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CORRELATING MUTATED ampC GENE EXPRESSION WITH AMPC BETA-LACTAMASE HYDROLYSIS ACTIVITY IN STENOTROPHOMONAS MALTOPHILIA

SHIT CHONG SENG

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By

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As a member of β-lactamase producing bacteria, *Stenotrophomonas maltophilia* gains attention because of its multiple drug resistance characteristic. L1 and L2 β-lactamases are the dominant β-lactamases produced by *S. maltophilia*. However, the production of other obscure β-lactamases by *S. maltophilia* has been reported as well. As a result, domination of β-lactam resistance by L1 and L2 β-lactamases is no longer valid. Thus, it is possible for *S. maltophilia* to produce AmpC β-lactamase. AmpC β-lactamase synthesized by *ampC* gene of *S. maltophilia* is predicted to have hydrolytic activity as published in the National Center for Biotechnology Information (NCBI) database. In addition, the presence of SXSK and YXN elements in the predicted amino acid sequence represented features of AmpC β-lactamase. This enzyme could confer resistance to cefepime (4^{th} generation cephalosporins) and other cephalosporins groups. Since little is known about the function of *ampC* gene, characterization of *ampC* gene profile in *S. maltophilia* is crucial.

A total of 78 isolates of *S. maltophilia* were collected from Universiti Kebangsaan Malaysia Medical Centre (UKMMC), Kuala Lumpur in year 2009. Most of the isolates were collected from ICU patients (62.1%) and the most frequent isolation site was from blood sample (39.4%). Great genetic diversity was determined among the clinical isolates by pulsed-field gel electrophoresis (PFGE). Only four antibiotics exhibited effectiveness against *S. maltophilia* infection which is co-trimoxazole (100%

susceptibility), polymyxin-B (100% susceptibility), ciprofloxacin (98% susceptibility), and cefepime (98% susceptibility).

A cefepime- and ceftazidime-resistant *S. maltophilia* strain was isolated with MIC values of ceftazidime (64 mg/L) and cefepime (48 mg/L). Thus, screening of *ampC* gene by Polymerase Chain Reaction (PCR) was performed since most of the cefepime resistance issue found in other bacteria such as *Escherichia coli*, *Acinetobacter baumannii*, and Enterobacteriacaea are related to overexpressed and/or mutated *ampC* gene. The presence of *ampC* gene was determined in most of the clinical isolates (68 out of 78 isolates, 87%) including *S. maltophilia* ATCC 13637. Nucleotides alignment showed ten nucleotides differences between cefepime-resistant isolate (CX isolate) and cefepime-sensitive *S. maltophilia* ATCC 13637. These mutations have resulted in four amino acids sequence changes. Noticeably, two identical mutation points were found in the predicted *ampC* amino acid of *S. maltophilia* k279a (published in NCBI database) and clinical *S. maltophilia* CX isolate compared to *S. maltophilia* ATCC 13637. Furthermore, SXSK and YXN elements which represented features of AmpC β-lactamase also were found in the amino acid sequences.

Characterization of *ampC* gene was carried out in order to figure out the function of the gene and its expressed protein. Gene characterization by cloning could prevent the overlapping function of L1 and L2 β-lactamase genes with the *ampC* gene. The expressed protein from *ampC* gene of CX isolate showed greater hydrolytic activity towards tested substrates compared to the expressed protein from *ampC* gene of *S. maltophilia* ATCC 13637 isolate. The first β-lactam ring antibiotic, penicillin; the second to the fourth generation of cephalosporins, cefoxitin, ceftazidime, and cefepime were chosen as substrates for kinetic assay.

Lastly, the expression level of *ampC* gene of clinical CX isolate and cefepime-sensitive *S. maltophilia* ATCC 13637 was compared. Relative quantitative real-time reverse transcriptase polymerase chain reaction (qPCR) assay was designed for gene expression study. Relative quantification qPCR showed a significant higher expression level of *ampC* gene in the clinical CX isolate compared to *S. maltophilia* ATCC 13637. In addition, L1 and L2 β-lactamase genes in clinical CX isolate and *S. maltophilia* ATCC 13637 were expressed at the same level.

In conclusion, this study demonstrated that although most of the clinical *S. maltophilia* isolates including *S. maltophilia* ATCC 13637 carry *ampC* gene, only mutated *ampC* gene resulting in changes of amino acids sequence does lead to cefepime resistance. Remarkable findings in this study especially the identical mutation points found in both clinical CX isolate and the *S. maltophilia* k279a could be an interesting exploration in future.

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MENGHUBUNGKAITKAN BERMUTASI EKSPRESI GEN *ampC* DENGAN AKTIVITI HIDROLISIS AMPC BETA-LAKTAMASE DALAM *STENOTROPHOMONAS MALTOPHILIA*

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Sebagai ahli kepada bakteria yang menghasilkan β-laktamase, *Stenotrophomonas maltophilia* dapat menarik perhatian dengan ciri resistansinya keatas pelbagai jenis antibiotik. L1 dan L2 β-laktamase adalah dominan β-laktamase yang dihasilkan oleh *S. maltophilia*. Tetapi, kemungkinan bakteria ini untuk menghasilkan β-laktamase samar-samar telah pun dilaporkan. Dengan demikian, adalah tidak sah lagi untuk L1 dan L2 β-laktamase dikenali sebagai dominan β-laktamase. Justeru, kemungkinan besar bagi *S. maltophilia* menghasilkan AmpC β-laktamase. Gen *ampC* yang menghasilkan AmpC β-laktamase diramal mempunyai aktiviti hidrolitik seperti yang diterbitkan pada Pusat Kebangsaan bagi Maklumat Bioteknologi (NCBI). Tambahan pula, kewujudan unsur-unsur SXSK dan YXN pada ramalan jujukan amino asid merupai ciri-ciri AmpC β-laktmase. Enzim ini dapat mengakibatkan resistensi keatas cefepime (cephalosporins generasi ke-empat) dan juga kumpulan cephalosporins yang lain. Hanya sedikit yang diketahui tentang fungsi gen *ampC*. Oleh itu, penerokaan profil gen *ampC* dalam *S. maltophilia* adalah penting.

Sebanyak 78 pencilan *S. maltophilia* dikumpulkan dari Pusat Perubatan Universiti Kebangsaan Malaysia (PPUKM), Kuala Lumpur pada tahun 2009. Kebanyakan pencilan adalah dikumpulkan daripada pesakit ICU (62.1%). Sampel darah (39.4%) merupai bahagian yang paling kerap untuk mendapatkan bakteria ini. Ketidakseragaman genetik telah dipastikan antara pencilan klinikal dengan

menggunakan pulsed-field gel elektroforesis (PFGE). Hanya empat antibiotik yang berkesan terhadap jangkitan *S. maltophilia* iaitu co-trimoxazole (100% kerentanan), polymyxin-B (100% kerentanan), ciprofloxacin (98% kerentanan), dan cefepime (98% kerentanan).

Satu cefepime- dan ceftazidime-resistan *S. maltophilia* telah dijumpai dengan nilai MIC keatas ceftazidime (64 mg/L) dan cefepime (48 mg/L). Oleh itu, pemeriksaan kewujudan gen *ampC* dilakukan kerana kebanyakan kes resistan keatas cefepime dijumpai pada bakteria lain contohnya *Escherichia coli*, *Acinetobacter baumannii*, dan Enterobacteriacaea adalah berkaitan dengan gen *ampC* yang berekspresi berlebihan dan/atau bermutasi. Kewujudan gen *ampC* telah didapati dalam kebanyakan pencilan klinikal (68 daripada 78 pencilan, 87%) termasuk *S. maltophilia* ATCC 13637. Terdapat perbezaan sebanyak sepuluh nukleotida ditemui antara cefepime-resistan isolat (CX isolat) dengan cefepime-sensitif *S. maltophilia* ATCC 13637 selepas penyelarasan nukleotida dilakukan. Mutasi ini mengakibatkan perubahan keatas empat jujukan asid amino. Yang menarik perhatian, terdapat dua titik mutasi yang serupa ditemui pada *ampC* asid amino yang diramalkan antara *S. maltophilia* k279a (telah diterbitkan dalam NCBI) dan klinikal *S. maltophilia* CX isolat jika berbanding dengan *S. maltophilia* ATCC 13637. Di samping itu, unsur-unsur SXSK dan YXN yang ditemui dalam jujukan asid amino juga merupai ciri-ciri AmpC β-laktmase.

Selepas itu, pencirian gen *ampC* telah pun dijalankan untuk memahami fungsi gen dan protein yang diekspresikan. Pencirian gen *ampC* dengan menggunakan teknik klon dapat mengelakkan kekeliruan antara fungsi gen L1 dan L2 β-laktamase dengan gen *ampC*. Protein yang diekspresi oleh gen *ampC* dari CX isolat menunjukkan aktiviti hidrolitik yang lebih besar terhadap substrat yang diuji berbanding dengan protein yang diekspresi oleh gen *ampC* dari *S. maltophilia* ATCC 13637 isolat. Substrat yang dipilih untuk pengujian kinetik termasuk antibiotik pertama yang mempunyai β-laktam struktur, iaitu penisilin; cephalosporins dari generasi kedua sampai ke generasi keempat, iaitu cefoxitin, ceftazidime dan cefepime.

Akhir sekali, perbandingan antara tahap ekspresi gen *ampC* dari klinikal CX isolat dan cefepime-sensitif *S. maltophilia* ATCC 13637 telah pun dijalankan. Relatif kuantitatif real-time reaksi berantai polimerase reverse transcriptase (qPCR) essei telah direka untuk pengajian ekspresi gen. Kuantifikasi relatif qPCR menunjukkan tahap ekspresi yang tinggi dari gen *ampC* klinikal CX isolate jika dibandingkan dengan *S. maltophilia* ATCC 13637. Di samping itu, gen L1 dan L2 β-laktamase di klinikal CX isolat dan *S. maltophilia* ATCC 13637 diekspresikan pada tahap yang sama.

Kesimpulannya, kajian ini menunjukkan bahawa walaupun kebanyakan klinikal *S. maltophilia* termasuk *S. maltophilia* ATCC 13637 mempunyai gen *ampC*. Tetapi, hanya gen *ampC* yang bermutasi yang mengakibatkan perubahan keatas jujukan asid

amino akan menyebabkan cefepime resistan. Penemuan yang menakjubkan dalam kajian ini terutamanya titik mutasi yang serupa terdapat dalam kedua-dua isolat iaitu klinikal CX isolat dan *S. maltophilia* k279a boleh menjadi penerokaan menarik pada masa akan datang.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the Degree of Doctor of Philosophy. The members of the Supervisor Committee were as follows:

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4CN	4-chloro-1-naphthol
APS	Ammonium persulfate
AST	Antibiotics susceptibility test
ATCC	American Type Culture Collection
A_{260} / A_{280}	Absorbance reading at OD_{260} / Absorbance reading at OD_{280}
bp	Base pair
BSI	Blood stream infection
Ca^{2+}	Calcium ion
cDNA	Complement Deoxyribonucleic acid
cfu	Colony-forming unit
CLSI	Clinical and Laboratory Standards Institute
CSF	Cerebrospinal fluid
C_t values	Threshold line generated by real-time PCR
ddH_2O	Double distilled water
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTPs	Deoxynucleotide triphosphates
E	Efficiency
EDTA	Ethylenediaminetetraacetic acid
EPIs	Efflux pump inhibitors
ERIC-PCR	Enterobacterial repetitive intergenic consensus PCR
ESBL	Extended Spectrum beta-lactamase

g	Gravity
G/C content	Guanine-cytosine content
h	Hour
HRP	Horseradish peroxidase
H_2O	Water
H_2O_2	Hydrogen peroxide
IAA	Indole-3-acetic acid
ICU	Intensive Care Unit
IMAC	Immobilized metal-ion affinity chromatography
IPTG	Isopropylthio-β-galactoside
kb	Kilo bases
kbp	Kilo base pair
kDa	Kilo Dalton
K_m	Substrate concentration required for effective catalysis to occur
K_{cat}	Catalytic rate
LB agar	Luria-Bertani agar
LPS	Lipopolysaccharides
M	Molar
MIC	Minimum inhibitory concentration
min	Minute
mg	Milli gram
mM	Milli molar
mg/L	Milli gram / liter
$MgCl_2$	Magnesium chloride
MLST	Multi locus sequence typing

mRNA	Messenger RNA
MW	Medical ward
µg	Micro gram
µL	Micro liter
µm	Micro meter
µmol	Micro mol
µM	Micro molar
NaCl	Sodium chloride
NCBI	National Centre for Biotechnology Information
NFGNB	Non-fermenting Gram-negative bacilli
ng	Nano gram
nm	Nano meter
NPA	Nasopharyngeal airway
NTA	nitrilotriacetic acid
OD	Optical density
PBS-T20	Phosphate Buffered Saline Tween-20
PCR	Polymerase chain reaction
PFGE	Pulsed-field gel electrophoresis
pH	Hydrogen ion concentration
pI	Isoelectric point
PVDF	Polyvinylidene fluoride
RAPD	Random amplified polymorphic DNA
rDNA	Ribosomal deoxyribonucleic acid
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid

rpm	Revolutions per minute
RT-qPCR	Reverse transcription quantitative PCR
R^2	Correlation coefficient
s	Second (time)
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium-dodecyl-sulfate polyacrylamide gel electrophoresis
SOC broth	Super Optimal broth with Catabolite repression broth
spp.	Species
ss-PCR	Species-specific polymerase chain reaction
SW	Surgical ward
[S]	Substrate concentration
T. A	Tracheal aspirate
TBE	Tris-Borate-EDTA
TEMED	Tetramethylethylenediamine
TNF-α	Tumour necrosis factor-alpha
tRNA	Transfer RNA
U	Unit
V	Voltage
V	Velocity
V_{max}	Maximum velocity
VAP	Ventilator acquired pneumonia
VIA medium	Vancomycin, Imipenem, and Amphotericin-B medium
VOCs	Volatile organic compounds

w/v	Weight per volume
X	Times
Zn^{2+}	Zinc ion
α	Alpha
β	Beta

CHAPTER 1

INTRODUCTION

Stenotrophomonas maltophilia is a Gram-negative bacterium that is omnipresent in the environment. They are found in aquatic system and also in the plant roots rhizosphere. Hence, *S. maltophilia* is often associated with plant biotechnology and served as a biological control agent to combat plant fungal infection (Elad *et al*., 1987; Kwok *et al*., 1987; Lambert *et al*., 1987; Berg *et al*., 1996) and in bioremediation (Nawaz *et al*., 1993; Binks *et al*., 1995; Wang *et al*., 1997). While this bacterium plays an attentiveness role in agriculture, cases of *S. maltophilia* infections in hospitals have been increased for the past few decades. At present, the route of transmission of *S. maltophilia* from natural environment to clinical setting is still unclear (Gerner-Smidt *et al*., 1995; Denton and Kerr, 1998). Studies have suggested that both clinical and environmental *S. maltophilia* are originated from the same strain. However, most of clinical isolates are hypermutator strains which have higher mutation frequencies due to the challenging clinical atmosphere. In addition, mutants adapted to such condition will produce more mutants due to selection pressure. This is indicated by the discovery of the impairment of *mutS* gene between the environmental and clinical isolates of *S. maltophilia* (Adamek *et al*., 2011; Turrientes *et al*., 2010).

The impact of *S. maltophilia* infection usually leads to bacteraemia and persist colonization in a localized environment such as Intensive Care Unit (ICU). For instance, this bacterium intermittently causes bacteraemia amongst ICU patients with significant case/fatality ratios (Valdezate *et al*., 2001). Besides, it has been recognized as the third most common non-fermenting Gram-negative bacilli (NFGNB) after *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (Wuest *et al*., 1995; Garrison *et al*., 1996; Muder *et al*., 1996; Sader and Jones, 2005; Tunger *et al*., 2007; LiPuma *et al*., 2007; Rattanaumpawan *et al*., 2013). The mortality rate of *S. maltophilia* reaches up to 26.7% due to its intrinsically resistance characteristic to most of the commonly used antibiotics including β-lactams, aminoglycosides, macrolides, and quinolones (Senol *et al*., 2002, Sader and Jones, 2005). Several risk factors may predispose the patients to *S. maltophilia* infection. For instance, prolonged hospital stays as well as prescription of broad spectrum antibiotics such as carbapenems are the main causes. *S. maltophilia* is known to be intrinsically resistant to carbapenems. Several studies have found that prior treatment with carbapenems is reported to be an important predisposing factor for colonization and infection with *S. maltophilia* (Elting *et al*., 1990; Sanyal and Mokaddas, 1999). Improper usage of broad spectrum antibiotics is also a factor to the emergence of *S. maltophilia* infection (Denton and Kerr, 1998). In addition, spreading through the abiotic surface of invasive devices such as central venous catheter has been responsible for the persistence of *S. maltophilia* infection (Jucker *et al*., 1996).

S. maltophilia is a nosocomial pathogen with limited pathogenic potential. It is rarely capable of causing disease in healthy individuals except those with immunocompromised state. Bacteraemic cases of *S. maltophilia* infection are diagnosed when this bacterium is found in the sterile sites such as in the blood stream. Otherwise, colonization is interpreted when it is isolated from a non-sterile site where co-colonization with other microorganisms is common (Jumaa *et al*., 2006). In most of the case studies, *S. maltophilia* causes bacteraemia, pneumonia, wound and urinary tract infections (Looney *et al*., 2009). Any parts of patient's body could be affected depending on the patient's condition, on-going treatment, and the route of infection (Schaumann *et al*., 2001; Apisarnthanarak *et al*., 2003). Following colonization, it could trigger a cascade of inflammatory responses such as secretion of interleukins by leukocytes and tumour necrosis factor-alpha (TNF-α) by macrophages which could contribute to systemic inflammation (Di Bonaventura *et al*., 2010; Adamek *et al*., 2011).

Genetic fingerprinting provides better discriminatory power among the strains of *S. maltophilia* isolates in terms of tracing the source of isolation and mode of transmission of *S. maltophilia* (Jumaa *et al*., 2006). Pulsed Field Gel Electrophoresis (PFGE) is still the best molecular tool used for genetic analysis of *S. maltophilia* isolates due to its greater discriminatory and reproducibility power for strain differentiation compared to other techniques such as random amplified polymorphic DNA (RAPD), enterobacterial repetitive intergenic consensus PCR (ERIC-PCR), and ribotyping. A study done by Valdezate *et al*. in year 2001, demonstrated that their *S. maltophilia* isolates had highly diversified genetic relatedness. Despite rare incidence of person-to-person transmission, cross infection has been reported (Rogues *et al*., 2001; Lanotte *et al*., 2003). Several preventive measures of *S. maltophilia* infection have been documented such as by disinfecting the medical devices, and maintaining good hygiene standard of water dispenser in hospitals since this bacterium is frequently isolated from wet environments (Rosenthal, 1974; Sacchetti *et al*., 2009). Treatment of *S. maltophilia* colonization and infection is decisive as only few antibiotics are available to treat *S. maltophilia* infection, for instance co-trimoxazole, cefepime, minocycline, and levofloxacin (Valdezate *et al*., 2001; Blanquer *et al*., 2008; Belvisi *et al*., 2009). The impact of antibiotic resistance caused by this bacterium would lead to longer hospital stays due to the medical treatment of patients could be more complicated by the limitation of antibiotic options and thus give rise in healthcare costs (Lerner, 1998).

Resistance to most of the currently used antibiotics are due to low outer membrane permeability, efflux pumps, L1 and L2 β-lactamases of *S. maltophilia* (Barbolla *et al*., 2004; Toleman *et al*., 2007). The low permeability of outer membrane could prevent the penetration of most antibiotics into the bacterial cell. On the contrary, *S. maltophilia* has two groups of efflux pumps which are SmeABC and SmeDEF, however only SmeC and SmeDEF are responsible for antibiotic resistance (Alonso and Martinez, 2000). Efflux pumps harbouring bacteria could exhibit significant antibiotic resistances through the association between its low membrane permeability and efflux pumps (Nikaido, 1998; Poole, 2000).

β-lactam resistance of *S. maltophilia* is due to two intrinsic β-lactamases, which are L1 and L2 β-lactamases. These β-lactamases produced by *S. maltophilia* can be categorized as group 3, class B for L1 β-lactamase; and group 2e, class A β-lactamase for L2 β-lactamase (Ambler, 1980; Walsh *et al*., 1994; Bush *et al*., 1995; Walsh *et al*., 1997; Avison *et al*., 2001). Virtually all classes of β-lactams antibiotics could be hydrolyzed by these two dominance β-lactamases (Denton and Kerr, 1998). Yet, these β-lactamases are expressed only during the exposure of bacterial cell to β-lactams (Avison *et al*., 2002; Gould *et al*., 2006). The isoelectric point (pI) value of L1 β-lactamase is slightly acidic (pI 6.0). Meanwhile, pI value of L2 β-lactamase is alkaline (pI 8.2) (Rosta and Mett, 1989; Felici *et al*., 1993). So far only two types of β-lactamases produced by *S. maltophilia* have been mentioned by researchers (Looney, 2005; Hu *et al*., 2009). Recently, the presence of other putative β-lactamases such as AmpC β-lactamase produced by *ampC* gene in *S. maltophilia* has been described by Crossman *et al*. in 2008. Furthermore, the distribution and function of *ampC* gene in *S. maltophilia* still remain unclear. Thus, the findings deserve further discussion.

According to Ambler structural classification, AmpC β-lactamase is a group 1, class C β-lactamase which could not be inhibited by clavulanic acid (Ambler, 1980; Bush *et al*., 1995; Jacoby, 2009). AmpC β-lactamase is the first bacterial enzyme which is reported to hydrolyze penicillin (Abraham and Chain, 1940). Its molecular weight ranges from 34 to 40 kDa and usually its pI value is more than 8.0, although a few of AmpC β-lactamases have lower pI value (less than 7.0) (Philippon *et al*., 2002; Power *et al*., 2006). The enzyme is an intracellular enzyme since it has been found to be located in the periplasm of bacterial cell. However, *Psychrobacter immobilis* produces extracellular AmpC β-lactamase (Feller *et al*., 1997). They have higher affinity towards cephalosporins although they hydrolyze penicillin. Cefepime, fourth generation cephalosporins, is believed to be an effective antibiotic to against AmpC β-lactamase producing bacteria since it has rapid penetration property across the bacterial outer membrane. In addition, cefepime is a weak AmpC β-lactamase inducer. Thus, it has been considered as a stable drug to target the bacterial cell wall (Neu *et al*., 1986; Nikaido *et al*., 1990; Sanders, 1993; Tamma *et al*., 2013). Even though cefepime is recommended as a potential drug to treat AmpC β-lactamase producing bacteria, cefepime-resistant bacteria have recently been reported. Cefepime resistance rate was 35.3% for the Gram negative bacteria (Chong *et al*., 2010). However, *S. maltophilia* could demonstrate cefepime resistance rate up to 58% by the association of its antimicrobial defence mechanisms such as cooperation among biofilm production, outer membrane, efflux pumps and β-lactamases (Arvanitidou *et al*., 2003). Resistant to cefepime also could be associated by the amino acid substitution in AmpC β-lactamase, and/or overproduction of the AmpC β-lactamase (Barnaud *et al*., 2004). Disposition of *mutS* gene in *S. maltophilia* might trigger mutation of its *ampC* gene resulting the cefepime resistance (Turrientes *et al.*, 2010).

Since most researchers have documented that only L1 and L2 β-lactamases of *S. maltophilia* are responsible for the resistance characteristics against cephalosporins, other findings could not just be ignored. For instance, presence of other putative β-lactamases has been identified and *ampC* gene which is responsible for AmpC β-lactamase in other microorganisms has been detected in *S. maltophilia* k279a according to NCBI database. It has been predicted that this enzyme could be a hydrolase. Uncertainty of whether the presence of *ampC* gene could be contributed to cephalosporins resistance in *S. maltophilia* prompted us to characterize this gene.

General Objectives:

The main objective in this study was to characterize the *ampC* gene in *Stenotrophomonas maltophilia* clinical isolates.

Specific objectives:

1. To identify isolates of *S. maltophilia* and examine the genetic relatedness among the clinical isolates of *S. maltophilia* and cefepime-sensitive *S. maltophilia* ATCC 13637.

2. To determine the presence of *ampC* gene and nucleotides changes among the clinical isolates of *S. maltophilia* and cefepime-sensitive *S. maltophilia* ATCC 13637.

3. To characterize the *ampC* gene of clinical cefepime-resistant *S. maltophilia* strain and cefepime-sensitive *S. maltophilia* ATCC 13637.

4. To compare the expression level of *ampC* gene of the clinical cefepime-resistant *S. maltophilia* strain and cefepime-sensitive *S. maltophilia* ATCC 13637.

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