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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

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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

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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 *sul1* 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

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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

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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

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108 sequences was done using the Parsnp program from the harvest suite, gubbins and RaxML
109 (18–20). Mutation detection in SXT resistant *S. maltophilia* strains was performed with the
110 Breseq 0.31.1 software (*S. maltophilia* D457 was used as reference genome) (21). All non-
111 synonymous mutations were analyzed with PROVEAN (Protein Variation Effect Analyzer)
112 (13) to predict the functional deleteriousness caused by the missense mutation. Plasmid
113 detection, typing and reconstruction were performed with the MOB-suite software (22).
114

115 **Predicted structure and molecular docking**

116 The secondary and tertiary structures of proteins were predicted by using the Raptor X server
117 (23–26). The wild-type amino acid sequence from the D457 strain was chosen as template for
118 homology modeling. We used autodock vina to calculate Gibbs free energy of binding (ΔG_{bind})
119 of ligands (trimethoprim) with targets. They were further converted to the predicted inhibition
120 constants (Ki_{pred}) with this formula (27):

121 $$Ki_{pred} = \exp([\Delta G_{bind}*1{,}000]/[R*T])$$

122 where R (gas constant) is 1.98 cal(mol*K)−1, and T (room temperature) is 298.15 Kelvin.
123

124 **Quantification of biofilm formation**

125 The biofilm assay was performed as previously described (28) but with slight modifications.
126 Overnight cultures of *S. maltophilia* in 5 mL Luria Bertani infusion medium reaching an
127 optical density at 620 nm equivalent to 1 (OD620) (approximately $1*10^9$ CFU/mL) were
128 transferred to the wells of a sterile flat-bottomed 96-well polystyrene microtitre plate and
129 incubated for 24h at 35°C +/- 2°C. Non adherent cells were subsequently removed by
130 washing twice with 200 µl of sterile distilled water. The amount of biofilm biomass was
131 assessed by crystal violet staining. Biofilms were stained with 125 µl of 1.0 % (w/v) crystal
132 violet for 15 min. The dye solution was discarded, and the plate was washed three times with
133 sterile distilled water and allowed to air-dry for 24 h at room temperature. Stained biofilms
134 were exposed to 30.0 % (v/v) acetic acid for 15 min, and the OD 620 of the extracted dye was

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

145

146 **RESULTS**

147 **Epidemiological data**

148 During the cohort conducted in the neonatal unit - (see Materials and Methods), 4 *S.*
149 *maltophilia* were isolated. A first *S. maltophilia* strain (MS MALDI-TOF score of 2.736) was
150 isolated in January, 07th 2015 from an environmental swab (476SM) realized on a water tap
151 out of the three sinks present in the neonatalogy unit. Fourteen days later a second *S.*
152 *maltophilia* strain (MS MALDI-TOF score of 2.120) was isolated from a rectal swab of a
153 premature newborn (517SM) admitted to the neonatal ICU. Subsequently, two strains of *S.*
154 *maltophilia* were isolated from the fingerprints of 2 nurses at day 47 (629SM) and 56
155 (646SM) after the first isolation from the water tap (MS MALDI-TOF scores of respectively
156 2.102 and 2.052) (Table 1).

157

158 **Antimicrobial susceptibility testing**

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

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161 (MNO), ceftazidime (CAZ) and ticarcillin-clavulanate (TCC). The antimicrobial
162 susceptibility testing results of the 4 strains are showed in table1.
163
164 Table1: Antimicrobial susceptibility testing of the four *S. maltophilia* strains. Abbreviations:
165 SXT: trimethoprim-sulfamethoxazole, TCC: Ticarcillin-clavulanate, CAZ: ceftazidim, LVX:
166 levofloxacin, MNO: minocycline, MEM: meropenem, ETP: ertapenem and IPM: imipenem.
167

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)

168

169 **Phylogenetic analysis and multilocus sequence-types**

170 A phylogenetic analysis based on whole genome sequences was performed using the Parsnp
171 program. The 4 isolates are identical and clustered with a *S. maltophilia* (LQQS01000001)
172 originating from Malaysia (figure 1). The sequence types (ST) of our four isolates were
173 determined with MLST *in silico* with the public database (https://pubmlst.org/smaltophilia/).
174 They all belonged to a new ST: ST-288.
175
176

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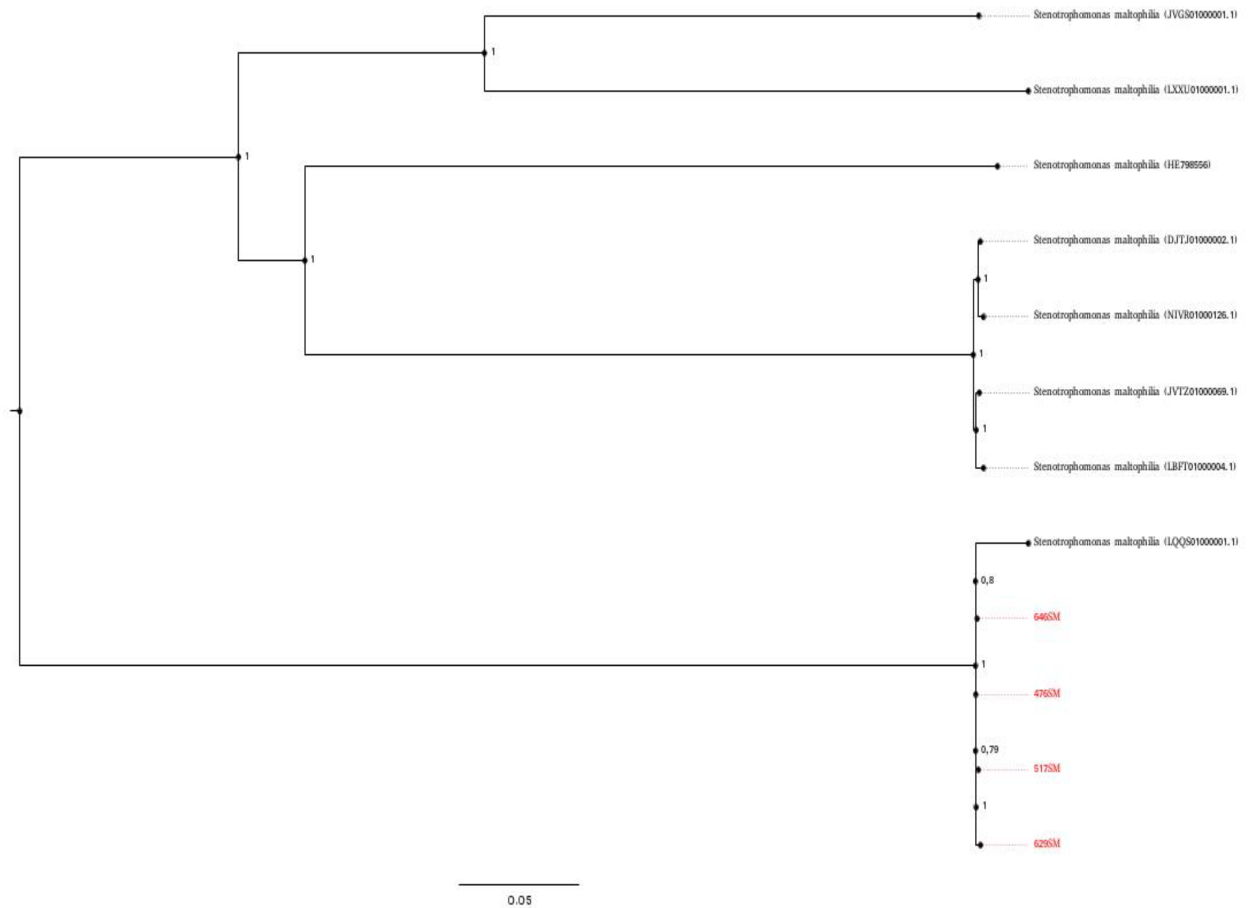
177



178

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

187

188 **Resistome analysis**

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

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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

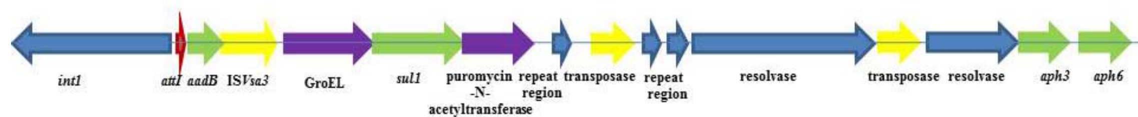
198 **Plasmid analysis**

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 founda 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 *sul*1, *aad*B, *aph*3, *aph*6 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 (*aph(3')-ii/aph(3')-xv*)	EC 2.7.1.95	
Aminoglycoside 2''-nucleotidyltransferase (*aph(2'')-ia (aadb* family))	EC 2.7.7.46	
Aminoglycoside 6-phosphotransferase (*aph(6)-ic/aph(6)-id*)	EC 2.7.1.72	
Class A beta-lactamase	EC 3.5.2.6	
Aminoglycoside 3''-phosphotransferase (*aph(3'')-i*)	EC 2.7.1.87	
Outer membrane channel TolC (*OpmH*)		efflux pump conferring antibiotic resistance
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		

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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 wth EmrAB-OMF efflux system		
Outer membrane low permeability porin, OprB family		gene modulating permeability to antibiotic
Hydrogen peroxide-inducible genes activator (*OxyR*)		regulator modulating expression of antibiotic resistance genes

214

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215 **Mutations and resistance to SXT**

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

224

225 **Predicted structure and molecular docking**

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

232

233 **Quantification of biofilm formation**

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

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241 modifications are probably the cause of weak biofilm production. The list of all mutations
242 detected is showed in supplementary material S1.
243 Table 4: Optical density (650nm) of biofilm formation of four strains
244

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

245

246 **Discussion**

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

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

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

269 The four isolates, which are genotypically identical, were resistant to SXT, one of the
270 therapeutic choices. The resistance of Gram-negative bacteria to sulfonamides is mainly
271 conferred by the acquisition of either *sul1* or *sul2*, encoding dihydropteroate synthases (31).
272 The *sul1* gene carried by class 1 integrons and sul2, which is linked to insertion sequence
273 common region (ISCR) elements, was identified in SXT-resistant *S. maltophilia* isolates (5,
274 32, 33). The resistance to trimethoprim in *S. maltophilia* is mainly conferred by the
275 dihydrofolate reductase *dfr* genes such as *dfrA1, dfrA5, dfrA12, dfrA17,* and *dfrA27* which are
276 usually located within class 1 integrons as part of various resistance gene cassettes. Both
277 types of *sul* and *dfr* genes can occur together in high-level SXT-resistant isolates (6, 34).
278 Moreover, the efflux pumps SmeDEF, TolCsm, and SmeYZ are associated with SXT
279 resistance (35–37). We found that the *sul1* gene was present but no *dfr* genes were detected,
280 pointing out the presence of another mechanism of resistance to trimethoprim. We were able
281 to show that a point mutation (A114T:GCC→ACC) in the dihydrofolate reductase gene (*folA*)
282 decreased the affinity of trimethoprim to the FolA protein ensuring therefore resistance to this
283 antibiotic.

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

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

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

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

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317 **Data availability:**

318 These whole genome shotgun projects have been deposited at DDBJ/ENA/GenBank. under
319 the accession VFJF00000000, VFJG00000000, VFJH00000000 and VFEX00000000
320 corresponding respectively for the strains.517SM, 629SM, 646SM and 476SM.

321

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

328

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