests. Twelve isolates from a previous study (Shittu et al., 2011) were also included in this investigation. Identification was confirmed by Matrix-Assisted Laser Desorption/Ionization-Time Of Flight analysis (MALDI-TOF). Susceptibility testing to penicillin (10 units), cefoxitin bromide (30 µg), doxycycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), chloramphenicol (30 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg) were determined using the disk diffusion method according to the Clinical Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute (CLSI), 2009).

DNA Extraction

*S. aureus* genomic DNA was extracted from an 18–24 h old culture on sheep blood agar using lysis buffer and lysis enhancer (StaphyType Kit, Alere Technologies GmbH, Jena, Germany) and processed using a DNeasy tissue kit (Qiagen, Hilden, Germany).

Molecular Typing of the Isolates

Typing of *S. aureus* was based on sequencing of the hypervariable region of the protein A gene (*spa*). The *spa* types were determined using the Ridiom StaphType software (Ridom GmbH, Würzburg, Germany, version 2.1.1) (Harmsen et al., 2003). Multilocus sequence typing (MLST) was performed for one isolate of each *spa* type (Enright et al., 2000), as a *spa* type usually belongs to one sequence type (ST) with few exceptions due to homoplasies (Basset et al., 2009, 2012). The allelic profiles and STs were also included in this investigation. Identification was confirmed by Matrix-Assisted Laser Desorption/Ionization-Time Of Flight analysis (MALDI-TOF). Susceptibility testing to penicillin (10 units), cefoxitin bromide (30 µg), doxycycline (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), chloramphenicol (30 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg) were determined using the disk diffusion method according to the Clinical Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute (CLSI), 2009).

DNA Microarray Hybridization

The DNA microarray of the StaphyType™ kit (Alere Technologies GmbH, Jena, Germany) was used in this study according to previously established protocols (Monecke et al., 2008). The isolates were grouped with various clonal complexes (CCs) by the imaging software Iconoclust based on comparison of hybridization profiles to a collection of reference strains previously characterized by MLST.
Splits Tree Analysis

The analysis identified four main clusters (CC5/CC25; CC8/CC97; CC1/CC7/CC80, and CC30/CC45) indicating the phylogenetic relationship between the isolates (Figure 2).
TABLE 1 | Characterization of the methicillin-susceptible S. aureus (MSSA) from Nigeria based on antibiotyping, microarray analysis, spa typing, and MLST.

| Isolate Number | Location | Sample/Clinical diagnosis | Antibiogram | Score (%) (Alere) | agr/Clonal complex (Alere) | spa type | MLST |
|----------------|----------|--------------------------|-------------|-------------------|----------------------------|----------|------|
| 11486_24       | Ile-Ife  | Wound Infection          | PEN         | 93.8              | agr_III/CC1                | t127     | ST1  |
| AB5_28         | Osogbo   | UTI                      | PEN, ERY(i) | 92.8              | agr_III/CC1                | t1127    | ST1  |
| Are_29         | Osogbo   | Semen                    | PEN         | 94.3              | agr_III/CC1                | t1127    | ST1  |
| MD16_4         | Not available | Not available        | PEN         | 94.3              | agr_III/CC1                | t1127    | ST1  |
| MD20_8*        | Maiduguri | Wound infection          | PEN, ERY(i), CC(i) | 93.5              | agr_III/CC1                | t521     | ST1  |
| 6056_34        | Osogbo   | Urine                    | PEN         | 93.9              | agr_III/CC1                | t110433   | ST1  |
| 5675_6         | Ile-Ife  | Abcess                   | PEN         | 91.8              | agr_II/CC5                 | t131     | ST5  |
| 5221_7         | Ile-Ife  | Urine                    | PEN, ERY(i), SXT(i) | 93.8              | agr_II/CC5                 | t131     | ST5  |
| D23_15         | Ile-Ife  | Pneumonia                | PEN         | 92.8              | agr_II/CC5                 | t131     | ST5  |
| D42_17         | Ile-Ife  | Adenocarcinoma           | PEN, ERY(i) | 92.4              | agr_II/CC5                 | t131     | ST5  |
| D46_18         | Ile-Ife  | Wound Infection          | PEN, ERY(i) | 92.2              | agr_II/CC5                 | t131     | ST5  |
| 1423_36        | Osogbo   | Urine                    | PEN, ERY(i) | 93.8              | agr_II/CC5                 | t142     | ST5  |
| D19_14         | Ile-Ife  | Not available            | PEN         | 93.5              | agr_II/CC5                 | t168     | ST5  |
| Asu29_27       | Osogbo   | Otitis media             | PEN, DO, ERY(i) | 91.9              | agr_II/CC5                 | t1127     | ST5  |
| 3211_30        | Osogbo   | Wound Infection          | PEN         | 92.9              | agr_II/CC5                 | t15235    | ST5  |
| 6773_11        | Ile-Ife  | Wound Infection          | PEN         | 93.6              | agr_II/CC7                 | t1091    | ST789 |
| N37_19         | Ile-Ife  | Erythematous lesion      | PEN, SXT    | 90                | agr_II/CC8                 | t1564    | ST2427 |
| UC45_37        | Ibadan   | Eye swab                 | PEN, GM, CHL, SXT | 91.3              | agr_II/CC8                 | t12668   | ST2427 |
| 55_40          | Ado-Ekiti | Wound Infection          | PEN, DO(ii), GM, CHL, SXT | 90.3              | agr_II/CC8                 | t12668   | ST2427 |
| O539_13*       | Lagos    | Semen/Infertility        | PEN, DO(ii), SXT | 91.7              | agr_II/CC8                 | t155     | ST8  |
| 11450_23       | Ile-Ife  | Sputum                   | PEN         | 92.9              | agr_II/CC15                | t104     | ST15 |
| 5169_1         | Ile-Ife  | Advanced Cancer          | PEN         | 94                | agr_II/CC15                | t104     | ST15 |
| 189_2          | Ile-Ife  | Blood                    | PEN, DO(ii), ERY(i) | 93.9              | agr_II/CC15                | t104     | ST15 |
| 4013_14*       | Ile-Ife  | Wound infection          | PEN         | 94.9              | agr_II/CC15                | t104     | ST15 |
| 5828_5         | Ile-Ife  | Abscess                  | susceptible to all antibiotics tested | 94.4              | agr_II/CC15                | t12216    | ST15 |
| MD7_3*         | Maiduguri | Semen/Infertility        | PEN, ERY(i) | 94.6              | agr_II/CC15                | t12216    | ST15 |
| MD19_11*       | Maiduguri | Wound infection          | PEN         | 94.4              | agr_II/CC15                | t12216    | ST15 |
| S13_6*         | Lagos    | Urinary Tract Infection  | PEN, ERY(i), SXT | 93.1              | agr_II/CC25                | t13772   | ST25 |
| 3925_32        | Osogbo   | Wound Infection          | PEN, ERY(i), SXT | 91.4              | agr_II/CC25                | t110183   | ST25 |
| 6073_3         | Not available | Not available       | PEN, DO    | 91.7              | agr_III/CC30               | t107     | ST30 |
| D30_16         | Ile-Ife  | Cholecystitis            | PEN         | 94.7              | agr_III/CC30               | t138     | ST30 |
| 6506_2         | Osogbo   | Wound Infection          | PEN, ERY(i), CC(i) | 91.4              | agr_III/CC30               | t138     | ST30 |
| NS7708_22      | Ile-Ife  | Nasal swab/screening    | PEN, ERY(i) | 94.7              | agr_III/CC30               | t138     | ST30 |
| 54_39          | Ado-Ekiti | Wound Infection          | PEN         | 94.4              | agr_III/CC30               | t138     | ST30 |
| S12_7*         | Lagos    | Wound infection          | PEN, ERY(i) | 93.8              | agr_III/CC30               | t138     | ST30 |
| OS41_10*       | Lagos    | Wound infection          | PEN         | 93.1              | agr_III/CC30               | t138     | ST30 |
| 6330_4         | Ile-Ife  | Osteomyelitis            | PEN         | 94.3              | agr_III/CC30               | t138     | ST30 |
| NS2907_21      | Ile-Ife  | Nasal swab/screening    | PEN, ERY(i), CC(i) | 91.8              | agr_III/CC45               | t109     | ST508 |
| 3950_33        | Osogbo   | Urine                    | PEN         | 91.5              | agr_III/CC45               | t110434   | ST508 |
| GDC_35         | Osogbo   | Semen                    | PEN         | 94.9              | agr_III/CC80               | t1934    | ST80 |
| MD14_2*        | Maiduguri | Wound infection          | PEN, DO(ii) | 92.9              | agr_II/CC97                | t1458    | ST97 |
| ZU_26          | Ile-Ife  | Unavailable              | PEN, ERY(i) | 89.3              | agr_II/CC11                | t1159    | ST121 |
| UC47_38        | Ibadan   | Eye swab                 | PEN, DO, ERY(i), CC(i) | 92.1              | agr_II/CC11                | t1159    | ST121 |
| W10_5*         | Ile-Ife  | Wound infection          | PEN, ERY(i) | 91.8              | agr_II/CC11                | t1314    | ST121 |
| MD_9*          | Maiduguri | Wound infection          | PEN, ERY(i), CC(i) | 92.1              | agr_II/CC11                | t1314    | ST121 |
| 6376_3         | Ile-Ife  | Abscess                  | PEN, DO(ii) | 93.1              | agr_II/CC11                | t12304    | ST121 |
| 6540_10        | Ile-Ife  | Bone Marrow Infection    | PEN         | 93.5              | agr_II/CC11                | t12304    | ST121 |
| NS2986_20      | Ile-Ife  | Nasal swab/screening    | PEN, DO, ERY(i), CC(i), SXT(i) | 92.8              | agr_II/CC11                | t12304    | ST121 |
| 3920_31        | Osogbo   | Aspirate                 | PEN         | 92.8              | agr_II/CC11                | t12304    | ST121 |
| D3_12          | Ile-Ife  | Cervical cancer          | PEN, ERY(i) | 94.6              | agr_II/ST152               | t1355     | ST152 |

(Continued)
TABLE 1 | Continued

| Isolate Number | Location     | Sample/Clinical diagnosis | Antibiogram | Score (%) (Alere) | agr/Clonal complex (Alere) | spa type | MLST     |
|----------------|--------------|----------------------------|-------------|------------------|----------------------------|----------|----------|
| D12_13         | Ile-Ife      | Ocular infection           | PEN         | 94.6             | agr_I/ST152                 | t365     | ST152    |
| W7.2_4*        | Ile-Ife      | Wound infection            | PEN         | 96.3             | agr_I/ST152                 | t365     | ST152    |

* S. aureus analyzed in a previous study; §: intermediate susceptibility; agr: accessory gene regulator; PEN: Penicillin; DO: Doxycycline; ERY: Erythromycin; CJ: Clindamycin; GEN: Gentamicin; CHL: Chloramphenicol; SXT: trimethoprim/sulfamethoxazole; CC: Clonal Complex; ST: Sequence type.

1 Spa types selected for Multilocus sequence typing (MLST); Sequence types (STs) of the remaining isolates were inferred from the derived MLST data.

**DISCUSSION**

We observed a complete agreement between DNA microarray analysis and MLST in the delineation of the isolates (Table 1), showing that the hybridization profile could be used to predict the lineages. Furthermore, the heterogeneous and divergent nature of the isolates observed in this study provided evidence on the overall higher diversity of MSSA compared with MRSA (Deurenberg and Stobberingh, 2008; Goering et al., 2008; Ghasemzadeh-Moghaddam et al., 2011; Ruffing et al., 2012; Blomfeldt et al., 2013; Rasmussen et al., 2013, 2014). In Nigeria, many diagnostic microbiology laboratories rely on the disc diffusion technique for antibiotic susceptibility testing, but this protocol does not provide information on the nature of resistance genes. The antibiotic susceptibility results observed in this study were in accordance with the corresponding resistance gene profiles by DNA microarray. MSSA isolates that exhibited full resistance to trimethoprim-sulfamethoxazole clustered with CC8 and CC25, but were dfrS1 negative indicating that a different mechanism could be attributed to resistance. A recent study (Nurjadi et al., 2014) has provided strong evidence that the dfrG gene is the predominant trimethoprim resistance determinant on S. aureus in Africa. Overall, resistant determinants for antibiotics, heavy metal and quaternary ammonium compounds were observed more often in CC8 than other CCs (Supplementary Materials 1, 3).

The accessory gene regulator (agr) and capsule typing methods are useful front-line tools for the characterization of S. aureus (Goerke et al., 2005). Hybridization signals for agr type I and IV were observed for one, three, and four isolates grouped with CC25, CC152, and CC121, respectively (Supplementary Materials 1, 2). This could be attributed to possible cross-hybridization as the alleles for the two agr types are closely related (Monecke et al., 2010). Our observations on CCs and agr groups were similar to previous reports on MSSA in five major African towns (Breurec et al., 2010), Gabon (Ateba Ngoa et al., 2012), and Nigeria (Ghebremedhin et al., 2009; Kolawole et al., 2013). In addition, our study also support the view (Wright et al., 2005; Holtfreter et al., 2007; Rasmussen et al., 2014) that an agr type may be detected in isolates which are assigned to genetically diverse CCs, whereas, it is also associated with specific CCs. The dominance of capsule type 8 in MSSA is consistent with data from Gabon (Schaumburg et al., 2011), Norway (Blomfeldt et al., 2013), and Sweden (Rasmussen et al., 2013, 2014).

Staphylococcal enterotoxins are typically encoded by genes located on mobile genetic elements (Baba et al., 2002). The egc cluster (seg+sei+sem+seo+seu) is located on the genomic island vSAβ and reported to be associated with specific clonal types regardless of the geographical strain distribution (Lindsay and Holden, 2006). In this investigation, the egc-enterotoxin gene cluster was a unique feature for CC5, CC25, CC30, CC45, and CC121. Previous studies have indicated that the cluster is predominately present in MSSA assigned with CC5, CC25, CC30, and CC45 (Van Trijp et al., 2010; Rasmussen et al., 2013). The seh gene is linked to the staphylococcal cassette chromosome mec (SCCmec) elements and reported to be restricted to the CC1 genomic background (Baba et al., 2002). Moreover, the seh gene has also been reported mainly in MSSA-CC30 (Blomfeldt et al., 2013). Nevertheless, our observation on seh-positive MSSA-CC1 is in agreement with previous reports (Chen et al., 2013; Rasmussen et al., 2013).

The genes associated with staphylococcal complement inhibitor (scn) and staphylokinase (sak) were also widely distributed across the CCs but CC15 isolates were sak gene negative. Virulence associated with the exfoliative toxins has been identified to cause epidermal cleavage in staphylococcal scalded skin syndrome (SSSS) and bullous impetigo (Ladhani et al., 1999). The exfoliative toxin D (ETD) is a 27-kDa protein which causes epidermal blisters in newborn mice (Yamasaki et al., 2006). The epidermal cell differentiation factors (EDIN) target and inhibit the small host protein RhoA, a master regulator of the host cell actin cytoskeleton (Inoue et al., 1991; Jaffe and Hall, 2005; Aktories, 2011). Furthermore, the edin-isofrom (edinB) and etd genes are located in tandem in a S. aureus etd pathogenicity island in a chromosome of etd-positive S. aureus strains (Yamaguchi et al., 2002). A strong association of the etd gene with invasive CC25 S. aureus isolates has also been
reported. In this study, all the isolates assigned with CC25 and CC80 were etd-positive, which is in agreement with a previous study in Nigeria (Shittu et al., 2011). Moreover, MSSA grouped with CC25, CC80, and CC152 were edinB positive but CC152 isolates were etd negative. Our observations were similar to a study on the distribution of the edin gene in S. aureus from diabetic foot ulcers (Messad et al., 2013). A study in MSSA bacteremia isolates in Sweden showed that the collagen binding protein (Cna) was detected in CC1, CC30, and CC45. Our report identified the gene in isolates assigned with CC1, CC30, CC45, CC121, and CC152.

Our study has a number of limitations. Although all isolates were of human origin, and the large majority was obtained from clinical samples, a clear distinction between commensal and clinical strains could not be made based on the available information. An association of isolates within the context of endemcity i.e., nosocomial vs. community associated infections, is also not clear. Furthermore, whereas the microarray analytical database is exhaustive, well-characterized, and validated with isolates from all continents, the attribution of CCs is based on the hybridization reactions and resulting microarray profile rather than gene sequencing, and a positive signal does not necessarily imply the presence of gene product (e.g., protein). In addition, the microarray method was unable to separate ST8 from ST2427.

This might be due to the close phylogenetic relation of both STs as they are single locus variants (ST8: 3–3–1–1–4–4–3 and ST2427: 3–3–297–1–4–4–3). Finally, with a collection of 52 isolates studied, and a large number of genes and genetic profile ascertained by microarray, the potential for individual statistical comparisons is limited. Yet, with this comprehensive genetic-analytical approach performed on a clinical isolate collection obtained from patients of various medical institutions in a sub-Saharan African country, Nigeria, a number of important observations could be made which clearly characterize and demarcate the clonal distribution as well as the virulence gene equipment.

More than one half (55.8%; n = 29) of these MSSA isolates were associated with a genetic background which is attributable to classic methicillin-resistant S. aureus (MRSA) clones. PVL-positive isolates were identified in seven of the 12 CCs. Moreover, toxin genes were observed to be distributed mainly with certain clonal types, and in agreement with previous investigations (Holtfreter et al., 2007; Monecke et al., 2008). Antibiotic resistance gene profiles of the isolates by the DNA microarray demonstrated concordant results with data on antibiotic susceptibility testing. The array-based, comprehensive approach has been shown to yield such diverse CC and gene specific results on an isolate collection from sub-Saharan Africa.
Overall, microarray analysis proved to be a useful tool to provide useful information on antibiotic resistance, population structure and various virulence factor profiles associated with infection and disease. It is assumed that these findings might be useful for a better understanding of clinical staphylococcal disease presentation, patient care and for assistance in outbreak investigation in health care institutions in a country such as Nigeria. Moreover, our study also underlines the need for further trials employing well-controlled, prospectively collected clinical isolates to delineate the genetic pathogen profile in conjunction with the clinical disease presentation in sub-Saharan Africa.

**AUTHOR CONTRIBUTIONS**

AS, UR, GP, FS, LM, and MH conceived the study, OO, KO, AR conducted the sample collection and preliminary identification of the isolates. AS performed the microarray technique, AS and UR analyzed the microarray data, and AS wrote the manuscript (with input from all authors). All authors read and approved the final version of the manuscript.

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**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fmicb.2015.01160

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**Conflict of Interest Statement**: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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