1 **Clonal transmission and new mechanism of resistance to trimethoprim-** 2 **sulfamethoxazole in *Stenotrophomonas maltophilia* strains isolated in a** 3 **neonatology unit at Antananarivo, Madagascar, deciphered by whole** 4 **genome sequence analysis**

5 Mamitina Alain Noah Rabenandrasana¹, Volaso Andrianoelina¹, Melanie Bonneault²,
6 Perlinot Herindrainy³, Benoit Garin⁴, Sebastien Breurec⁵, Elisabeth Delarocque-Astagneau²,
7 Zafitsara Zo Andrianirina⁶, Vincent Enouf⁷, Bich-Tram Huynh², Lulla Opatowski², Jean-Marc
8 Collard¹

9
10 ¹*Experimental Bacteriology Unit, Institut Pasteur, Antananarivo, Madagascar.*

11 ²*Biostatistique, Biomathématique, Pharmaco-épidémiologie et Maladies Infectieuses (B2PHI), Institut*
12 *Pasteur, Inserm, Université de Versailles–Saint-Quentin-en-Yvelines (UVSQ), Paris, France.*

13 ³*Epidemiology and Clinical Research Unit, Institut Pasteur, Antananarivo, Madagascar.*

14 ⁴*Laboratoire Immuno-Hématologie CHU, CHU Pointe-à-Pitre, Abymes, Guadeloupe, France.*

15 ⁵*Faculté de médecine, Institut Pasteur de la Guadeloupe, Pointe-à-Pitre, Guadeloupe*

16 ⁶*Pediatric Ward, Centre Hospitalier de Soavinandriana, Antananarivo, 97159, Madagascar.*

17 ⁷*Pasteur International Bioresources network (PIBnet), Plateforme de Microbiologie Mutualisée (P2M),*
18 *Institut Pasteur, Paris, France.*

19

20 Corresponding author:

21 Mamitina Alain Noah Rabenandrasana, rabalainnoah@gmail.com

22

23 **ABSTRACT**

24 *Stenotrophomonas maltophilia* has been recognized as an emerging multidrug resistant
25 organism in hospital settings due to its resistance to a broad range of antimicrobial agents.
26 These include β -lactams and aminoglycosides, afforded by the existence of intrinsic and

27 acquired resistance mechanisms. Trimethoprim/sulfamethoxazole (SXT) is recommended as
 28 one of the best treatment choices against *S. maltophilia* infections; however increasing
 29 resistance to SXT has complicated the treatment. From July 2014 to March 2015, individuals
 30 and surfaces from a neonatology ward in Antananarivo, Madagascar, were longitudinally
 31 followed to assess the transmission of bacteria resistant to antibiotics between neonates,
 32 individuals (parents and nurses) and ward environments. Four *S. maltophilia* strains were
 33 successively isolated from a water-tap (N=1), from feces obtained from a newborn (N=1), and
 34 nursing staff (N=2). Antimicrobial susceptibility testing and whole genome sequencing were
 35 performed on each isolate. Based on coregenome alignment, all strains were identical and
 36 belonged to the new sequence type ST-288. They were resistant to trimethoprim-
 37 sulfamethoxazole, carbapenems and intermediate to levofloxacin. Each isolate carried the
 38 *aadB*, *strA*, *strB* and *sulI* genes located in a class I integron but variants of the *dfrA* gene were
 39 absent. We assessed by PROVEAN analysis the single nucleotide mutations found in *folA*, *folC*
 40 and *folM* genes and only the mutation in *folA* (A114T:GCC→ACC) has an effect on the activity
 41 of trimethoprim. Our findings demonstrated the prolonged presence of SXT-resistant *S.*
 42 *maltophilia* in a clinical setting with consecutive transfers from the environment to a newborn
 43 and staff based on the isolation dates. We also hypothesized that single nucleotide mutations
 44 in *folA* could be responsible for trimethoprim resistance.

45

46 INTRODUCTION

47 *Stenotrophomonas maltophilia* is a non-fermentative Gram-negative bacterium generally
 48 found throughout the environment (soil, sewage, plants). It is also occasionally isolated in
 49 hospitals where this bacterium is currently regarded as an important opportunistic pathogen. It
 50 causes a large range of clinical syndromes such as bacteraemia, sepsis, pneumonia,
 51 meningitis, endocarditis, septic arthritis, urinary infections, and endophthalmitis (1, 2),
 52 especially in hospitalized immunocompromized patients or patients with underlying disease.
 53 *S. maltophilia* has been recognized as one of the leading nosocomial multidrug resistant

54 organisms due to its resistance to a broad range of antimicrobial agents, including β -lactams
 55 and aminoglycosides, afforded by the existence of intrinsic and acquired resistance
 56 mechanisms (3). However it remained susceptible to fluoroquinolones, polymyxins and
 57 trimethoprim/sulfamethoxazole (SXT) (4).
 58 SXT in association with ticarcillin and clavulanic acid is traditionally recommended as one of
 59 the first choices against *S. maltophilia* infections but fluoroquinolones (also in association
 60 with other antibiotics) are an attractive option due to their *in vitro* activity (5). However,
 61 increasing resistance to SXT has complicated the treatment and resistance determinants such
 62 as *sul* and *dfrA* genes, class 1 integrons and mobile genetic elements have been reported to
 63 contribute to SXT resistance (6–8). The aim of this study was to establish the link between 4
 64 SXT resistant *S. maltophilia* isolates collected in a neonatology unit and to decipher the
 65 genetic basis of resistance to SXT.

66

67 MATERIALS AND METHODS

68 Study design

69 The longitudinal study was conducted in the neonatal intensive care unit in CENHOSOA
 70 hospital in Antananarivo, Madagascar (08/27/2014–03/06/2015) and was already described in
 71 Bonneault *et al.* 2019 (9). Briefly, 22 newborns (NBs) were included in the cohort and were
 72 followed until discharge or death. Average unit stays lasted 18 days. All health-care workers
 73 (HCWs) and NBs' accompanying family members (FMs; usually the mother, involved in the
 74 basic infant care, except for one child who had four distinct accompanying FMs) were also
 75 followed. In total 22 NBs, 21 HCWs and 24 FMs were included in the study. At enrollment, a
 76 rectal swab was obtained from the NB and a stool sample from the FM to detect E-ESBL
 77 colonization. Rectal swabs were systematically obtained from the NB on a weekly basis. For
 78 stays <7 days in the unit, a stool sample was obtained the day of discharge. Stools were also
 79 collected from the FMs and from the HCWs every week. Environmental swabbing was
 80 performed at the beginning, middle and the end of the investigation. The study was approved

81 by the Madagascar Public Health Ministry Ethics Committee (Reference number: 040–
82 MSANP/CE).

83

84

85 **Bacteriological analyses**

86 All samples were cultivated on CHROMagar™ ESBL (CHROMagar, Paris, France) and each
87 colony morphotype was identified by mass spectrometry (MS) MALDI-TOF (Bruker
88 Daltonics, Bremen, Germany). Antimicrobial susceptibility testing was performed on each
89 isolate according to the standard disc methods described in the 2018 CASFM guidelines. In
90 this study, we only focused on *S. maltophilia* positive samples.

91

92 **Whole genome sequencing (WGS) & Bioinformatic analysis**

93 DNA extraction was performed on 5 mL of liquid cultures grown overnight at 37°C in a Luria
94 Bertani infusion medium by using the Cador Pathogen Extraction Kit (Indical Bioscience) on
95 the Qiacube HT (QIAGEN, France) device according to the manufacturer's protocol for
96 Gram-negative bacteria. DNA quantity and purity was assessed by using Nanodrop
97 2000/200C (Thermo Fisher Scientific, Waltham, MA, USA). Library preparation was
98 conducted by using the Nextera XT DNA Sample Kit (Illumina, San Diego, CA, USA). WGS
99 was performed on a NextSeq 500 platform (Illumina) by using 2 × 150-bp runs. FqCleaner
100 version 3.0 was used to eliminate adaptor sequences (10, 11), reduce redundant or
101 overrepresented reads (12), correct sequencing errors (13), merge overlapping paired reads,
102 and discard reads with Phred scores (measure of the quality of identification of nucleobases
103 generated by automated DNA sequencing) <20. Illumina reads *de novo* assembly was
104 performed using Spades (14). Acquired resistance genes were detected by the resfinder
105 software (15). The genomes were annotated by using prokka and PATRIC web server (16,
106 17). The sequence types (ST) were determined *in silico* with the public database for *S.*
107 *maltophilia* (<https://pubmlst.org/smaltophilia/>). Phylogenetic analysis based on whole genome

sequences was done using the Parsnp program from the harvest suite, gubbins and RaxML (18–20). Mutation detection in SXT resistant *S. maltophilia* strains was performed with the Breseq 0.31.1 software (*S. maltophilia* D457 was used as reference genome) (21). All non-synonymous mutations were analyzed with PROVEAN (Protein Variation Effect Analyzer) (13) to predict the functional deleteriousness caused by the missense mutation. Plasmid detection, typing and reconstruction were performed with the MOB-suite software (22).

Predicted structure and molecular docking

The secondary and tertiary structures of proteins were predicted by using the Raptor X server (23–26). The wild-type amino acid sequence from the D457 strain was chosen as template for homology modeling. We used autodock vina to calculate Gibbs free energy of binding (ΔG_{bind}) of ligands (trimethoprim) with targets. They were further converted to the predicted inhibition constants ($K_{i_{\text{pred}}}$) with this formula (27):

$$K_{i_{\text{pred}}} = \exp([\Delta G_{\text{bind}} * 1,000] / [R * T])$$

where R (gas constant) is 1.98 cal(mol*K)⁻¹, and T (room temperature) is 298.15 Kelvin.

Quantification of biofilm formation

The biofilm assay was performed as previously described (28) but with slight modifications. Overnight cultures of *S. maltophilia* in 5 mL Luria Bertani infusion medium reaching an optical density at 620 nm equivalent to 1 (OD₆₂₀) (approximately 1*10⁹ CFU/mL) were transferred to the wells of a sterile flat-bottomed 96-well polystyrene microtitre plate and incubated for 24h at 35°C +/- 2°C. Non adherent cells were subsequently removed by washing twice with 200 µl of sterile distilled water. The amount of biofilm biomass was assessed by crystal violet staining. Biofilms were stained with 125 µl of 1.0 % (w/v) crystal violet for 15 min. The dye solution was discarded, and the plate was washed three times with sterile distilled water and allowed to air-dry for 24 h at room temperature. Stained biofilms were exposed to 30.0 % (v/v) acetic acid for 15 min, and the OD 620 of the extracted dye was

subsequently measured. The average OD values was calculated for all tested strains and negative controls, since all tests are performed in triplicate and repeated three times. Second, the cut-off value (OD_c) was established. It is defined as three standard deviations (SD) above the mean OD of the negative control: OD_c=average OD of negative control + (3×SD of negative control). OD values of a tested strain are expressed as average OD value of the strain reduced by OD_c value (OD=average OD of a strain -OD_c). OD_c value is calculated for each microtiter plate separately. Based upon the previously calculated OD values: OD ≤OD_c = no biofilm producer; OD_c ≤OD ≤2 × OD_c = weak biofilm producer; 2 × OD_c≤OD≤4×OD_c = moderate biofilm producer; 4OD_c<OD = strong biofilm producer. All tests were performed in triplicate and repeated three times.

RESULTS

Epidemiological data

During the cohort conducted in the neonatal unit - (see Materials and Methods), 4 *S. maltophilia* were isolated. A first *S. maltophilia* strain (MS MALDI-TOF score of 2.736) was isolated in January, 07th 2015 from an environmental swab (476SM) realized on a water tap out of the three sinks present in the neonatology unit. Fourteen days later a second *S. maltophilia* strain (MS MALDI-TOF score of 2.120) was isolated from a rectal swab of a premature newborn (517SM) admitted to the neonatal ICU. Subsequently, two strains of *S. maltophilia* were isolated from the fingerprints of 2 nurses at day 47 (629SM) and 56 (646SM) after the first isolation from the water tap (MS MALDI-TOF scores of respectively 2.102 and 2.052) (Table 1).

Antimicrobial susceptibility testing

All 4 isolates were resistant to cotrimoxazole (SXT), ertapenem (ETP), meropenem (MEM) and imipenem (IPM), intermediate to levofloxacin (LVX) and sensitive to minocycline

(MNO), ceftazidime (CAZ) and ticarcillin-clavulanate (TCC). The antimicrobial susceptibility testing results of the 4 strains are showed in table1.

Table1: Antimicrobial susceptibility testing of the four *S. maltophilia* strains. Abbreviations: SXT: trimethoprim-sulfamethoxazole, TCC: Ticarcillin-clavulanate, CAZ: ceftazidim, LVX: levofloxacin, MNO: minocycline, MEM: meropenem, ETP: ertapenem and IPM: imipenem.

Strains	Origin	Isolation date	Sample	inhibition zone diameters (mm)							
				SXT	TCC	CAZ	LVX	MNO	MEM	ETP	IPM
476SM	Watertap	January 07, 2015	Environemental swab	6 (R)	35 (S)	22 (S)	21 (I)	25 (S)	6 (R)	6 (R)	6 (R)
517SM	Baby	January 21, 2015	Rectal swab	6 (R)	37 (S)	23 (S)	21 (I)	27 (S)	6 (R)	6 (R)	6 (R)
646SM	Nursing staff1	February 23, 2015	Fingerprint	6 (R)	36 (S)	23 (S)	21 (I)	24 (S)	6 (R)	6 (R)	6 (R)
629SM	Nursing staff2	March 04, 2015	Fingerprint	6 (R)	34 (S)	21 (S)	21 (I)	26 (S)	6 (R)	6 (R)	6 (R)

Phylogenetic analysis and multilocus sequence-types

A phylogenetic analysis based on whole genome sequences was performed using the Parsnp program. The 4 isolates are identical and clustered with a *S. maltophilia* (LQQS01000001) originating from Malaysia (figure 1). The sequence types (ST) of our four isolates were determined with MLST *in silico* with the public database (<https://pubmlst.org/smaltophilia/>). They all belonged to a new ST: ST-288.

Figure 1: Phylogenetic tree based on coregenome alignment of four *S. maltophilia* including representative genomes from NCBI genbank. Sequences were aligned using ParSNP, genetic recombination were removed using Gubbins and phylogenetic inferences were obtained using the maximum likelihood method within raxML software. Bootstrap values are expressed by decimal of 1 000 replicates with a parameter test and shown at the branching points. The branches of the tree are indicated by the genus and species name of the type strains followed by the NCBI gene accession numbers. The four *S. maltophilia* isolates from this study are represented in red color.

Resistome analysis

Multiple antimicrobial, heavy-metal and arsenic resistance markers were identified in the chromosome of each isolate (Table 2). The resistome of these 4 strains revealed the presence of 27 antibacterial-resistant genes using the resfinder software and PATRIC webserver. The

192 isolates possess two different types of β -lactamases, i.e., Amber class A genes (L2 family),
193 Amber class B metallo- β -lactamase (MBL) genes (L1 family), and five aminoglycoside
194 inactivation enzymes: *aph(2'')-ia*, *aph(3'')-i*, *aph(3')-ii/aph(3')-xv*, *aph(6)-ic/aph(6)-id*. We
195 also identified 9 efflux pumps conferring antibiotic resistance and two regulators modulating
196 expression of antibiotic resistance genes

197

198 Plasmid analysis

199 Mobsuite software enabled us to identify and reconstruct two plasmids: an IncP conjugative
200 plasmid harboring the aminoglycoside 6-phosphotransferase gene (*sph* gene) and which
201 presented 86% nucleotidic identities with the plasmid p35734 found in *E. cloacae*. Moreover
202 we found a non-transferable plasmid with a RND efflux transporter conferring resistances to
203 cobalt, zinc, cadmium and which presented 99% nucleotidic identities with the plasmid
204 pLMG930 found in *Xanthomonas euvesicatoria*. As we used the short-reads sequencing
205 technique, we were unable to circularize the plasmids.

206

207 Genetic environment of resistance genes

208 All isolates carried class I integrons that contains *sul1*, *aadB*, *aph3*, *aph6* genes and the entire
209 heat shock protein gene *groEL* upstream the *sul1* gene (Figure 2).

210

211 Figure2: Structure of class I integrons in the *S. maltophilia* isolated in the neonatology unit.

212 Table 2: Resistome of *S. maltophilia* isolated in the neonatology unit

Function	Enzymes commission numbers	Classification
Subclass B3 beta-lactamase (L1 family)	EC 3.5.2.6	antibiotic inactivation enzyme
Putative aminoglycoside 6-phosphotransferase		
Aminoglycoside 3'-phosphotransferase (<i>aph(3')-ii/aph(3')-xv</i>)	EC 2.7.1.95	
Aminoglycoside 2"-nucleotidyltransferase (<i>aph(2'')-ia (aadb family)</i>)	EC 2.7.7.46	
Aminoglycoside 6-phosphotransferase (<i>aph(6)-ic/aph(6)-id</i>)	EC 2.7.1.72	
Class A beta-lactamase	EC 3.5.2.6	
Aminoglycoside 3"-phosphotransferase (<i>aph(3'')-i</i>)	EC 2.7.1.87	efflux pump conferring antibiotic resistance
Outer membrane channel TolC (<i>OpmH</i>)		
Multidrug efflux system EmrAB-OMF, membrane fusion component EmrA		
Macrolide-specific efflux protein MacA		
Multidrug efflux system MdtABC-TolC, inner-membrane proton/drug antiporter MdtB (RND type)		
Macrolide export ATP-binding/permease protein MacB		

213 Table 2: Continued

Function	Enzymes commission numbers	Classification
Multidrug efflux system EmrAB-OMF, inner-membrane proton/drug antiporter EmrB (MFS type)		efflux pump conferring antibiotic resistance
Multidrug efflux system MdtABC-TolC, inner-membrane proton/drug antiporter MdtC (RND type)		
Multidrug efflux system MdtABC-TolC, membrane fusion component MdtA		
Outer membrane factor (OMF) lipoprotein associated with EmrAB-OMF efflux system		
Outer membrane low permeability porin, OprB family		gene modulating permeability to antibiotic
Hydrogen peroxide-inducible genes activator (<i>OxyR</i>)		regulator modulating expression of antibiotic resistance genes

214

Mutations and resistance to SXT

When using *S. maltophilia* D457 as reference genome a total of 327 deletions, 286 insertions and 2133 substitutions were detected with the Breseq 0.31.1 software. In all isolates, single nucleotide mutations in genes encoding enzymes involved in folic acid metabolism which can be inactivated by trimethoprim were detected in *folA* (A114T:GCC→ACC), *folC* (L339V:TTG→GTG) and *folM* (A72P:GCC→CCC) which encode respectively the dihydrofolate reductase, dihydrofolate synthase and FolM alternative dihydrofolate reductase. Only the mutation in *folA* has an effect on the activity of the protein as assessed by PROVEAN analysis

Predicted structure and molecular docking

The Gibbs free energy of binding (ΔG_{bind}) between the FolA protein (mutated and wild-type) and trimethoprim was calculated and values of - 6.9 kcal/mol and -7.1 kcal/mol were found for the mutated and the wild-type proteins, respectively. The predicted inhibition constants ($K_{\text{i,pred}}$) between trimethoprim and the FolA wild-type protein was 3.59×10^{-6} and 8.39×10^{-6} between trimethoprim and the mutated FolA indicating a reduced susceptibility to trimethoprim.

Quantification of biofilm formation

The amount of biofilm biomass was assessed by crystal violet staining as explained in table 4. All 4 isolates had an average OD_{650nm} value of 0.145 (OD_c= 0.086), meaning they are weak biofilm producers. Additionally, we detected in the four isolates modifications in genes involved in biofilm production: 43 substitutions in the *rmlA* gene, 32 substitutions in the *spgM* gene, two insertions and three substitutions in the intergenic region between the *manA* and *spgM* genes, two substitutions in the intergenic region between the *rfbB* and *rmlA* genes and one substitutions in the intergenic region between the *rpfC* and *rpfF* genes. These genetic

modifications are probably the cause of weak biofilm production. The list of all mutations detected is showed in supplementary material S1.

Table 4: Optical density (650nm) of biofilm formation of four strains

ID strain	517SM	476SM	629SM	646SM	Standard deviation	OD _{negative}
test 1	0,212125	0,11725	0,174125	0,1245	0,00698851	0,08659053
test2	0,174125	0,19625	0,125375	0,100625	0,01431034	0,08368101
test3	0,13575	0,135625	0,124	0,12375	0,00484031	0,08952092
Average	0,174	0,14970833	0,14116667	0,11629167	0,00871305	0,08659749

Discussion

The prevalence of *S. maltophilia* has increased in hospitals worldwide simultaneously with the emergence of a myriad of other antibiotic resistant bacteria (29). Here, we demonstrated, based on WGS phylogeny, the clonal transmission of *S. maltophilia* resistant to SXT in a neonatology unit, which might be explained by its ecology and fitness in hospitals wards and/or by poor hygiene management. The determination of a new ST is in accordance with the high plasticity and capacity of this bacterium to adapt to specific niches and develop new characteristics. We showed in our study that the same strain of *S. maltophilia* was isolated four times on a period of two months in a neonatal ward of a hospital in Antananarivo, confirming its ability to persist and spread in the medical environment. Selective pressure imposed by specific conditions in a hospital environment could promote the survival of certain STs with an adaptive advantage for this specific setting and lead afterwards to their clonal spread.

The reduced susceptibility of *S. maltophilia* to most antibiotics can be attributed to both intrinsic and acquired resistances. The proteins mediating intrinsic resistance in *S. maltophilia* include chromosomally encoded multidrug efflux pumps such as SmeABC, SmeDEF, SmeYZ, SmeOP-TolCSm, antibiotic-inactivating enzymes (L1/L2 β -lactamases

and aminoglycoside inactivating enzymes), and the chromosomally encoded Qnr pentapeptide repeat proteins (30) which are present in most if not all strains of *S. maltophilia*, as in our Madagascan strains, suggesting that they did not arise during the recent evolution of resistance caused by antibiotic therapy. In addition, *S. maltophilia* can acquire mechanisms to increase its resistance pattern through horizontal gene transfer via plasmids, and subsequently by recombination processes with integrons, transposons and genomic islands (GIs).

The four isolates, which are genotypically identical, were resistant to SXT, one of the therapeutic choices. The resistance of Gram-negative bacteria to sulfonamides is mainly conferred by the acquisition of either *sul1* or *sul2*, encoding dihydropteroate synthases (31). The *sul1* gene carried by class 1 integrons and *sul2*, which is linked to insertion sequence common region (ISCR) elements, was identified in SXT-resistant *S. maltophilia* isolates (5, 32, 33). The resistance to trimethoprim in *S. maltophilia* is mainly conferred by the dihydrofolate reductase *dfr* genes such as *dfrA1*, *dfrA5*, *dfrA12*, *dfrA17*, and *dfrA27* which are usually located within class 1 integrons as part of various resistance gene cassettes. Both types of *sul* and *dfr* genes can occur together in high-level SXT-resistant isolates (6, 34). Moreover, the efflux pumps SmeDEF, TolCsm, and SmeYZ are associated with SXT resistance (35–37). We found that the *sul1* gene was present but no *dfr* genes were detected, pointing out the presence of another mechanism of resistance to trimethoprim. We were able to show that a point mutation (A114T:GCC→ACC) in the dihydrofolate reductase gene (*folA*) decreased the affinity of trimethoprim to the F_{olA} protein ensuring therefore resistance to this antibiotic.

We have also shown the presence of two plasmids, one of which harbored heavy metal resistance genes and which was almost identical to the pLMG930 plasmid found in *Xanthomonas euvesicatoria*, a bacterial spot-causing xanthomonads. This indicates a high probability of dissemination of the strain in different ecological niches, notably those contaminated with heavy metals. It is well known that the presence of both metals and antibiotic resistance genes play a major role in the persistence, selection and spread of

antibiotic- and metal-resistant bacteria in anthropogenic environments heavily contaminated with detergents, heavy metals and other antimicrobials [29, 30]. In developing countries, rivers, lakes and lagoons are often contaminated with untreated hospital and industrial effluents and also by urban storm-water containing anthropogenic pollutants due to intensive uncontrolled urbanization. These are optimal conditions for bacterial development and the spread of antibiotic-resistant bacteria.

Biofilm formation in bacteria is a multifactorial event that depends on surface characteristics, motility of strains, genes involved in biofilm formation, and other factors, and is usually correlated with a higher level of resistance to antibiotics and disinfectants (38). Different factors influence the physiology of biofilm formation in *S. maltophilia*, namely the SmeYZ efflux pump that confers resistance to antimicrobials (37), the iron level in the media (39), and histidine kinase and BfmAK system (40). Interestingly, our study revealed a negative correlation between the simultaneous presence of genes involved in biofilm formation such as *spgM*, *rmlA* and *rpfF* genes and the biofilm production. This correlation could be due to the mutations identified in the three previous genes. However, a clone was detected along a period of two months in the neonatology ward pointing out a persistence of the strain in this environment.

In conclusion, this work represents the first characterization at the genomic level of SXT resistant *S. maltophilia* strains circulating in a neonatology ward of a hospital in Antananarivo, Madagascar. We also pointed out the possible role of a point mutation in the *folA* gene conferring resistance to trimethoprim. Clonal relatedness between strains indicated the transmission and the persistence of *S. maltophilia* in the hospital setting and the threat it could represent for newborns, especially for preterms.

Data availability:

These whole genome shotgun projects have been deposited at DDBJ/ENA/GenBank. under the accession [VFJF000000000](#), [VFJG000000000](#), [VFJH000000000](#) and [VFEX000000000](#) corresponding respectively for the strains.517SM, 629SM, 646SM and 476SM.

Acknowledgments: We would like to thank Tatianah Sehen Rivomanantsoa, the field investigator, and the staff of pediatric and neonatology units' at the CENHOSOA Hospital, Antananarivo, Madagascar. We would also like to thank the program “Actions concertées inter-pasteuriennes” (ACIP: grant no. A-22-2013) which supported this work and staff of the “Plateforme de Microbiologie Mutualisée (P2M)” at Institut Pasteur Paris where the whole genome sequencing was performed.

References

1. Denton M KK. 1998. Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. Clin Microbiol Rev 11:57–80.
2. Looney WJ, Narita M, Mühlemann K. 2009. *Stenotrophomonas maltophilia* : an emerging opportunist human pathogen. Lancet Infect Dis 9:312–323.
3. JS B. New strategies against *Stenotrophomonas maltophilia*: a serious worldwide intrinsically drug-resistant opportunistic pathogen. Expert Rev Anti Infect Ther 12:1–4.
4. Yu Lin, Wang Marco R, Scipione, Yanina Dubrovskaya JP. Monotherapy with Fluoroquinolone or Trimethoprim-Sulfamethoxazole for Treatment of *Stenotrophomonas maltophilia*. Infect Antimicrob Agents Chemother 58:176–182.
5. Barbolla R, Catalano M, Orman BE, Famiglietti A, Vay C, Smayevsky J, Centrón D PSA. 2004. Class 1 integrons increase trimethoprim-sulfamethoxazole MICs against epidemiologically unrelated *Stenotrophomonas maltophilia* isolates. Antimicrob Agents Chemother 48:666–669.
6. Hu LF, Chen GS, Kong QX, Gao LP, Chen X, Ye Y LJB. 2016. Increase in the

prevalence of resistance determinants to trimethoprim/sulfamethoxazole in clinical

Stenotrophomonas maltophilia isolates in China. PLoS One 11.

7. Chatelut M, Dournes JL, Chabanon G MN. 1995. Epidemiological typing of *Stenotrophomonas* (*Xanthomonas*) *maltophilia* by PCR. J ClinMicrobiol 33:912–914.

8. Chang YT, Lin CY, Chen YH HPR. 2015. Update on infections caused by *Stenotrophomonas maltophilia* with particular attention to resistance mechanisms and therapeutic options. Front Microbiol 6:893.

9. Bonneault; M, Andrianoelina; VH, Herindrainy; P, Rabenandrasana; MAN, Garin; B, Breurec; S, Delarocque-Astagneau; E, Guillemot; D, Andrianirina; ZZ, Collard; J-M, Huynh; BT, Opatowski; L. 2019. Transmission Routes of Extended-Spectrum Beta-Lactamase – Producing Enterobacteriaceae in a Neonatology Ward in Madagascar 00:1–8.

10. Criscuolo A BS. 2013. AlienTrimmer: a tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads. Genomics 102:500–506.

11. Crusoe MR, Alameldin HF, Awad S, Boucher E, Caldwell A CR. 2015. The khmer software package: enabling efficient nucleotide sequence analysis. F1000Res 4:900.

12. Liu Y, Schröder J SB. 2018. Musket: a multistage k-mer spectrum-based error corrector for Illumina sequence data. Bioinformatics 29:308–315.

13. Choi Y, Sims GE, Murphy S, Miller JR CAP. 2012. Predicting the Functional Effect of Amino Acid Substitutions and Indels. PLoS One 7.

14. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham SON, Prjibelski AD, Pyshkin A V, Sirotkin A V, Vyahhi N, Tesler G, Alekseyev MAXA, Pevzner PA. 2012. and Its Applications to Single-Cell Sequencing 19:455–477.

15. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes 2640–

2644.

16. Wattam AR, Davis JJ, Assaf R, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC , the all-bacterial Bioinformatics Database and Analysis Resource 45:535–542.
17. Seemann T. 2014. Prokka : rapid prokaryotic genome annotation 30:2068–2069.
18. Stamatakis A. 2014. RAxML version 8 : a tool for phylogenetic analysis and post-analysis of large phylogenies 30:1312–1313.
19. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR. 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins 43.
20. Treangen TJ, Ondov BD, Koren S, Phillippy AM. 2014. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes 1–15.
21. DE D, JE. B. 2014. Identification of mutations in laboratory-evolved microbes from next-generation sequencing data using breseq. *Methods Mol Biol* 1151:165–188.
22. Nash JR, E. JH. 2018. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. *Microb Genomics* 4.
23. Jian Peng JX. Raptorx: Exploiting structure information for protein alignment by statistical inference. *Predict Methods Reports, PROTEINS* 79.
24. Peng JI XJ. 2011. A multiple-template approach to protein threading. *Proteins* 79:1930.
25. Xu J, ; MS, Feng; W, Jinbo; Z. 2013. Protein threading using context-specific alignment potential. *Bioinformatics* 29:257–265.
26. O. Trott AJO. 2010. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J Comput*

- 398 Chem 31:455–461.
- 399 27. Sergey Shityakov CF. 2014. In silico predictive model to determine vector-mediated
400 transport properties for the blood–brain barrier choline transporter. Adv Appl
401 Bioinforma Chem 7:1–14.
- 402 28. Srdjan Stepanovic ´, Dragana Vukovic ´, Veronika Hola, Giovanni Di Bonaventura,
403 Slobodanka Djukic ´ IC ´Irkovic ´, Ruzicka. F. 2007. Quantification of biofilm in
404 microtiter plates: overview of testing conditions and practical recommendations for
405 assessment of biofilm production by staphylococci. APMIS 115:891–899.
- 406 29. Haowa Madi, Jovanka Lukić, Zorica Vasiljević, Marjan Biočanin MK, Branko Jovčić
407 JL. 2016. Genotypic and Phenotypic Characterization of *Stenotrophomonas maltophilia*
408 Strains from a Pediatric Tertiary Care Hospital in Serbia. PLoS One 11:10.
- 409 30. Sanchez MB, Hernandez A MJL. 2009. *Stenotrophomonas maltophilia* drug resistance.
410 Futur Microbiol 4:655–660.
- 411 31. Rådström P SG. 1988. RSF1010 and a conjugative plasmid contain sulII, one of two
412 known genes for plasmid-borne sulfonamide resistance dihydropteroate synthase.
413 Antimicrob Agents Chemother 32:1684–1692.
- 414 32. Toleman MA, Bennett PM, Bennett DM, Jones RN WTR. 2007. Global emergence of
415 trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated
416 by acquisition of sul genes. Emerg Infect Dis 13:559–565.
- 417 33. Chung HS, Kim K, Hong SS, Hong SG, Lee K CY. 2015. The sul1 gene in
418 *Stenotrophomonas maltophilia* with high-level resistance to
419 trimethoprim/sulfamethoxazole. Ann Lab Med 35:246–249.
- 420 34. Hu LF, Chang X, Ye Y, Wang ZX, Shao YB, Shi W, Li X LJB. 2011.
421 *Stenotrophomonas maltophilia* resistance to trimethoprim/sulfamethoxazole mediated
422 by acquisition of sul and dfrA genes in a plasmid-mediated class 1 integron. Int J
423 Antimicrob Agents 37:230–234.
- 424 35. Huang YW, Hu RM YTC. 2013. Role of the pcm-tolCsm operon in the multidrug

resistance of *Stenotrophomonas maltophilia*. J Antimicrob Chemother 68:1987–1993.

36. Sánchez MB MJ. 2015. The efflux pump SmeDEF contributes to trimethoprim-sulfamethoxazole resistance in *Stenotrophomonas maltophilia*. Antimicrob Agents Chemother 59:4347–4348.

37. Lin YT, Huang YW, Chen SJ, Chang CW YTC. 2015. The SmeYZ efflux pump of *Stenotrophomonas maltophilia* contributes to drug resistance, virulence-related characteristics, and virulence in mice. Antimicrob Agents Chemother 59:4067±4073.

38. BalcaÂzar JL, Subirats J BCM. The role of biofilms as environmental reservoirs of antibiotic resistance. Front Microbiol 6:1216.

39. GarcõÂa CA, Alcaraz ES, Franco MA P de RBN. 2015. Iron is a signal for *Stenotrophomonas maltophilia* biofilm formation, oxidative stress response, OMPs expression, and virulence. Front Microbiol 6:926.

40. Zheng L, Wang FF, Ren BZ, Liu W, Liu Z QW. 2016. Systematic Mutational Analysis of Histidine Kinase Genes in the Nosocomial Pathogen *Stenotrophomonas maltophilia* Identifies BfmAK System Control of Biofilm Development. Appl Env Microbiol 82:2444±2456.