Multiple antibiotics resistant among environmental isolates of *Stenotrophomonas maltophilia*

**ETINOSA O. IGBINOSA, FAITH E. OVIASOGIE**

Department of Microbiology, Faculty of Life Sciences, University of Benin, Private Mail Bag 1154, Benin City 300001, Nigeria

**ABSTRACT:** In this study we assessed the functionality of integrons, melanin-like pigment and biofilm formation on multidrug resistance among environmental isolates of *Stenotrophomonas maltophilia*. Marked resistances were noted against aztreonam (60%), cefepime (68%), ceftazidime (77%), ciprofloxacin (72%), gentamicin (65%), meropenem (75%), piperacillin/tazobactam (65%) in *S. maltophilia*. Ticarcillin/clavulanic acid (66%) and trimethoprim/sulfamethoxazole (75%) were the active antibiotics against *S. maltophilia*. Class 1 integron was significantly detected in 56.3% (54/96) of *S. maltophilia* strains. Integron-positive strains were significantly resistant to cefepime (69%), ceftazidime (78%), ciprofloxacin (74%), gentamicin (65%), and meropenem (72%). Gene cassettes arrays within integrons were identified as aminoglycoside resistance genes *aacA4, aadA2, aadB, aacC4,* and *aacA6-Ib*; β-lactams resistance genes *blaTEM, blaOXA,* and *blaCARB,* chloramphenicol resistance genes *cmlA* and *catB2,* quaternary ammonium compound (QAC) resistance genes *smr* and *qac,* and multi-gene cassettes: *smr/aacA4* and *blaTEM/aac6-II/aadA5.* High-pigment-producing *S. maltophilia* strains revealed significant correlation with resistance to cefepime, ceftazidime, ciprofloxacin, levofloxacin and piperacillin/tazobactam. Biofilm formation was not significant with resistance to cefepime, levofloxacin, meropenem, ticarcillin/clavulanic and trimethoprim/sulfamethoxazole. Our findings characterize the significant roles of integrons, melanin-like pigment and biofilm formation in the multidrug resistance of *S. maltophilia*. The range of antibiotics resistance genes and mobile genetic elements found suggests that the organism could potentially act as a reservoir of drug resistance determinants in environmental and clinical settings, which is an issue of public health concern. © JASEM

http://dx.doi.org/10.4314/jasem.v18i2.16

Introduction: *Stenotrophomonas maltophilia* (formerly known as *Pseudomonas maltophilia*, *Xanthomonas maltophilia*) is a ubiquitous species from the gamma sub-division of Proteobacteria (Hayward et al., 2010). *S. maltophilia* is a Gram-negative opportunistic pathogen, and increasing incidence of nosocomial and community-acquired *S. maltophilia* infections is of particular concern for immunocompromised individuals, as this bacterial pathogen is associated with a significant fatality rate (Jumaa et al., 2006; Brooke, 2012). *S. maltophilia* is an environmental bacterium found in aqueous habitats, plant rhizospheres, animals, foods, contaminated medical care fluids, and water sources (Ryan et al., 2009). Infections of *S. maltophilia* can occur in a range of organs and tissues; the organism is commonly found in respiratory tract infections (Brooke, 2012).

*S. maltophilia* has emerged as one of the most frequently found bacteria in cystic fibrosis (CF) patients (Waters et al., 2007). *S. maltophilia* has been associated with infections of the eyes (Penland and Wilhelmus, 1996), urinary and respiratory tracts infections (Vartivarian et al., 1996). *S. maltophilia* possess endogenous β-lactamase production and low outer membrane permeability resulting to its resistant to many broad-spectrum of antibiotics including penicillins, carbapenems, and aminoglycosides (Gilligan and Whittier, 1999). The molecular mechanisms underlying pathogenicity of *S. maltophilia* are mainly unknown. Although its high capacity to adhere to various surfaces with biofilm formation or its intrinsic resistance to majority of commonly used antibiotics are factors that positively contribute to the infection process (Looney et al., 2009).

*S. maltophilia* exhibits variety of mechanisms that singly or collectively contribute to its multidrug resistance (MDR) status. Intrinsic resistance includes inducible efflux pumps (Li and Nikaido, 2004; Liaw et al., 2010) and multiple β-lactamase expression (Avison et al., 2002) but not mutations in the quinolone resistance-determining region (Valdezate et al., 2005). In addition, *S. maltophilia* can acquire resistance through integrons, transposons, and plasmids (Barbolla et al., 2004; Liaw et al., 2010). Class 1 integrons have been characterized from *S. maltophilia* strains isolated in Argentina and Taiwan, which indicates that they contribute to trimethoprim/sulfamethoxazole (TMP/SMX) resistance through the *sul1* gene carried as part of the 3’ end of the class 1 integron (Barbolla et al., 2004). Integrons are located on transposons or plasmids that facilitate the rapid spread of integrons to other strains and bacterial species (Jones et al., 1997). In this

*Corresponding author: E-mail: eigbinosa@gmail.com*
study we examine the functionality of integrons, melanin-like pigment and biofilm formation on multidrug resistance (MDR) among environmental isolates of *S. maltophilia*.

**MATERIALS AND METHODS**

*Collection of samples:* Samples were collected from different environmental settings in Benin City, Nigeria which includes:- abattoir (runoff water and effluent); hospital environment (wastewater outlets, pollutant dumpsite and reservoir water) and animal farms (feeds, water and effluent discharge).

*Isolation of *S. maltophilia* isolates:* *Stenotrophomonas maltophilia* strains were isolated from environmental samples; samples were process and incubated overnight using Nutrient broth (Merck, South Africa). Aliquots of the broth cultures were spread on Mueller-Hinton agar (Merck, South Africa) and imipenem disks (Mast Diagnostics, Merseyside, United Kingdom) were applied on the bacterial lawn. Tiny colonies observed in the inhibition zone after 18 h of growth at 30°C were carefully picked and purified (Bollet et al., 1995). All pure isolates were identified by phenotypic characteristics and standard biochemical reaction using API 20 NE test kit (bioMerieux, France). Isolate inoculation into API 20 NE strips was performed as recommended by the manufacturer (bioMerieux, France). After incubation for 48 h at 30°C, results were read and analysed using the Analytical Profile Index (API) database (V4.1) with the apilweb™ identification software. Only confidence levels expressed as 'excellent identification' or 'very good identification' was considered in this study. All candidate isolates were confirmed by polymerase chain reaction with *S. maltophilia*-specific primers SM1f/SM4 as describe by Adamek et al. (2011).

*Antimicrobial susceptibility testing:* Antimicrobial susceptibilities were determined by the agar dilution method as described by the Clinical and Laboratory Standards Institute (CLSI, 2005, 2008). The following antibiotics were used included: aztreonam (ATM); cefepime (FEP); ceftazidime (CAZ); ciprofloxacin (CIP); gentamicin (GEN); levofloxacin (LEV); meropenem (MER); piperacillin/tazobactam (TZP); ticarcillin/clavulanic acid (TIM) and trimethoprim/sulfamethoxazole (SXT). Control strains for susceptibility testing included *Pseudomonas aeruginosa* ATCC 19582, *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739.

*Genomic DNA preparation:* *S. maltophilia* isolates from an overnight culture were resuspended in 200μl of sterile deionized water, boiled for 15 min and centrifuged for 5 min at 12,000 × g. The supernatant was stored at -20°C for further use as genomic DNA template for PCR.

*Detection of Integron by polymerase chain reaction (PCR):* Class 1 integrons were detected with class 1 integrase specific primers (5'-ACATGTG ATGGCGA CGCACGA-3' and 5'-ATTTCGTCC TGGC TGGCGA-3'). Gene cassettes within integrons were amplified with primers specific for the integron 5'CS (5'-GGCATC CAAGCA GCAAG-3') and 3'CS (5'-AACGAGGCTTACG-3') (Pluy et al., 2000). The amplicons were sequenced and sequence comparisons were made using the BLAST program.

*Pigment bioassay:* A melanin-like pigment bioassay was carried out as described by Wang et al. (2000). *S. maltophilia* were cultured in L-tyrosine-containing agar to facilitate the observation of brown pigment production. Pigment formation were recorded as 0, 1+, 2+ or 3+, with increased intensity and intensity equal to or greater than 2+ designated as high pigment expression.

*Biofilm bioassay:* The biofilm bioassay was carried out as described by Di Bonaventura et al. (2004). *S. maltophilia* were cultured overnight in Luria-Bertani medium, cultures were aseptically transferred to the wells of polystyrene microtitre plates. The plates were incubated at 37°C for 10 h and subsequently stained with crystal violet.

**RESULTS**

The results of susceptibility testing (Table 1) showed that most of the *S. maltophilia* strains were resistant to aztreonam (60%), cefepime (68%), ceftazidime (77%), ciprofloxacin (72%), gentamicin (65%), levofloxacin (58%), meropenem (75%), piperacillin/tazobactam (65%). Ticarcillin/ clavulanic acid (66%) and trimethoprim/sulfamethoxazole (75%) were the active antibiotics against *S. maltophilia* (Table 1).

Fifty-five environmental isolates of multiple antibiotic resistance (MAR) [multiple antibiotic resistance, defined as resistant to four or more of six categories of antibiotics tested, including cephalosporins, β-lactams, β-lactamase inhibitors, carbapenems, monobactams, aminoglycosides, quinolones and trimethoprim/sulfamethoxazole (SXT)] and 41 non-MAR (i.e. resistant to three or less of the six categories of antibiotics tested). Among the antibiotics tested, MAR and non-MAR isolates reveal slight differences in resistance to aztreonam (91% vs. 76%), ciprofloxacin (100% vs. 75%), gentamicin (93% vs. 73%), meropenem (89% vs. 73%), and piperacillin/tazobactam (84 vs. 80%) respectively (Table 2).
Fifty-four strains 56.3% (54/96) were significantly detected with class 1 integrase (Table 1). Integron-positive strains were significantly resistant to cefepime (69%), ceftazidime (78%), ciprofloxacin (74%), gentamicin (65%), and meropenem (72%) (Table 1). Also the MAR strains 71% (39/55) possessed class 1 integron than the non-MAR strains 15/41 (37%) (Table 3).

The identified gene cassettes within class 1 integrons included aminoglycoside resistance genes aacA4, adaA2, adaB, aacC4, and aacA6’-Ib; β-lactams resistance genes blaIMP, blaOXA, and blaCARB; chloramphenicol resistance genes cmlA and catB2; quaternary ammonium compound (QAC) resistance genes smr and qac; and multi-gene cassettes: smr/aacA4 and blaIMP/blaOXA6-II/aadA5.

A good number of MAR isolates produced a higher level of pigment than non-MAR isolates (82% vs. 15%) (Table 3). High-pigment-producing S. maltophilia strains revealed significant correlation with resistance to cefepime, ceftazidime, ciprofloxacin, levofloxacin and piperacillin/tazobactam (Table 4). Aztreonam, gentamicin and meropenem were not significant with pigment producing strains (Table 4). MAR isolates had an average OD₅₄₀ₙₐₜ value of 0.85 ± 0.21 compared with a value of 0.25 ± 0.01 for non-MAR isolates (P < 0.002) (Table 3). Biofilm formation was not significant with resistance to ciprofloxacin, levofloxacin, meropenem, ticarcillin/clavulanic and trimethoprim/sulfamethoxazole (Table 4).

**DISCUSSION**

The rapid spread of antibiotic resistance genes among bacterial isolates is an increasing problem in infectious disease control. In the study, we examined the roles of integrons, melanin and biofilm formation in relation to multidrug resistance in S. maltophilia. A significant correlation was established between the mechanisms studied and multidrug resistance. Studies have revealed that conserved DNA sequence, integron, may be carried on these episomal genetic structures (Stokes and Hall, 1989; Rowe-Magnus and Mazel, 1999). Integrons possess two conserved segments separated by a variable region that includes integrated cassettes, which often include antibiotic resistance genes (Recchia and Hall, 1995). Many resistance genes are present as gene cassettes within integrons, which may themselves be located on transmissible plasmids and transposons (Recchia and Hall, 1995).

Antimicrobial Surveillance Program isolates indicated that the newer fluoroquinolones demonstrated good efficacy; the most active were levofloxacin (6.5% resistance) and gatifloxacin (14.1%) (Sader and Jones, 2005). The resistance to levofloxacin was higher in our study (58% resistance) compare to previous the study of Sader and Jones (2005). A recent study encompassing data from Europe, Latin America and North America indicates that the level of resistance to TMP/SMX is 3.8%; however, previous studies indicate that the level is higher in Latin America than North America (Gales et al., 2001; Fedler et al., 2006). The current drug of choice for treating S. maltophilia infections is trimethoprim-sulfamethoxazole, but resistance is seen in S. maltophilia isolates due to a mobile determinant (Toleman, et al., 2007; Nicodemo and Paez, 2007). Other drugs with reasonable activity against S. maltophilia are minocycline and newer fluoroquinolones (Nicodemo and Paez, 2007). However, mutants resistant to these last resort drugs are readily selected in vitro. One mutation may be sufficient to cause resistance to these drugs, and worryingly, this mutation can be selected for in the presence of a front-line antimicrobial such as amikacin (Gould and Avison, 2006). Although surveillance studies are few, resistance to TMP/SMX appears to be emerging, and recent in vitro modeling studies have shown that combination therapies of TMP/SMX plus ciprofloxacin and TMP/SMX plus tobramycin exhibit a greater killing capacity then TMP/SMX alone (Zelenitsky et al., 2005; Al-Jasser, 2006).

The class 1 integron (56.3%) positive isolates obtained in this study is slightly similar to that of Liaw et al. (60%) (2010) but higher than that reported by Chang et al. (22%) (2004). Such variation could be attributed to the different origins of the samples collected. The finding that the most common gene cassette carried by class 1 integron was comprised of aminoglycoside resistance determinants may account for the significant difference in susceptibility to gentamicin between the integron-positive and integron-negative isolates (strains). Integrons were significantly associated with resistance to certain antibiotics, including aminoglycosides (gentamicin), quinolones (levofloxacin and ciprofloxacin), and beta-lactam agents. This is not surprising, since many antibiotic resistance gene cassettes encoding resistance to a wide range of antibiotics have been reported previously (Sallen et al., 1995; Rowe-Magnus and Mazel, 1999).

Integrons capture genes as part of a genetic element known as a gene cassette. Most cassettes within integrons with known functions confer antibiotic or quaternary ammonium compound (QAC) resistance. Most of the integron arrays contained more than one resistance gene cassette, which can mediate resistance to multiple antibiotics. Increasing proportions of isolates containing class 1 integrons were detected in S. maltophilia (Barbolla et al., 2004; Toleman et al., 2007). Among the 17 SXT-resistant isolates, 15 (88%) carried class 1 and 2 integrons of them possessed...
sul1 genes. This report confirms the increased incidence of class 1 integron in SXT-resistant clinical isolates of *S. maltophilia* (Barbolla et al., 2004; Toleman et al., 2007). Toleman *et al.* (2006) indicates that resistance genes are linked to insertion sequence common region (ISCR) elements, which are DNA sequences found beyond and close to the 3′ conserved sequences of class 1 integrons. These ISCR elements have been identified in numerous Gram-negative bacteria and a few Gram-positive bacteria, and are responsible for the mobility and dissemination of many antibiotic resistance genes, including extended spectrum β-lactamase, carbapenemase genes, aminoglycoside, chloramphenicol, quinolone as well as trimethoprim resistance genes (Toleman *et al.*, 2006). The use of quaternary ammonium compounds (QAC) in the natural environment has the potential to select for antibiotics resistance (Gaze *et al.*, 2005). The frequent occurrence of the *qac* gene cassette within class 1 integron in the present study implies that multidrug resistance may result from the introduction of biocides into hospital settings.

Melanin-like pigment has been shown to protect cells from environmental attack in bacteria and fungi (Coyne and al-Harthi, 1992; Gomez and Nosanchuk, 2003). In this study, it was revealed that pigment production in *S. maltophilia* was associated with antibiotic resistance. Our findings show that cell with and without pigment formation indicates that pigment-bearing cells were more resistant to antibiotics. The MAR phenotype and biofilm formation of *S. maltophilia* observed gives a clearer insight why the bacterium is persistent, and difficult to eradicate.

In conclusion this study revealed that all resistance determinants contributed to the multidrug resistant phenotype of *S. maltophilia*. Along with the presence of pigment production, biofilm formation and class 1 integron may possibly play important roles leading to multidrug resistance of *S. maltophilia*. The varieties of antibiotics resistance genes and mobile genetic elements found suggest that the organism could potentially act as a reservoir of drug resistance determinants in an environmental and clinical settings, which is an issue of public health concern.

### Table 1: The relationship between antibiotic susceptibility profile and integrons in *S. maltophilia*

| Antibiotic | Antibiotic profile (n = 96) | Integron-positive isolates (n = 54) | Integron-negative isolates (n = 42) | P-value |
|------------|-----------------------------|------------------------------------|------------------------------------|---------|
|            | R n (%)                     | S n (%)                            | R n (%)                            | S n (%) |
| ATM        | 58 (60)                     | 38 (40)                            | 32 (59)                            | 22 (41) |
|            | 26 (62)                     | 16 (38)                            |                                    |         |
| FEP        | 65 (68)                     | 31 (32)                            | 37 (69)                            | 17 (31) |
|            | 28 (67)                     | 14 (33)                            |                                    |         |
| CAZ        | 74 (77)                     | 22 (23)                            | 42 (76)                            | 12 (22) |
|            | 32 (76)                     | 10 (24)                            |                                    |         |
| CIP        | 69 (72)                     | 27 (28)                            | 40 (74)                            | 14 (26) |
|            | 29 (69)                     | 13 (31)                            |                                    |         |
| GEN        | 62 (65)                     | 34 (35)                            | 35 (65)                            | 19 (35) |
|            | 27 (64)                     | 15 (36)                            | < 0.001                            |         |
| LEV        | 56 (58)                     | 40 (42)                            | 31 (57)                            | 23 (43) |
|            | 25 (60)                     | 17 (40)                            | < 0.001                            | < 0.001 |
| MER        | 72 (75)                     | 24 (25)                            | 39 (72)                            | 15 (28) |
|            | 33 (79)                     | 9 (21)                             | < 0.001                            | < 0.001 |
| TZP        | 62 (65)                     | 34 (35)                            | 30 (56)                            | 24 (44) |
|            | 32 (76)                     | 10 (24)                            | < 0.001                            | < 0.001 |
| TIM        | 33 (34)                     | 63 (66)                            | 14 (26)                            | 40 (74) |
|            | 19 (45)                     | 25 (55)                            | 0.021                              |         |
| SXT        | 24 (25)                     | 72 (75)                            | 12 (22)                            | 42 (78) |
|            | 12 (29)                     | 30 (71)                            | < 0.001                            | < 0.001 |

Value in parenthesis represents percentage profile

**Legend:** ATM-aztreonam; FEP-cefaclor; CAZ-cefazidime; CIP-ciprofloxacin; GEN-gentamicin; LEV-levofoxacin; MER-meropenem; TZP-piperacillin/tazobactam; TIM-ticarcillin/clavulanic acid and SXT-trimethoprim/sulphamethoxazole; R-resistant; S-susceptible

### Table 2: Susceptibility profile of multiple antibiotic resistant (MAR) and non-MAR *S. maltophilia* strains

| Antibiotic | Range | MIC₇₀ (µg/ml) | MAR (n =55) | Non-MAR (n =41) | P-value |
|------------|-------|--------------|-------------|----------------|---------|
|            |       | R n (%)      | S n (%)     | R n (%)        | S n (%) |
| ATM        | 0.5 - 0.256 | > 256 | 50 (91) | 5 (9) | 256 | 31 (76) | 10 (24) | < 0.001 |
| FEP        | 0.5 - 0.256 | 256 | 52 (95) | 3 (5) | 64 | 8 (20) | 33 (80) | < 0.001 |
| CAZ        | 0.5 - 0.256 | > 256 | 54 (98) | 1 (2) | >256 | 12 (29) | 29 (71) | 0.002 |
| CIP        | 4 - 128 | 128 | 55 (100) | 0 (0) | 8 | 31 (75) | 10 (24) | 0.005 |
| GEN        | 0.5 - 0.256 | > 256 | 51 (93) | 4 (7) | 64 | 30 (73) | 11 (27) | 0.001 |
| LEV        | 4 - 128 | 64 | 45 (82) | 10 (18) | 8 | 5 (12) | 36 (88) | < 0.001 |
| MER        | 0.5 - 0.256 | > 256 | 49 (89) | 6 (11) | 256 | 30 (73) | 11 (27) | < 0.001 |
| TZP        | 0.5 - 0.256 | > 256 | 46 (84) | 9 (16) | >256 | 33 (80) | 8 (20) | < 0.001 |
| TIM        | 4 - 128 | >128 | 21 (38) | 34 (61) | 16 | 1 (2) | 40 (98) | < 0.001 |
| SXT        | 0.0625/1.1875 | 16/304 | 20 (36) | 35 (64) | 2/38 | 1 (2) | 40 (98) | 0.001 |

Value in parenthesis represents percentage profile

**ETINOSA O. IGBINOSA, FAITH E. OVIASOGIE**
Table 3: Correlation profile of multiple antibiotic resistant with class 1 integron, formation of pigment and biofilm in environmental strains of \textit{S. maltophilia}

| Variables          | Class 1 integron | Pigment | Biofilm |
|--------------------|------------------|---------|---------|
|                    | +ve (n = 54)     | -ve (n = 42) | H (n = 51) | L (n = 45) | Mean (S.D) OD$_{540nm}$ |
| MAR (n = 55)       | 39 (71)          | 16 (29) | 45 (82) | 10 (18) | 0.85 ± 0.21 |
| Non-MAR (n = 41)   | 15 (37)          | 26 (63) | 6 (15)  | 35 (85) | 0.25 ± 0.01 |
| P - value          | 0.001            | 0.001   | <0.001  | <0.001  | <0.002 |

Values in parentheses indicate MAR and non-MAR percentage profile

Legend: +ve, presence of integrons; -ve, absence of integrons; H, high expression; L, low and no expression; S.D. standard deviation; OD$_{540nm}$, optical density at 540nm

Table 4: Effect of pigment production and biofilm formation on antibiotic susceptibility profile of environmental strains of \textit{S. maltophilia}

| Antibiotic | Pigment high (or +ve (n = 51)) | Pigment low (or -ve (n = 45)) | P -value | Biofilm high (n = 42) | Biofilm low (n = 54) | P -value |
|------------|---------------------------------|-------------------------------|----------|-----------------------|----------------------|----------|
| R          | S                               | n (%)                        | n (%)    | R                     | S                    | n (%)    |
| ATM        | 44 (86)                         | 7 (14)                       | 35 (78)  | 10 (22)               | ns                   | 40 (95)  |
| FEP        | 45 (88)                         | 6 (12)                       | 25 (56)  | 20 (44)               | 0.002                | 38 (90)  |
| CAZ        | 47 (92)                         | 4 (8)                        | 14 (31)  | 31 (69)               | 0.001                | 35 (83)  |
| CIP        | 42 (82)                         | 9 (18)                       | 21 (47)  | 24 (53)               | 0.005                | 22 (52)  |
| GEN        | 40 (78)                         | 11 (22)                      | 37 (82)  | 8 (18)                | ns                   | 39 (93)  |
| LEV        | 31 (61)                         | 20 (39)                      | 15 (33)  | 30 (67)               | 0.001                | 28 (67)  |
| MER        | 44 (86)                         | 7 (14)                       | 37 (82)  | 8 (18)                | ns                   | 36 (86)  |
| TZP        | 41 (80)                         | 10 (20)                      | 11 (24)  | 34 (76)               | 0.002                | 33 (79)  |
| TIM        | 12 (24)                         | 39 (76)                      | 5 (11)   | 40 (89)               | 0.001                | 19 (45)  |
| SXT        | 9 (18)                          | 42 (82)                      | 8 (18)   | 37 (82)               | ns                   | 10 (24)  |

Value in parenthesis represents percentage profile

- High, optical density at 540 nm (OD$_{540nm}$) >0.4; low, OD$_{540nm}$ ≤0.1

Legend: ATM-aztreonam; FEP-cefepime; CAZ-ceftazidime; CIP-ciprofloxacin; GEN-gentamicin; LEV-levofoxacin; MER-meropenem; TZP-piperacillin/tazobactam; TIM-ticarcillin/clavulanic acid and SXT-trimethoprim/sulfamethoxazole; R-resistant; S-susceptible; ns- not significant

REFERENCES

Adamek M, Overhage J, Bathe S, Winter J, Fischer R, Schwartz T (2011). Genotyping of environmental and clinical Stenotrophomonas maltophilia isolates and their pathogenic potential. \textit{PLoS ONE} 6(11): e27615. doi:10.1371/journal.pone.0027615

Al-Jasser AM (2006). \textit{Stenotrophomonas maltophilia} resistant to trimethoprim sulfamethoxazole: an increasing problem. \textit{Ann Clin Microbiol Antimicrob} 5:23-26.

Avison MB, Higgins CS, Ford PJ, von Heldrech CJ, Walsh TR, Bennett PM (2002). Differential regulation of L1 and L2 β-lactamase expression in \textit{Stenotrophomonas maltophilia}. \textit{J Antimicrob Chemother} 49:387-389.

Barbolla R, Catalano M, Orman BE, Famiglietti A, Vay C, Smayevsky J, et al. (2004). Class 1 integrons increase trimethoprim/sulfamethoxazole MICs against epidemiologically unrelated \textit{Stenotrophomonas maltophilia} isolates. \textit{Antimicrob Agents Chemother} 48:666-669.

Bollet C, Davin-Regli A, De Micco P (1995). A simple method for selective isolation of \textit{Stenotrophomonas maltophilia} from environmental samples. \textit{Appl Environ Microbiol} 61:1653-1654.

Brooke JS (2012). \textit{Stenotrophomonas maltophilia}: an emerging global opportunistic pathogen. \textit{Clin Microbiol Rev} 2:2-41.

Chang LL, Chen HF, Chang CY, Lee TM, Wu WJ (2004). Contribution of integrons, and SmeABC and SmeDEF efflux pumps to multidrug resistance in clinical isolates of \textit{Stenotrophomonas maltophilia}. \textit{Antimicrob Agents Chemother} 48:3759-3767.

ETINOSA O. IGBINOSA, FAITH E. OVIASOGIE
**Stenotrophomonas maltophilia. J Antimicrob Chemother 53:518-521.**

Gomez BL, Nosanchuk JD (2003). Melanin and fungi. *Curr Opin Infect Dis* 16:91-96

Gould VC, Avison MB (2006). SmeDEF-mediated antimicrobial drug resistance in *Stenotrophomonas maltophilia* clinical isolates having defined phylogenetic relationships. *J Antimicrob Chemother* 57:1070-1076

Hayward AC, Fegan N, Fegan M and Stirling GR (2010). *Stenotrophomonas* and *Lyso bacter*: ubiquitous plant-associated gamma-proteobacteria of developing significance in applied microbiology. *J Appl Microbiol* 108:756-770.

Coyne VE, al-Harthi L (1992). Induction of melanin biosynthesis in *Vibrio cholerae*. *Appl Environ Microbiol* 58:2861-2855.

Di Bonaventura G, Spedicato I, D’Antonio D, Robuffo I, Piccolomini R (2004). Biofilm formation by *Stenotrophomonas maltophilia*: modulation by quinolones, trimethoprim-sulfamethoxazole, and ceftazidime. *Antimicrob Agents Chemother* 48:151-160.

Fedler KA, Biedenbach DJ, Jones RN (2006). Assessment of pathogen frequency and resistance patterns among paediatric patient isolates: report from the 2004 SENTRY Antimicrobial Surveillance Program on three continents. *Diagn Microbiol Infect Dis* 56:427-36.

Gales AC, Jones RN, Forward KR, Linares J, Sader HS, Verhoef J (2001). Emerging importance of *Acinetobacter* species and *Stenotrophomonas maltophilia* as pathogens in severely ill patients: geographic patterns, epidemiological features, and trends in the SENTRY Antimicrobial Surveillance program (1997-1999). *Clin Infect Dis* 32(Suppl):S104-113.

Gaze WH, Abdouslam N, Hawkey PM, Wellington EMH (2005). Incidence of class I integrons in a quaternary ammonium compound-polluted environment. *Antimicrob Agents Chemother* 49:1802-1807

Gilligan PH, Whittier S (1999). *Burkholderia, Stenotrophomonas, Ralstonia, Brevundimonas, Comamonas, and Acidovorax*, p. 526-538. In P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.). Manual of clinical microbiology, 7th ed. American Society for Microbiology Press, Washington, D.C.

Li X-Z, Nikaido H (2004). Efflux-mediated drug resistance in bacteria. *Drugs* 64:159-204.

Liaw S-J, Lee Y-L, Hsueh P-R (2010). Multidrug resistance in clinical isolates of *Stenotrophomonas maltophilia*: roles of integrons, efflux pumps, phosphoglucomutase (SpgM), and melanin and biofilm formation. *Int’l J Antimicrob Agents* 35: 126-130.

Looney WJ, Narita M, Muhlemann K (2009). *Stenotrophomonas maltophilia*: an emerging opportunistic human pathogen. *Lancet Infect Dis* 9:312-323.

Nicodemo AC, Paez JI (2007). Antimicrobial therapy for *Stenotrophomonas maltophilia* infections. *Eur J Clin Microbiol Infect Dis* 26:229-237

Penland RL, Wilhelmus KR (1996). *Stenotrophomonas maltophilia* ocular infections. *Arch Ophthalmol* 114:433-436.

Ploy MC, Denis F, Courvalin P, Lambert T (2000). Molecular characterization of integrons in *Acinetobacter baumannii*: description of a hybrid class 2 integron. *Antimicrob Agents Chemother* 44:2684-2688.
Recchia GD, Hall RM (1995). Gene cassettes: a new class of mobile element. *Microbiol* 141:3015-3027.

Rowe-Magnus DA, Mazel D (1999). Resistance gene capture. *Curr Opin Microbiol* 2:483-488

Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, et al. (2009) The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. *Nat Rev* 7: 514-525

Sader HS, Jones RN (2005). Antimicrobial susceptibility of uncommonly isolated non-enteric gram-negative bacilli. *Int’l J Antimicrob Agents* 25:95-109.

Sallen B, Rajoharison A, Desvarenne S, Mabilat C (1995). Molecular epidemiology of integron-associated antibiotic resistance genes in clinical isolates of Enterobacteriaceae. *Microb Drug Resist* 1:195-202

Stokes HW, Hall RM (1989). A novel family of potentially mobile DNA elements encoding site-specific gene-integration functions: integrons. *Mol Microbiol* 3:1669-683

Toleman MA, Bennett PM, Walsh TR (2006). ISCR elements: novel gene-capturing systems of the 21st century? *Microbiol Mol Biol Rev* 70: 296-316

Toleman MA, Bennett PM, Bennett DM, Jones RN, Walsh TR (2007). Global emergence of trimethoprim/sulfamethoxazole resistance in *Stenotrophomonas maltophilia* mediated by acquisition of *sul* genes. *Emerg Infect Dis* 13:559-565.

Valdezate S, Vindel A, Saéz-Nieto JA, Baquero F, Canton R (2005). Preservation of topoisomerase genetic sequences during *in vivo* and *in vitro* development of high-level resistance to ciprofloxacin in isogenic *Stenotrophomonas maltophilia* strains. *J Antimicrob Chemother* 56:220-223.

Vartivarian, S. E., K. A. Papadakis, and E. J. Anaissie.1996. *Stenotrophomonas maltophilia* urinary tract infection. A disease that is usually severe and complicated. Arch. Intern. Med.156:433-435.

Wang G, Aazaz A, Peng Z, Shen P (2000). Cloning and over expression of a tyrosinase gene mel from *Pseudomonas maltophilia*. *FEMS Microbiol Lett* 185:23-27.

Waters VJ, Gómez MI, Soong G, Amin S, Ernst RK, Prince A (2007). Immunostimulatory properties of the emerging pathogen *Stenotrophomonas maltophilia*. *Infect Immun* 75:1698-1703.

Zelenitsky SA, Iacovides H, Ariano RE, Harding GK (2005). Antibiotic combinations significantly more active than monotherapy in an *in vitro* infection model of *Stenotrophomonas maltophilia*. *Diagn Microbial Infect Dis* 51:39-43

---

ETINOSA O. IGBINOSA, FAITH E. OVIASOGIE