INTRODUCTION

Stenotrophomonas maltophilia is an aerobic gram negative bacillus that is widely distributed in nature including various aquatic environments, soil and plants. *S.maltophilia* is an organism of low virulence and frequently colonizes fluids used in the hospital setting (e.g. irrigation solutions, intravenous fluids) and patient secretions (respiratory secretions, urine, wound exudates). *S.maltophilia* usually considered as an opportunistic pathogen that frequently encountered with the use of invasive medical devices that bypass normal host defenses ***(Bruke, 2008).***

Stenotrophomonas maltophilia is the third most common gram negative bacilli encountered in clinical specimens after *Entero-bacteriaceae* and *acinetobacter*. Previous reports have demonstrated that the frequency of infections caused by this species may be increasing most likely due to the prolonged hospitalization and partly associated with a greater use of broad spectrum antibiotics ***(Garcia et al., 2008).***

Stenotrophomonas maltophilia are slightly smaller (٠,٧um) than other members of the genus especially *pseudomonas*. They are motile due to polar flagella and grow well on MacConkey agar producing pigmented colonies similar to *pseudomonas* ***(Bailey and Scotts, 2007).***

Stenotrophomonas maltophilia is catalase positive, oxidase negative which distinguishes it from most members of the genus *pseudomonas*. *S.maltophilia* has a specific positive reaction for extracellular DeoxyriboNucleic acid (DNAase). *S.maltophilia* is reliably identified by commercial Analytical Profile Index (API) system or by conventional biochemical and phenotypic characteristics ***(Bailey and Scotts, 2007).***

Stenotrophomonas maltophilia can lead to nosocomial infections in immunocompromised patients (catheter related infections, bacteremia, respiratory infections, urinary tract infection, wound infections, miscellaneous infections of other body sites ***(Ansari et al., ٢٠٠٧).***

Stenotrophomonas maltophilia is usually intrinsically resistant to multiple antimicrobials, including carbapenams, aminoglycosides and quinolones leaving trimethoprim-sulfamethoxazole as the primary drug of choice for infections caused by this species. Although few other agents such as minocycline, ticarcillin/ clavulanic acid and chloramphenicol may be used as alternatives according to Clinical Laboratory Standard Institute (CLSI) recommendations ***(CLSI, 2008).***

AIM OF THE WORK

The aim of the present study is to identify and differentiate *Stenotrophomonas maltophilia* and it's associated risk factors among different pseudomonal isolates that were recovered from immunocompromised patients.

Chapter (**I**)

STENOTROPHOMONAS SPECIES

Historical aspect

Bacterium bookeri, now known as *S.maltophilia*, was first isolated in ١٩٤٣ and was subsequently classified as a member of the genus *Pseudomonas* in ١٩٦١ on the basis of its flagellar characteristics. In ١٩٨٣, the new name *Xanthomonas maltophilia* was proposed on the strength of ribosomal RiboNucleic Acid (rRNA) homology data. In ١٩٩٣, the genus *Stenotrophomonas maltophilia* was created to accommodate *Xanthomonas maltophilia* (formly *Pseudomonas maltophilia*) ***(Looney, 2009)***. Several recent studies have shown that there is considerable genetic diversity within *Stenotrophomonas maltophilia* and that this species consists of at least nine genomic groups ***(Koneman's et al., 2006).***

Taxonomic Classification

Stenotrophomonas maltophilia belongs to bacteria, proteobacteria, gammaproteobacteria, xanthomonadales, xanthomonadaceae, Stenotrophomonas maltophilia ***(Gilligan et al., 2003)***.

Table (١): Showing taxonomic classification of *S.maltophilia*

Kingdom:	Bacteria
Phylum:	Proteobacteria
Class:	gammaproteobacteria
Order:	xanthomonadales
Family:	xanthomonadaceae
Genus:	stenotrophomonas
Species:	Stenotrophomonas Maltophilia

(Gilligan et al., 2003)

Stenotrophomonas maltophilia is the only member of the genus *Stenotrophomonas* (*Stenos*, Greek: narrow; *trophos*, Greek: one who feeds; *monas*, Greek: a unit, monad; i.e., a unit feeding on few substrates; and *malt*, Old English: malt; *philos*, Greek: friend; i.e., a friend of malt). The genus was proposed in ١٩٩٣ by Palleroni and Bradbury ***(Palleroni and Bradbury, 1993)*** after many years of debate regarding the appropriate taxonomic position of this organism. The type strain was isolated in ١٩٥٨ by Hugh from an oropharyngeal swab from a patient with an oral carcinoma and named *Pseudomonas maltophilia* ***(Hugh and Leifson, 1963)***. Subsequently, Hugh and Ryschenkow reclassified "*Bacterium bookeri*," which had been isolated from pleural fluid in ١٩٤٣ by J.L. Edwards, as *P. maltophilia*. *Pseudomonas melanogena* isolated from Japanese rice paddies in ١٩٦٣ was later recognized as

Pseudomonas maltophilia, as were strains which had initially been characterized as *Pseudomonas alcaligenes* by Komagata ***(Komagata et al., 1974)***.

Similarly, strains of *Alcaligenes faecalis* as described by Ulrich and Needham ١٩٥٣ were subsequently reclassified by Hugh and Ryschenko ١٩٦١ as *P. maltophilia*. Later, the use of DeoxyriboNucleic Acid DNA-rRNA hybridization techniques revealed the presence of five rRNA homology groups in the genus *Pseudomonas*, as it then was, and showed that the rRNA cistrons of the *P. maltophilia* type strain American Type Culture Collection (ATCC) ١٣٦٣٧ were most similar to those of three strains of *Xanthomonas* ***(Palleroni et al., 1973*)**.

This information was used by Swings et al. in ١٩٨١ to propose that *P. maltophilia* be reclassified in the genus *Xanthomonas* as *X. maltophilia* **(*Swings et al., 1983*)**. In addition, they cited several other factors to support their case. These included the Guanine-plus-Cytosine (G+C) content (*P. maltophilia*, ٦٣ to ٦٧,٥٪; *Xanthomonas*, ٦٣ to ٧٠٪); comparative enzymology, particularly the absence of Nicotinamide Adenine Dinucleotide Phosphate (NADP)-linked dehydrogenases; the occurrence of the same type of ubiquinones (*P. maltophilia* and *Xanthomonas* spp. both possess ubiquinones with eight isoprene units, whereas all

other *Pseudomonas* strains possess nine units); and similar cellular fatty acid composition. A subsequent analysis of whole cell proteins patterns appeared to support this proposal ***(VanZyl and Steyn, 1990)***. Additional evidence from isoelectric focusing studies of outer membrane esterase confirmed that *P. betle* and *P. hibiscicola* were synonyms of *X. maltophilia* ***(Van den Mooter and Swings, ١٩٩٠; Singer et al., ١٩٩٤)***. However, the proposed reclassification of *P. maltophilia* as *X. maltophilia* did not meet with universal approval ***(Palleroni, 1984)***, and the controversy about the taxonomic status of this bacterium in the genus *Xanthomonas* remained unresolved ***(Bradbury, ١٩٨٤)***.

The controversy was partly because some of the evidence put forward by ***Swings et al. (1983)*** was subsequently contradicted by the findings of later studies. During an extensive DNA-rRNA hybridization study of the genus *Pseudomonas*, different melting temperatures were reported for the same ٢٧ strains of *Xanthomonas* used in the proposal of ***Swings et al.*** despite the use of identical experimental conditions and the same *X. campestris* type strain as reference rRNA. The range for *X. maltophilia* was ٧٦,٥ to ٧٨°C, almost overlapping the range for the genus *Xanthomonas* reported by Swings et al. (٧٨,٨ to ٨١,٥°C)

but further removed from that (٨٠,٠ to ٨١,٥°C) reported later by De Vos and De Ley ***(De Vos and De Ley, 1983)***.

In addition, using polyamine and fatty acid analysis, demonstrated that *X. maltophilia* possessed profiles distinct from other species within the genus *Xanthomonas*. Furthermore, the possession of ubiquinones with eight isoprene units was shown not to be limited to *X. maltophilia* and other members of the genus *Xanthomonas* by Oyiazu and Komagata, who cited that they were found in several other *pseudomonads* including *P. avenae* and *P. palleroni* of rRNA group III ***(Yang et al., 1993)***.

These factors, along with supplementary evidence, were used to request a reinterpretation of the taxonomic position of *X. maltophilia* ***(Van Zyl and Steyn, 1992)***. This was supported by experiments with a *Xanthomonas*-specific ١٦S rDNA sequence as the primer for Polymerase Chain Reaction (PCR). *Xanthomonads* were recognized by the presence of a single ٤٨٠-bp PCR fragment; however, *X. maltophilia* strains produced additional PCR fragments, leading the author to conclude that *X. maltophilia* does not belong to the genus *Xanthomonas* ***(Maes, 1993).***

Continuing dissatisfaction with the classification of this organism finally gave rise to the proposal in ١٩٩٣ to

create the new genus *Stenotrophomonas* with *S. maltophilia* as the sole member. Later, ***Nesme et al.*** confirmed the distinction between *S. maltophilia* and members of the genus *Xanthomonas* by using restriction mapping of PCR-amplified ١٦S rRNA genes ***(Nesme et al., 1995)***.

Drancourt et al. proposed a new species; *Stenotrophomonas africana*, which is biochemically identical to *S. maltophilia* except for its inability to assimilate cis-aconitate. Genotypic analysis, however, revealed only ٣٥٪ DNA homology between the two species ***(Drancourt et al., 1997)***.

The genus *Stenotrophomonas* currently consists of four species, only one of which, *S. maltophilia*, is known to cause infection in human beings ***(Coenye et al., 2004)***. Genetic analysis suggests that *S. maltophilia* has adapted to human colonization, having lost certain plant pathogenic traits and gained potential human virulence factors. The closest sequenced relatives of *S. maltophilia* are the plant pathogenic *xanthomonads* ***(Crossman et al., 2008)***.

Stenotrophomonas maltophilia isolates from environmental and clinical sources represent a number of genomic groups (defined by DNA fingerprinting, DNA hybridization, and ١٦S rDNA sequencing). Three groups A, B, and C have been recognized so far through ١٦S rRNA sequencing, with group A strains showing the highest similarity to the *S. maltophilia*-type strain ***(Gould et al., ٢٠٠٤)***. Analysis of a collection of clinical isolates from

three continents showed that most clinical isolates fall into group A (٤٤٪) or B (٣٤٪), with high genetic homology within group A and considerable genetic heterogeneity within group B. It is possible that group A strains share certain characteristics that favour the development of infection. It has also been postulated that isolates from specific genomic groups [defined by *gyrB* Restriction Fragment Length Polymorphism (RFLP) analysis] might be better adapted to colonizing the respiratory tracts of patients with Cystic Fibrosis (CF) ***(Gould et al., 2006)*.**

Chapter (**II**)

EPIDEMIOLOGY AND SPECTRUM OF DISEASE

Natural habitat:

Stenotrophomonas maltophilia is a bacterium that can occur in almost any aquatic or humid environment, including the drinking water supply. Although not highly virulent, *S. maltophilia* can be the cause of serious human infections. Several factors make this bacterium a cause for concern for modern medicine ***(Cervia et al., 2008)*.**

It is an environmental organism found in water (including natural waters, water treatment plants, and chlorinated distribution networks), in soil, and on plants. It has been isolated from human and animal faeces, frozen fish, woodland ticks, and raw milk. In the hospital environment, *S.maltophilia* has been found as a contaminant of numerous medical devices, eidetic tubes, chlorhexidine-cetrimide disinfectant, and sterile water ***(Meyer et al., 2006)***.

Incidence:

A study was done at the National Cancer Institute (NCI) (Egypt, Cairo), regarding the microbial spectrum of

gram-negative bacteria in various infection sites from patients with malignant tumors, the incidence of *S.maltophilia* within clinical isolates was ٧,٩٪ ***(Ashour and El-Sharif, 2009).***

Also, a study of Health care Associated Infection (HAI) incidence according to Center for Diseases Control and Prevention (CDC) revealed that microbiological analysis of ٢٠ lower respiratory tract samples collected from patients on mechanical ventilators was *Stenotrophomonas maltophilia* (٧,٣٪) ***(CDC, 2008).***

Surveys from several continents document an increasing isolation rate for *S.maltophilia* that probably reflects an increasing population of patients at risk as a result of advances in medical technologies and treatment. For example, in England and Wales the annual number of blood isolates increased between ٢٠٠٠ and ٢٠٠٦ by ٩٣٪ up to ٧٧٣ cases, and a Taiwanese tertiary-care hospital reported an ٨٣٪ increase from ٥,٣ to ٩,٨ episodes per ١٠٠٠٠ discharges from ١٩٩٩ to ٢٠٠٤. But the rate of *S. maltophilia* isolation varies between hospitals and geographic regions ***(Tan et al., 2008)***.

A Spanish study (late ١٩٩٠s) reported isolation rates between ٣,٤ and ١٢,١ per ١٠٠٠٠ admissions. A German study in ٣٤ Intensive-Care Units (ICUs) between ٢٠٠١ and

٢٠٠٤ showed an increasing rate of *S. maltophilia* infections in some units٧,٦ per ١٠٠٠ patient-days. In CF patients, *S. maltophilia* has been isolated with increasing frequency from the respiratory tract, but prevalence rates vary considerably between centers, with a mean of ٤-٦٪ and peaks of ١٠-٢٥٪ ***(Tan et al., 2008).***

The prevalence of *Stenotrophomonas maltophilia* is evidenced by the fact that, it is the third most common gram negative bacillus encountered in clinical specimens. Community acquired infections with *Stenotrophomonas maltophilia* can occur, but the vast majority of infections are HAI ***(Baily and Scott's, 2007)***.

Route of Transmission:

Unraveling the transmission routes of microorganisms is generally difficult, as multiple routes are possible, including direct patient-to-patient contact, contact between patients and healthy carriers of the bacterium (e.g., hospital personnel) who acquired colonization from other patients or from the environment, and direct contact between the patient and environmental sources ***(Looney, ٢٠٠٥)***.

Risk Factors:

As *S. maltophilia* is an opportunistic pathogen, all risk factors for acquiring this infection are associated with a severely compromised health status, medical treatment involving indwelling devices such as intravascular catheters and ventilation tubes, exposure to broad-spectrum antimicrobials, and long hospital stays. Chronic Obstructive Pulmonary Disease (COPD) and the duration of antibiotic therapy have been found to be independent risks for ICU-acquired *S. maltophilia* ***(Nseir et al., 2006)***.

Blood-Stream Infection (BSI) due to *S .maltophilia* is more likely in the presence of a Central-Venous Catheter (CVC) during treatment in hospital increases the risk of relapse. In addition, in patients with cancer, profound Chemotherapy-Induced Neutropenia (CIN) (fewer than ١٠٠ cells per µL) of long duration, mucositis, and recipient of total parenteral nutrition have been associated with *S. maltophilia* BSI. Prolonged mechanical ventilation predisposes to *S. maltophilia* pneumonia. In critically-ill trauma patients a high injury-severity score and pulmonary contusions were independent predictors for multiple episodes of late-onset *S. maltophilia* Ventilator-Associated Pneumonia (VAP); single episodes of VAP were associated with tracheostomy. Colonization and infection with *S. maltophilia* is favoured by

exposure to broad-spectrum antimicrobials carbapenems, higher-generation cephalosporins such as ceftazidime and cefepime, and quinolones; the risk increases with duration of administration and the number of antimicrobials given ***(Tunger et al., 2007).***

Pathogenesis and virulence factors:

Clinical experience has shown that whole-genome sequencing of *S. maltophilia* does not reveal whether the organism is highly virulent ***(Aisenberg et al., 2007).*** Nevertheless, several factors may promote the ability of this bacterium to colonize the respiratory tract and plastic surfaces (such as catheters and endotracheal tubes). These factors include a positively charged surface and flagella and fimbrial adhesions; the latter have been associated with biofilm formation ***(Waters et al., 2007)***. Biofilms exhibit phenotypic characteristics that are distinct from planktonic organisms, including increased resistance to host immune defences and antimicrobial compounds ***(Passerini de Rossi et al., 2007)***.

In most clinical situations, however, isolation of *S. maltophilia* will represent colonization or contamination rather than true infection, and it has been difficult in many instances to substantiate a causative role of *S. maltophilia*