Review of Literature
Appreciation of the threat to public health associated with chronic kidney disease (CKD) has been a relatively recent phenomenon. For example, in the United States, it was estimated that 324,826 patients were receiving dialysis, 128,131 patients had a functioning renal transplant at the end of 2003, and clinically problematic (non-end-stage) CKD was at least an order of magnitude more common than end-stage disease. (US Renal Data System 2005). Although advances in the management of later stage CKD have occurred, the prognostic associations and resource consumption of CKD remain sobering. Thus, mortality rates for dialysis patients in the United States remain greater than 20% per year, despite an associated cost exceeding $27 billion in 2003. (US Renal Data System 2005). This is considerably higher than the 1-year mortality rates reported for European and Japanese dialysis populations (16 and 7%, respectively) in the Dialysis Outcomes and Practice Patterns Study, with disparities evident even when adjustment was made for patient demographics and comorbidities. (Goodkin et al. 2004).

According to World Health organization (WHO) Global Burden of Disease project, diseases of the kidney and urinary tract contribute to global burden with approximately 850,000 deaths every year and 115,010,107 disability adjusted life years. CKD 12th leading cause of death and 17th cause of disability. (WHO 2006). This global prevalence, however, may be grossly underestimated for a number of reasons. Patients with CKD are at high risk for cardiovascular disease (CVD) and cerebrovascular disease (CBVD), and they are more likely to die of CVD than to develop end stage renal failure.

Moreover, patients with CVD often develop CKD during the course of their disease, which may go unrecognized. Therefore, an unknown proportion of people whose death and disability attributed to CVD have kidney disease as well. (Schieppati et al. 2005) Moreover, most epidemiological data (prevalence, incidence, patient demography, morbi-
dity, and mortality) on CKD are derived from renal registries. However, most registries record data of patients who are at late stage of kidney disease. Much less is known about the prevalence of the earlier stages of the CKD. Indeed, it has been acknowledged that the majority of the individuals at early stages of CKD have gone undiagnosed and under treated. (Schieppati et al. 2005)

The Indian Scenario: The population of India exceeds one billion and is projected to become the major reservoir of chronic diseases like diabetes and hypertension. Since 25–40% of these subjects may develop CKD, the end stage renal disease (ESRD) burden will rise and the health care system would need to take care of them.

India is experiencing a rapid health transition with large and rising burdens of chronic diseases, which are estimated to account for 53% of all deaths and 44% of disability adjusted life years lost in 2005. Even in rural India, chronic non-communicable diseases are emerging as the leading cause of death (Joshi et al. 2006). India leads the world with the largest number of diabetics earning the term of "diabetes capital of the world". According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of diabetics in India currently around 40.9 million is expected to rise to 69.9 million by 2025 unless urgent preventive measures are practiced. (Mohan et al. 2007) Changes in lifestyle and urbanization resulted in obesity, hypertension and diabetes, which are associated with increased risk of CKD.

For a long time, it has been presumed that nearly 100,000 new patients with ESRD in India require renal replacement therapy every year based on data from tertiary referral centres. (Kher et al. 2002) There are only three population based studies in India that reported on the magnitude of CKD. (Mani et al. 2005, Modi et al. 2006). In a prevention program started at community level in Chennai, the reported prevalence of CKD was 0.86% in the study population and 1.39% in the control region. This study was limited since it studied only the rural population around Chennai in the state of Tamil Nadu and only those with urinary abnormalities or a positive response to a questionnaire were subjected to
blood testing, with a potential to underestimate the true prevalence of CKD, which was defined as estimated glomerular filtration rate (GFR) less than 80 ml/min by MDRD (Modification of diet in renal diseases) formula. (Mani et al. 2005)

Assuming the uniform incidence all over the country, approximately 152,000 new ESRD patients would require renal replacement therapy (RRT) every year in India. (Modi et al. 2006) The resources and skill for taking care of this large case load are currently inadequate.

On basis of the recent survey of the ICMR (Indian Council of Medical Research), it is estimated that prevalence of diabetes in adults is 3.8% and 11.8% in rural and urban areas, respectively. (WHO 2006) Moreover, prevalence of hypertension has been reported to range from 20–40% and 12–17% in urban and rural adults, respectively. (Gupta et al. 2004)

The risk of cardiovascular disease in patients with CKD far exceeds general population estimates and a stepwise association between risk and level of kidney function has been seen in most studies. Indeed, following stratification by age, gender, race, and the presence or absence of diabetes, cardiovascular mortality in dialysis patients is between 10 and 20 times higher than in the general population, (Foleys et al. 1998) accounting for approximately half of all deaths in these patients. Patients with CKD appear to develop cardiovascular disease at much younger ages than matched controls and are much more likely to die from cardiovascular causes than to develop end-stage renal disease. In summary, a broad consensus has emerged that CKD is a state of high cardiovascular risk. However, the evidence supporting aggressive management of traditional risk factors such as diabetes, hypertension, and cholesterol in CKD populations is less than convincing. For instance, lipid-lowering therapy with fluvastatin failed to show net benefit in one large trial of transplant patients. (Holdass et al. 2003)

Another landmark study, Die Deutsche Diabetes Dialyse (4D) trial, showed that atorvastatin had no effect on the composite primary outcome
of cardiac death, non-fatal myocardial infarction, and non-fatal stroke in maintenance haemodialysis patients with type II diabetes. (Wanner et al. 2005)

**Definition and Classification**

Chronic kidney disease has been defined according to the criteria listed in table below.

<table>
<thead>
<tr>
<th>Table. Definition of Chronic Kidney Disease Criteria</th>
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<tr>
<td>1. Kidney damage for ≥3 months, as defined by structural of functional abnormalities of the kidney, with or without decreased GFR, manifest by either:</td>
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<tr>
<td>- Pathological abnormalities; or</td>
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<tr>
<td>- Markers of kidney damage, including abnormalities in the composition of the blood or urine, or abnormalities in imaging tests</td>
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<tr>
<td>2. GFR &lt;60 mL/min/1.73 m² for ≥3 months, with or without kidney damage</td>
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All individuals with GFR <60 mL/min/1.73 m² for ≥3 months are classified as having chronic kidney disease, irrespective of the presence or absence of kidney damage. The rationale for including these individuals is that reduction in kidney function to this level or lower represents loss of half or more of the adult level of normal kidney function, which may be associated with a number of complications.

Individuals with GFR 60 to 89 mL/min/1.73 m² without kidney damage are classified as “decreased GFR.” Decreased GFR without recognized markers of kidney damage is very frequent in infants and older adults, and is usually considered to be “normal for age.” The age-related decline in GFR in adults is accompanied by pathological findings of global glomerular sclerosis and cortical atrophy. The consequences of declining GFR with age have not been carefully studied. It is interesting to speculate whether the increasing incidence of end-stage renal disease in the elderly could be due, in part, to age-associated decline in GFR.
Proteinuria as a marker of kidney damage - Proteinuria is an early and sensitive marker of kidney damage in many types of chronic kidney disease. Albumin (molecular weight [MW] = 68,000 daltons) is the most abundant urine protein in most types of chronic kidney disease. Low molecular weight (LMW) globulins are the most abundant urine proteins in some types of chronic kidney disease. In this and later guidelines, the term proteinuria includes albuminuria, increased urinary excretion of other specific proteins, and increased excretion of total urine protein. On the other hand, the term albuminuria has been used only when referring to increased urinary albumin excretion. Older laboratory methods, such as the urine dipstick or acid precipitation, detect most urine proteins. Microalbuminuria refers to excretion of small but abnormal amounts of albumin, which requires recently developed, more sensitive laboratory methods that are now widely available. (Levev et al. 2005).

Decreased GFR is associated with a wide range of complications in other organ systems, manifested by high blood pressure, laboratory abnormalities, and symptoms. Severity of complications worsens as level of GFR declines. The Work Group defined categories of decreased GFR as mild (Stage 2, 60 to 89 mL/min/1.73 m²), moderate (Stage 3, 30 to 59 mL/min/1.73 m²), and severe (Stage 4, 15 to 29 mL/min/1.73 m²). Although these definitions are arbitrary, evidence compiled in later guidelines supports these broad categories and cut-off levels.

The incidence and the prevalence of reported ESRD have doubled in the past 10 years in the United States. Data from the 2000 Annual Data Report of the USRDS documents the incidence of ESRD in 1998 of more than 85,000, or 308 per million individuals per year at risk. The point prevalence of ESRD on December 31, 1998 was more than 320,000, or 1,160 per million population, of whom 72% were treated by dialysis (230,000 patients, or 835 per million population) and 28% had functioning kidney transplants (90,000 patients, or 325 per 100,000). The number of individuals with GFR <15 mL/min/1.73 m² not on dialysis has not been estimated reliably.
There are a number of limitations to the proposed definition and classification of chronic kidney disease. The Work Group believes that these limitations should be identified, but does not think that they invalidate the proposal. Instead, these limitations should serve to stimulate further research to refine the definition and classification.

**ELECTOLYTES/ENDOCRINIAL/URINE ANALYSIS**

1. The kidneys are the key organs to maintain the balance of the different electrolytes in the body and the acid-base balance. Progressive loss of kidney function results in a number of adaptive and compensatory renal and extra renal changes that allow homeostasis to be maintained with glomerular filtration rates in the range of 10-25 ml/min. With glomerular filtration rates below 10 ml/min, there are almost always abnormalities in the body's internal environment with clinical repercussions.

2. Water Balance Disorders: In advanced chronic kidney disease (CKD), the range of urine osmolality progressively approaches plasma osmolality and becomes isostenuric. This manifests clinically as symptoms of nocturia and polyuria, especially in tubulointerstitial kidney diseases. Water overload will result in hyponatremia and a decrease in water intake will lead to hypernatremia. Routine analyses of serum Na levels should be performed in all patients with advanced CKD. Except in edematous states, a daily fluid intake of 1.5-2 liters should be recommended.

Hyponatremia does not usually occur with glomerular filtration rates above 10 ml/min. If it occurs, an excessive intake of free water should be considered or no osmotic release of vasopressin by stimuli such as pain, anaesthetics, hypoxemia or hypovolemia, or the use of diuretics. Hypernatremia is less frequent than hyponatremia in CKD. It can occur because of the provision of hypertonic parenteral solutions, or more frequently as a consequence of osmotic diuresis due to inadequate water intake during intercurrent disease, or in some circumstance that limits access to water (obtundation, immobility).
3. Sodium Balance Disorders: In CKD, fractional excretion of sodium increases so that absolute sodium excretion is not modified until glomerular filtration rates below 15 ml/min. Total body content of sodium is the main determinant of extracellular volume and therefore disturbances in sodium balance will lead to clinical situations of volume depletion or overload: Volume depletion due to renal sodium loss occurs in abrupt restrictions of salt intake in advanced CKD. It occurs more frequently in certain tubulointerstitial kidney diseases (salt losing nephropathies).

Volume overload due to sodium retention can occur with glomerular filtration rates below 25 ml/min and leads to edema, arterial hypertension and heart failure. The use of diuretics in volume overload in CKD is useful to force natriuresis. Thiazides have little effect in advanced CKD. Loop diuretics are effective and should be used in higher than normal doses. The combination of thiazides and loop diuretics can be useful in refractory cases. Weight and volume should be monitored regularly in the hospitalized patient with CKD.

4. Potassium Balance Disorders: In CKD, the ability of the kidneys to excrete potassium decreases proportionally to the loss of glomerular filtration. Stimulation of aldosterone and the increase in intestinal excretion of potassium are the main adaptive mechanisms to maintain potassium homeostasis until glomerular filtration rates of 10 ml/min.

The main causes of hyperkalemia in CKD are the following: Use of drugs that alter the ability of the kidneys to excrete potassium: ACEIs, ARBs, NSAIDs, aldosterone antagonists, nonselective beta-blockers, heparin, trimetoprim, calcineurin inhibitors. Determination of serum potassium two weeks after the initiation of treatment with ACEIs/ARBs is recommended. Routine use of aldosterone antagonists in advanced CKD is not recommended. Abrupt reduction in glomerular filtration rate: Constipation, Prolonged fasting, metabolic acidosis.
A low-potassium diet is recommended with GFR less than 20 ml/min, or GFR less than 50 ml/min if drugs that raise serum potassium are taken). In the absence of symptoms or electrocardiographic abnormalities, review of medications, restriction of dietary potassium and use of oral ion exchange resins are usually sufficient therapeutic measures). If symptoms and/or electrocardiographic abnormalities are present, the usual parenteral pharmacological measures should be used (10% calcium gluconate, insulin and glucose, salbutamol, resins, diuretics)). Parenteral bicarbonate and ion exchange resins in enemas are not recommended as first-line treatment. Haemodialysis should be considered in patients with glomerular filtration rates below 10 ml/min.

5. Acid-Base Disorders in CKD: Moderate metabolic acidosis (Bic 16-20) mEq/L is common with glomerular filtration rates below 20 ml/min, and favors bone demineralization due to the release of calcium and phosphate from the bone, chronic hyperventilation, and muscular weakness and atrophy. Its treatment consists of administration of sodium bicarbonate, usually orally (0.5-1 mEq/kg/day), with the goal of achieving a serum bicarbonate level of 22-24 mmol/L). Limitation of daily protein intake to less than 1 g/kg/day is also useful). Use of sevelamer as a phosphate binder aggravates metabolic acidosis since it favors endogenous acid production and therefore acidosis should be monitored and corrected if it occurs. Hypocalcaemia should always be corrected before metabolic acidosis in CKD. Metabolic acidosis is an infrequent disorder and requires exogenous alkali administration (bicarbonate, phosphate binders) or vomiting.

6. Kidney failure, also known as renal failure, can be either acute or chronic. Acute renal failure has a sudden onset, usually hours to days, and can be caused by trauma, infection or an obstruction. With acute renal failure, if the underlying cause is corrected, kidney function returns. According to "Fluids and Electrolytes
Demystified,” chronic renal failure involves progressive and irreversible loss of kidney function.

Phosphorus and Calcium

Phosphorus and calcium levels are affected by renal failure. Because of the reciprocal relationship between phosphorus and calcium, the retention of phosphorus in renal failure causes a decrease in the level of calcium. Low levels of calcium cause muscle spasms, seizures and abnormal heart rhythms. The presence of high serum levels of phosphorus for extended periods of time may lead to additional complications. As calcium levels remain low, skeletal demineralization starts to occur and calcium deposits occur in vascular cells causing hardened arterial walls. This, in turn, can lead to enlargement of the left ventricle of the heart, high blood pressure and ultimately cardiac failure.

Chronic kidney disease (CKD) is a very real and growing problem, as indicated by demographic trends. The total number of treated patients has markedly increased during the last 30 years, and CKD currently affects approximately 19 millions of adult Americans, with an incidence that is increasing rapidly (Snyder and Pendergraph et al. 2005). This trend is caused by a growing percentage of elderly people in the population as well as by technical progress and broader availability of dialysis therapy. An increasing number of diabetic patients are also an important factor.

CKF is associated with many kinds of metabolic changes caused by the kidney disease and also attributable to dialysis treatment. Phenomena such as accumulation or deficit of various substances and dysregulation of metabolic pathways combine in the pathogenesis of these changes (Cibulka et al. 2005).

Disturbed synthesis of some crucial metabolic regulators (e.g. erythropoietin, active vitamin D) in kidneys also plays an important role. All of the above mentioned factors lead to many serious complications for CKD patients during the course of predialysis and dialysis. All accelerate the development of atherosclerosis, malnutrition inflammation complex syndrome (MICS), anemia, hyperparathyroidism, and other serious

**ACID-BASE BALANCE**

Acid–base disorder is commonly observed in the course of CKF. Metabolic acidosis is noted in a majority of patients when GFR decreases to less than 20 to 25 % of normal. The degree of acidosis approximately correlates with the severity of CKF and usually is more severe at a lower GFR. Metabolic acidosis can be of the high-anion-gap type, although the anion gap can be normal or only moderately increased even with stages 4 or 5 of CKF (Kraut and Kurtz et al. 2005). In mild chronic renal insufficiency, metabolic acidosis is the result of a reduced ability to reabsorb bicarbonate, to excrete ammonia, and to eliminate titratable acid excretion (hyperchloremic, normal anion gap acidosis). In more severe renal insufficiency, organic and other conjugate anions of acids (nonvolatile acids) cannot be sufficiently excreted, and elevated anion gap acidosis appears (Kovacic et al. 2003). Acidosis resulting from advanced renal insufficiency is called uremic acidosis. The level of GFR at which uremic acidosis develops varies depending on a multiplicity of factors.

In general, metabolic acidosis is rare when the GFR is greater than 20–25 ml/min (Oh et al. 2004). Several adverse consequences have been associated with uremic acidosis; including muscle wasting, bone disease, abnormalities in growth hormone and thyroid hormone secretion, impaired insulin sensitivity, and exacerbation of beta-2 micro globulin accumulation (Kraut and Kurtz et al. 2005). Other complications include negative nitrogen balance, anorexia, fatigue, impaired function of the cardiovascular system, hyperkalemia, and altered gluconeogenesis and triglyceride metabolism (Kovacic et al. 2003).

In dialysis patients, treatment of acidosis relies on the gain of alkali from the dialysate either as bicarbonate in hemodialysis or as lactate in peritoneal dialysis (Oh et al. 2004).
PROTEIN METABOLISM

A strict low-protein diet can have a negative effect on nitrogen balance in the predialysis period. A safe low-protein diet should contain a minimum 0.6 g of protein/kg/day. Disorders in protein metabolism in the dialysis period are usually caused by combined (protein and energy) malnutrition that can be termed uremic 2007 Metabolic Disorders in Chronic Kidney Failure 699 malnutrition. It is present in approximately 20-50 % of patients on dialysis and is characterized by insidious loss of somatic protein stores (reflected in lean body mass and serum creatinine) and visceral protein concentrations (reflected in serum albumin and prealbumin concentrations) (Ikizler et al. 2004). Urinary losses of protein and losses of amino acids during a dialysis session may also play a role. Metabolic acidosis is an important factor that markedly contributes to negative nitrogen and total body protein balance in CKF (Kovacic et al. 2003, Mehrotra et al. 2003).

It has been demonstrated that the presence of uremic malnutrition increases mortality and morbidity in chronic dialysis patients (Kopple 1994, Ikizler et al. 1999). It is very often combined with a chronic inflammation state in the malnutrition inflammation complex syndrome (MICS) (Kalantar-Zadeh et al. 2003).

CARBOHYDRATE METABOLISM

Disorders of carbohydrate metabolism are also very frequent in CKD. Diabetics represent about 35 % of all patients on dialysis therapy. Furthermore, non-diabetic CKD patients often have glucose intolerance, probably because of peripheral insulin resistance (Alvestrand et al. 1997).

Insulin resistance is primarily detectable when the GFR is below 50 ml/min. Reduced insulin-mediated non oxidative glucose disposal is the most evident defect of glucose metabolism, but impairments of glucose oxidation, the defective suppression of endogenous glucose production, and abnormal insulin secretion also contribute to uremic glucose intolerance (Rigalleau and Gin et al. 2005).

Accumulating nitrogenous uremic toxins seem to be the dominant cause of a specific defect in insulin action, and identification of these
toxins is progressing, particularly in the field of carbamoylated amino acids. The consequences of CKF, such as exercise intolerance, anemia, metabolic acidosis, secondary hyperparathyroidism, or vitamin D deficiency, also indirectly play a role (Rasic-Milutinovic et al. 2000, Rigalleau and Gin et al. 2005).

It has been reported that insulin resistance may be related to arterial hypertension (Ferrannini et al. 1987) and may contribute to high cardiovascular morbidity and mortality in patients with CKF (Shoji et al. 2001, Shinohara et al. 2002).

The underlying mechanism can be an impaired synthesis of nitric oxide (NO) in the endothelium of patients with CKD. It was reported that appropriately functioning endothelial NO synthase (eNOS) is important for the control not only of arterial pressure but also of glucose and lipid homeostasis (Duplain et al. 2001).

**LIPID METABOLISM**

Serum triglycerides (TG) are elevated in CKF because of enhanced production of TG-rich lipoproteins such as very-low-density lipoproteins (VLDL) in the liver (Attman et al. 1993) and also because of dysfunction of TG degradation resulting from insufficient mitochondrial beta-oxidation of fatty acids. It can be caused by a deficit of L-carnitine, which is frequently present, especially in hemodialysis patients (Guarnieri et al. 1992, Cibulka et al. 2005).

Modifications contribute to impaired LDL receptor mediated clearance from the plasma and promote prolonged circulation. HDL particles are structurally altered during the states of inflammation. The contribution of this complex atherogenic form of dyslipidemia to cardiovascular disease (CVD) in patients with renal disease is at present not clear. Some studies have reported negative results regarding the predictive power of serum lipids for the development of CVD (Wanner and Krane et al. 2002).

Recent findings have suggested that the development of MICS is responsible for this phenomenon. Therefore, hypercholesterolemia, obesity, and increased blood levels of creatinine and homocysteine
appear to be protective and paradoxically associated with a better outcome in patients with CKF (Kalantar-Zadeh et al. 2003, 2005).

It is well known that HDL cholesterol levels are inversely correlated with the risk of atherosclerosis. In addition to its role in reverse cholesterol transport, HDL has the ability to protect LDL particles against oxidation. The underlying mechanism by which HDL inhibits LDL oxidation is partly enzymatic. There is increasing evidence that paraoxonase 1 (PON1) could be involved in this process (Mackness et al. 1993).

It was proved that serum PON1 activity is reduced in CKF patients. The possible causes can include reduced HDL levels, altered HDL sub fraction distribution, reduced PON1 concentration and different PON1 phenotype distributions. Another possible explanation could be that PON1 activity is inhibited in an uremic environment. Generally, reduced serum PON1 activity could also contribute to the accelerated development of atherosclerosis in CKF patients (Dirican et al. 2004).

It has been demonstrated that the low-molecular-weight Apo (a) phenotype independently predicted coronary artery disease occurrence in a cohort of 440 unselected hemodialysis (HD) patients in a prospective study over five years (Kronenberg et al. 1999). The major problems are primarily renal anemia and renal bone disease (renal osteodystrophy).

**RENAL ANEMIA**

Renal anemia, which is often associated with fatigue and cognitive and sexual dysfunction, has a significant impact on the quality of life of patients with CKF. Anemia has also been identified as an important etiologic factor in the development of left ventricular hypertrophy, an independent risk factor for heart failure and a predictor of mortality in HD patients (Golper et al. 2003).

The major cause of renal anemia in CKF is an inadequate production of the glycoprotein hormone erythropoietin (EPO) because of a reduction in functional kidney parenchyma (Santoro et al. 2002).

Furthermore, free radicals elicited from leukocytes by their contact with the dialysis membrane cause hemolysis with consecutive anemia in
CKF patients on extracorporeal renal replacement therapy (Eiselt et al. 1999).

There are numerous other metabolic derangements associated with uremia that can affect the production and survival of red blood cells (e.g. some uremic toxins, parathormone, protein malnutrition) (Eschbach and Adamson 1985, Golper et al. 2003).

The introduction of recombinant human EPO (rHuEPO) has revolutionized the treatment of anemia in CKF. The vast majority of patients respond well to treatment, but 5-10 % of patients show some resistance to rHuEPO, the most common causes of which are considered due to iron deficiency (Santoro et al. 2002) and the development of MICS (Kalantar-Zadeh et al. 2003).

These problems are partly related to the deficit of L-carnitine in HD patients (dialysis-related carnitine disorder). This disorder is a functional metabolic deficiency common in chronic HD patients and can have a negative impact on erythrocyte production and survival. Laboratory studies examining the influence of carnitine on red blood cell function and clinical trials in HD patients support the use of L-carnitine in the setting of rHuEPO hypo responsiveness (Golper et al. 2003).

RENAL OSTEODYSTROPHY

Renal osteodystrophy, which is the term used to describe the skeletal abnormalities of many CKD patients, is a multifactorial disorder of bone remodeling. It encompasses a heterogeneous group of disorders from states of high bone turnover to states of low bone turnover. High-turnover bone disease or osteitis fibrosa represents the manifestations of secondary hyperparathyroidism (SHPT) on bone. Low bone turnover syndromes are represented by the increasingly prevalent a dynamic bone or less commonly, by osteomalacia. These disorders may occur in combination or alternately, each may predominate in any given patient (Gonzalez and Martin et al. 2001).

The deficit of calcitriol causes an inadequate absorption of calcium in the small intestine, with resulting hypocalcaemia. Retention of inorganic phosphate may deteriorate this situation because phosphates
impair the activity of 1-α-hydroxylase even more. Long-lasting hypocalcaemia and coincidental hyperphosphatemia lead to the stimulation of parathyroid glands and subsequent SHPT, which causes decalcification of bones. Chronic Metabolic Disorders in Chronic Kidney Failure 701 metabolic acidosis intensifies this harmful process (Kraut and Kurtz et al. 2005). All of these factors are closely interrelated, and while one or more of them may predominate in a particular patient during the course of CKF, much overlap occurs (Gonzalez and Martin et al. 2001).

Moreover, hyperphosphatemia has been recognized as an important risk factor for CVD mortality in patients with CKF. It is a direct cause of vascular calcification (Giachelli et al. 2005, Lund et al. 2006).

Calcitriol or other 1-α-hydroxylated vitamin D sterols should not be used to treat vitamin D deficiency (National Kidney Foundation 2004).

**CARDIOVASCULAR DISEASE**

CVD is the leading cause of death in patients with CKF. For every registry reporting national dialysis data in Europe, the U.S., Japan, and elsewhere, about 50 % of deaths are attributed to CVD (Foley et al. 1998). In comparison with the general population, dialysis patients have a more than 20-fold increased risk of cardiovascular death (Levey and Eknoyan et al. 1999).

The risk of CVD and associated mortality increases in proportion to the decrease in GFR. It is significantly higher if GFR has fallen below approximately 75 ml/min. The evidence suggests that the damage is already far progressed when patients reach ESRD; thus, effective intervention must be started much earlier (Diaz-Buxo and Woods et al. 2006). Patients with albuminuria and normal GFR are also at increased risk (Go et al. 2004).

Evaluation for traditional risk factors, including high lipid levels, hypertension, smoking, and sedentary lifestyle, is essential (Snyder and Pendergraph et al. 2005). The KDOQI (Kidney Disease Outcomes Quality Initiative) recommended a blood pressure goal of 130/80 mm Hg in patients with normal urinary albumin concentrations, and a blood
pressure goal of 125/75 mm Hg in patients with excretion of more than 1 g of protein/24 h (National Kidney Foundation 2002).

The KDOQI guidelines on managing dyslipidemias in CKD patients recommend an LDL cholesterol goal of less than 100 mg/dl (2.60 mmol/l) (National Kidney Foundation 2006). However, as mentioned above, some CKD patients with the lowest cholesterol levels are the most likely to die of CVD because low levels of cholesterol are associated with nontraditional cardiac risk factors of malnutrition and are markers of MICS (Liu et al. 2004, Kalantar-Zadeh et al. 2005).

Additional cardiac risk factors specific to CKF include volume overload, hyperparathyroidism, uremia, anemia, endothelial dysfunction, and, especially in HD patients, oxidative stress (Eiselt et al. 1999).

**OXIDATIVE STRESS**

Oxidative stress is the state in which the production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defense system in cells and tissues. ROS are free radicals, highly reactive substances with an unpaired electron in the outer orbital, and other related reactive compounds (such as hydrogen peroxide and hypochlorous acid) that can attack lipids, proteins, and nucleic acids and alter the structure and function of these macromolecules (Klaunig et al. 1998, Eiselt et al. 1999).

Free radicals originate from leucocytes, which are activated during the contact with the dialysis membrane, and also from erythrocyte iron released as a consequence of hemolysis (Eiselt et al. 1999).

Intravenous administration of iron often used as a supplement of EPO treatment can also contribute to oxidative stress, increasing free radical production by the so-called Fenton reaction (Lim et al. 1999).

Co-administration of ascorbic acid with the goal of 702 Cibulka and Racek Vol. 56 mobilizing iron stores further stimulates free radical formation, possibly by reduction of Fe (III) ions to more dangerous Fe (II) compounds (Eiselt et al. 2006).
DIALYSIS-RELATED AMYLOIDOSIS

Dialysis-related amyloidosis (DRA) is a frequent complication of CKF and long-term renal replacement therapy. Increased carbonyl compounds derived from autoxidation of both carbohydrates and lipids modify proteins in uremia, leading to augmentation of advanced glycation end-products and advanced lipoxidation end-product production. Thus, uremia might be a state of carbonyl overload with potentially damaging proteins (carbonyl stress) (Miyata et al. 1999).

HYPERHOMOCYSTEINEMIA AND ENDOTHELIAL DYSFUNCTION

Hyperhomocysteinemia is present in the majority of CKF patients. They have plasma concentration of homocysteine (Hcy) elevated 3 to 4 times above normal (Suliman et al. 2001). The causes are still not clear, but the possibilities include defective renal or extra renal metabolism as a result of uremic toxicity (Perna et al. 2004).

In the general population, hyperhomocysteinemia is considered to be an independent risk factor for the development of CVD (Racek et al. 2005).

As mentioned above, reverse epidemiology of traditional cardiovascular risk factors can occur in CKF, so that increased Hcy levels appear to be paradoxically associated with a better clinical outcome. The development of MICS is responsible for this phenomenon (Kalantar-Zadeh et al. 2005).

Plasma Hcy concentration is higher in CRF patients with normal nutritional status than in malnourished patients. Plasma Hcy was inversely correlated with subjective global nutritional assessment and positively correlated with serum albumin and protein intake. Thus, serum albumin concentration is a strong determinant of plasma Hcy in patients with CRF, which may contribute to the lower Hcy levels in malnourished patients (Suliman et al. 2001).

On the other hand, the toxicity of Hcy results from the structural modification of proteins and DNA. Disruption of DNA methylation has been demonstrated to occur as a result of hyperhomocysteinemia and is associated with vascular damage (Perna et al. 2005).
Hcy could be a principal candidate for endothelial dysfunction in patients with CRF. Hyperhomocysteinemia may impair endothelial function by the generation of oxygen species and decreased NO bioavailability. Normal endothelium is characterized by an intact and appropriately functioning eNOS-NO system in which eNOS is constitutively active and constantly generates small amounts of NO (Nedeljkovic et al. 2003).

NO mediates normal endothelial and vessel wall functions including antithrombosis, endothelial permselectivity, and vasomotor tone. In addition, NO suppresses cellular proliferation (including vascular smooth muscle cells) and has a quenching effect on inflammation. The function of the eNOS-NO system is impaired in patients with CKD (Kone et al. 1997). However, the precise mechanisms underlying the link between hyperhomocysteinemia and impaired endothelial function in CRF remain unclear (Takamitsu and Nakanishi et al. 2001).

Some authors propose that accumulation of asymmetric dimethylarginine (ADMA), an endogenous inhibitor of NO synthase, is the missing connecting link. Hcy is produced during ADMA synthesis and can alter ADMA catabolism mainly by inhibiting dimethylarginine dimethylaminohydrolase (Dayal and Lentz et al. 2005).

ADMA levels are elevated in CKD patients, and ADMA is a candidate as a new uremic toxin (De Deyn et al. 2003). In some studies, an increased level of ADMA has been identified as an independent predictor of mortality in patients with CKD (Ravani et al. 2005).

**SEPESIS IN CKD**

Sepsis is a potentially deadly medical condition characterized by a whole-body inflammatory state (called a systemic inflammatory response syndrome or SIRS) caused by severe infection. (Levy et al. 2003)

Septicemia (also septicaemia or septicaemia ) is a related medical term referring to the presence of pathogenic organisms in the bloodstream, leading to sepsis. The term has not been sharply defined. It has been inconsistently used in the past by medical professionals, for
example as a synonym of bacteremia, causing some confusion. (Wilson et al. 2007).

Common symptoms of sepsis include those related to a specific infection, but usually accompanied by high fevers, hot, flushed skin, elevated heart rate, hyperventilation, altered mental status, swelling, and low blood pressure. In the very young and elderly, or in people with weakened immune systems, the pattern of symptoms may be atypical, with hypothermia and without an easily localizable infection. (Levy et al. 2003, Jui et al. 2011)

In the United States, severe sepsis contributes to more than 200,000 deaths per year. (Munford et al. 2012). To guide therapy, a central venous catheter and an arterial catheter may be placed; measurement of other hemodynamic variables (such as cardiac output, mixed venous oxygen saturation or stroke volume variation) may also be used. Sepsis patients require preventive measures for deep vein thrombosis, stress ulcers and pressure ulcers, unless other conditions prevent this. Some might benefit from tight control of blood sugar levels with insulin (targeting stress hyperglycemia). (Dellinger et al. 2008)

The use of corticosteroids is controversial. (Patel et al. 2012). Activated drotrecogin alfa (recombinant activated protein C), originally marketed for severe sepsis, has not been found to be helpful, and has recently been withdrawn from sale. (Marti Carvail et al. 2012)

In addition to symptoms related to the provoking infection, sepsis is frequently associated with either fever or hypothermia, rapid breathing, elevated heart rate, confusion, and edema. (Levy et al. 2003).

The most common primary sources of infection resulting in sepsis are the lungs, the abdomen and the urinary tract. (Dolin et al. 2010) No source is found in one third of cases. (Dolin et al. 2010) The infectious agents are usually bacteria but can also be fungi and viruses. (Dolin et al. 2010). While gram-negative bacteria were previously the most common cause of sepsis, in the last decade, gram-positive bacteria, most commonly staphylococci are thought to cause more than 50% of cases of sepsis. (Hirasawa et al. 2009).
In those with sepsis it is recommended that blood cultures be drawn before antibiotics are given. (Dellinger et al. 2008)

According to the American College of Chest Physicians and the Society of Critical Care Medicine, there are different levels of sepsis:

( Wilson et al. 2007).

- Systemic inflammatory response syndrome (SIRS) is the presence of two or more of the following: abnormal body temperature, heart rate, respiratory rate or blood gas, and white blood cell count.
- Sepsis is defined as SIRS in response to an infectious process. It however can be triggered by many things other than sepsis. (Soong et al. 2012 Jun).
- Severe sepsis is defined as organ dysfunction due to an infection.
- Septic shock is severe sepsis plus persistently low blood pressure following the administration of intravenous fluids. (Dellinger et al. 2008)

Infection can be suspected or proven (by culture, stain, or polymerase chain reaction (PCR)), or a clinical syndrome pathognomonic for infection. Specific evidence for infection includes WBCs in normally sterile fluid (such as urine or cerebrospinal fluid (CSF)); evidence of a perforated viscus (free air on abdominal x-ray or CT scan; signs of acute peritonitis); abnormal chest x-ray (CXR) consistent with pneumonia (with focal opacification); or petechiae, purpura, or purpura fulminans.

**END-ORGAN DYSFUNCTION**

Examples of end-organ dysfunction include the following: (Abraham et al. 2007).

- Lungs: acute lung injury (ALI) (PaO$_2$/FiO$_2$ < 300) or acute respiratory distress syndrome (ARDS) (PaO$_2$/FiO$_2$ < 200)
- Brain: encephalopathy symptoms: agitation, confusion, coma; cause: ischemia, hemorrhage, micro thrombi, micro abscesses, multifocal necrotizing leukoencephalopathy
- Liver: disruption of protein synthetic function: manifests acutely as progressive coagulopathy due to inability to synthesize clotting factors, disruption of metabolic functions: manifests as cessation of
bilirubin metabolism, resulting in elevated unconjugated serum bilirubin levels

- Kidney: oliguria and anuria, electrolyte abnormalities, volume overload
- Heart: systolic and diastolic heart failure, likely due to cytokines that depress myocyte function, cellular damage, manifest as a troponin leak (although not necessarily ischemic in nature)

More specific definitions of end-organ dysfunction exist for SIRS in pediatrics. (Goldstein et al. 2005)

- Cardiovascular dysfunction (after fluid resuscitation with at least 40 ml/kg of crystalloid)
- Respiratory dysfunction (in the absence of cyanotic heart disease or known chronic lung disease)
- Neurologic dysfunction
- Hematologic dysfunction
- Renal dysfunction
  - serum creatinine ≥ 2 times the upper limit of normal for age or 2-fold increase in baseline creatinine in patients with chronic kidney disease
- Hepatic dysfunction (only applicable to infants > 1 month)
  - total serum bilirubin ≥ 4 mg/dl, OR
  - alanine aminotransferase (ALT) ≥ 2 times the upper limit of normal

Consensus definitions, however, continue to evolve, with the latest expanding the list of signs and symptoms of sepsis to reflect clinical bedside experience (Levy et al. 2003).

Sepsis is caused by a combination of factors related to the invading organism(s) and the host (pre-disposing illnesses, genetics, and immune system).

Bacteremia is the presence of viable bacteria in the bloodstream. Likewise, the terms viremia and fungemia simply refer to viruses and fungi in the bloodstream. These terms say nothing about the consequences this has on the body. For example, bacteria can be introduced into the bloodstream during toothbrushing. (Lockhart et al.
2008). This form of bacteremia almost never causes problems in normal individuals. However, bacteremia associated with certain dental procedures can cause bacterial infection of the heart valves (known as endocarditis) in high-risk patients. (Wilson et al. 2007) Conversely, a systemic inflammatory response syndrome can occur in patients without the presence of infection, for example in those with burns, polytrauma, or the initial state in pancreatitis and chemical pneumonitis. (Bone et al. 1992).

Approximately 20–35% of people with severe sepsis and 30–70% of people with septic shock die. Lactate is a useful method of determining prognosis with those who have a level greater than 4 mmol/L having a mortality of 40% and those with a level of less than 2 mmol/L having a mortality of less than 15%. (Soong et al. 2012)

Some people may experience severe long-term cognitive decline following an episode of severe sepsis, but the absence of baseline neuropsychological data in most sepsis patients makes the incidence of this difficult to quantify or to study. (Jackson et al. 2009)

Sepsis causes millions of deaths globally each year. (Dellinger et al. 2008) In the United States sepsis affects approximately 3 in 1000 people a year. (Soong et al. 2012) Due to its rarely being reported as a primary diagnosis (often being a complication of cancer or other illnesses), the incidence, mortality, and morbidity rates are likely underestimated.

Pfeiffer coined the term endotoxin at the beginning of the 20th century to denote the pyrogenic principle associated with Vibrio cholerae. It was soon realised that endotoxins were expressed by most and perhaps all Gram negative organisms. The lipopolysaccharide character of enteric endotoxins was elucidated in the 1944 by Shear the molecular character of this material was determined by Luderitz et al. in 1973. (Luderitz et al. 1973).

It was discovered in 1965 that a strain of C3H/HeJ mice were immune to the endotoxin induced shock. (Heppner et al. 1965). The genetic locus for this effect was dubbed Lps. These mice were also found to be hypersusceptible to infection by Gram negative bacteria. (O'Brein et
al. 1980) These observations were finally linked in 1998 by the discovery of the Toll-like receptor gene 4 (TLR 4).

A large international collaboration was established to educate people about sepsis and to improve patient outcomes with sepsis, entitled the "Surviving Sepsis Campaign". The Campaign has published an evidence-based review of management strategies for severe sepsis, with the aim to publish a complete set of guidelines in subsequent years. (Dellinger et al. 2008 Jan).

Chronic kidney disease (CKD) is a major public health problem affecting approximately 11.5% of US adults (Coresh et al. 2007). CKD is associated with poor outcomes including an increased risk of kidney failure, cardiovascular disease and mortality (Coresh et al. 2007, James et al. 2010, Go, et al. 2004, Warnock et al. 2010) These associations may result from the presence of traditional risk factors as well as biochemical abnormalities such as increased inflammatory factors, endothelial dysfunction and enhanced coagulation. (Go et al. 2004)

Microbial infection may result in bacteremia, triggering exaggerated inflammation and sepsis. (Dellinger et al. 2008) Infection, bacteremia and sepsis are major sources of morbidity and mortality in patients with end-stage renal disease receiving chronic hemodialysis therapy (Sarnak et al. 2000, Powe et al. 1999) For example, using the US Renal Data System, Powe et al. (Powe et al. 1999) found that during 7 years of follow-up, 11.7% of all hemodialysis patients and 9.4% of peritoneal dialysis patients had at least one episode of septicemia, and Sarnak and Jaber (Sarnak et al. 2000) found that the sepsis mortality was 100- to 300-times higher for chronic dialysis patients than the general public. Hypothesized reasons for this association include increased susceptibility to infection, the presence of comorbidities such as diabetes, and repetitive exposure to pathogens during hemodialysis.

In contrast to the approximately 500,000 dialysis patients, over 20 million US adults have CKD (Coresh et al. 2007, Kahn et al. 2006). An important unanswered question is whether the increased risk of infection and infection-associated mortality extends from dialysis patients to the
much larger population of patients with predialysis CKD. An association between earlier stages of CKD and infection or infection-associated mortality has important implications for clinical care, pointing to infection prevention and mitigation as important strategies for reducing mortality in this population.

Chronic kidney disease (CKD) is fast emerging as a major public health problem in the 21st century. According to the National Kidney Foundation Disease Outcomes Quality Initiative guidelines define CKD as kidney damage or a glomerular filtration rate of less than 60 mL/min per 1.73 m² for at least 3 months. Three intermediary stages follow, with kidney failure or end-stage renal disease (ESRD), as the final stage, defined by a glomerular filtration rate of less than 15 mL/min per 1.73 m².1. (National Kidney Foundation: K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Kidney Disease Outcome Quality Initiative. Am J Kidney Dis 39:S1-S246, 2002)

Many people within the large group of CKD patients are also affected by cardiovascular disease (CVD) as well as infectious complications. CVD is in fact twice as common in CKD patients as in the general population, and it advances at twice the rate. (Collins et al. 2003)

Although a great deal of attention has been paid to CVD, not enough has been said about infectious complications, which closely follow CVD in frequency and seriousness. The 2 complications appear to be closely linked; infection is an inflammatory state and as such may be implicated in the development of atherosclerotic disease, (Ross et al. 1999, Ishani et al. 2005) and the risk of the developing CVD is increased in the 6 months after an infection. These immunologic abnormalities are complicated by the use of immunosuppressive drugs to treat and control underlying diseases and exacerbated by nutritional deficiencies, the dialysis procedure, and the disruption of cutaneous and mucosal barriers to infection. (Kessler et al. 1993, Ann Pharmacother et al. 1998)
The infectious complications in the non-ESRD population will also be discussed and compared with the event rates for dialysis patients and the non-CKD population.

**INFECTIONOUS COMPLICATIONS**

The incidence of the commonly seen infectious complications is approximately 3 times greater among CKD patients who have not yet initiated dialysis than in the general population, with UTI, pneumonia, and sepsis in descending order of prevalence. Notably, raw death rates after infection follow a different pattern in the CKD and non-CKD patient groups, with sepsis, pneumonia, and UTI in descending order of prevalence for both. The higher UTI susceptibility in the CKD group may be explained, in part, by a greater incidence of urinary obstructions, which in turn leads to infections. (Ishani et al. 2005) commonly seen in those with benign prostatic hypertrophy, kidney stones and urinary tract cancers. (Appel et al. 2000) Patients with ESRD

The urinary tract, which may not be recognized as an important source of infection among dialysis patients because of their minimal urine output, (D’Agata et al. 2000) is responsible for the highest rates of hospitalization followed by pulmonary infections. Other potential sources of infection include the skin, the dialysis water treatment system, and dialyzer reuse, which can cause septicemia in a small minority of patients. Hemodialysis patients, during the normal course of treatment, are exposed to several infectious risks, and the majority of patients require at least 1 hospitalization every year for treatment of infections. (U.S. Renal Data System: USRDS 1997 Annual Data Report. Bethesda, Maryland, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 1997) the type of vascular access in use plays an important role in the subsequent development of bloodstream infections.

Central venous catheters significantly increase the risk of bacteremia in hemodialysis patients. (Powe et al. 1999); those with temporary catheters have been shown to have a 50% higher risk of septicemia than patients with a native fistula. Maximizing the use of
arteriovenous fistula as hemodialysis access is likely to lower infection risk. Old, nonfunctional, clotted prosthetic arteriovenous grafts have recently been recognized as a frequent cause of bacteremia and morbidity among hemodialysis patients. (Nassar et al. 2001)

Infected grafts should be surgically excised without delay and systemic antibiotics delivered. At present, there are no prospective data to address the question of routine excision of old grafts; a high-risk population should be identified and actively managed. Chronic hemodialysis has been recognized as a risk factor for the development of infective endocarditis (IE) since the early 1960s, (Doulton et al. 2003) and mortality rates for IE in the hemodialysis population are high. The study by Strom et al. (Strom et al. 2000) put the relative risk of IE in dialysis patients compared with the general population at 16.9. Prevention and early detection of this complication are imperative. Compared with hemodialysis patients, in whom the predominant morbidity is from cardiovascular complications, peritoneal dialysis patients are more often hospitalized for infections, with peritonitis secondary to catheter tunnel infections as the most common cause of morbidity. (U.S. Renal Data System: USRDS 2005 Annual Data Report. Bethesda, MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2005) Peritonitis usually results from decreased host phagocytic efficiency with depressed phagocytosis and bactericidal capacity of peritoneal macrophages. (Von Graevenitz et al. 1992)

During episodes of peritonitis, fluid movement is reversed, away from the lymphatics and peritoneal membrane and toward the cavity. As a result, bloodstream infections are rare, yet there is no mistaking the impact of these infections on peritoneal membrane function with the loss of ultrafiltration capacity and subsequent difficulties in fluid removal. Most peritonitis episodes are caused by gram-positive bacteria. (von Graevenitz et al. 1992) Staphylococcus aureus or epidermitis are common, and often associated with a catheter infection, frequently requiring catheter removal for resolution. S. aureus infections in peritoneal dialysis patients are especially common in nasal carriers, as the bacteria move
from the nasal reservoir to the hands and skin and from there to the access site.

Compared with hemodialysis and peritoneal dialysis patients, kidney transplant recipients are at the lowest risk for infection, although United States Renal Data System data indicate that the annual percentage of mortality secondary to sepsis is 20-fold higher in kidney transplant recipients than in the general population. (Sarnak et al. 2000).

**INFECTIONS IN CKD PATIENTS**


Compared with the non-CKD population, the rates of pneumonia are 3 times greater in the CKD population and 5 times greater in the dialysis population. Of particular interest, the length of hospital stays for pneumonia in the CKD and dialysis populations are very similar and 4 to 6 times longer than those in the non-CKD population. In fact, pneumonia as a complication in the CKD population appears to be more severe than previously appreciated. Bacteremia/sepsis patterns are also quite different when comparing the non-CKD, CKD, and dialysis populations. (U.S. Renal Data System: USRDS 2003 Annual Data Report. Bethesda MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2003)

Currently, only 56% of dialysis and transplant patients receive influenza vaccinations each year despite the Centers for Disease Control and Prevention (CDC) Healthy People 2010 target objective of 90%. Equally surprising are the low rates of influenza vaccinations in those
less than 65 years old. In light of a recent study by Gilbertson et al. showing significantly lower risk of infectious hospitalizations and infectious death in patients who receive influenza vaccinations, perhaps performance measures for providers should be implemented to address this gap in a potentially effective treatment. Rates of pneumococcal vaccination are also surprising low, at only 13.5% per year. (U.S. Renal Data System: USRDS 2005 Annual Data Report. Bethesda, MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2005).

Tracking these vaccinations is very difficult because no integrated set of information is transmitted to dialysis units from hospitals, nursing homes, or skilled nursing facilities, or from primary care physician offices. Pneumococcal vaccinations may be more important than previously considered. Recent reports indicate reduced rates of severe pneumococcal infections in the general population during a period of increased vaccinations of children with new pneumococcal vaccine, a so-called herd immunity. (Eickhoff et al. 1961)

Repeated pneumococcal vaccination may be nonproblematic; the CDC has not reported adverse effects of vaccinations given within 2 years, although the package insert labeling recommends revaccination every 5 years. (Zhou et al. 2003)

There is little doubt that the CKD and ESRD populations are at substantial risk for pneumonia, yet it appears little is being done to prevent this major source of morbidity and mortality with known vaccinations. Acute hepatitis B virus (HBV) infection in uremic patients on dialysis is generally mild or asymptomatic, but these patients have a higher incidence of chronic HBV infection compared with immunocompetent persons. (Szmuness et al. 1974, Szmuness et al. 1981).

Infection control measures and the use of hepatitis B vaccine have significantly reduced the annual incidence of HBV infection among patients on dialysis, from 3.0% to 0.05% between 1976 and 1997. (Tokars et al. 1998)
Preventive strategies to reduce the future incidence of IE in long-term dialysis patients should be used and include scrupulous attention to asepsis in line insertion and toileting, antibiotic locks in dialysis catheters. (Vercaigne et al. 2002)

Vaccination against *staphylococci* (though uremic patients characteristically respond poorly to this immunization), (Shinefield et al. 2002) and consideration of using antibiotic-impregnated dual-lumen tunneled catheters when available. (Polderman et al. 2002).

Despite improvements in infection-control practices and dialysis techniques, bacterial and viral infections are a major cause of morbidity and mortality among patients on longterm hemodialysis or peritoneal dialysis; many of these deaths may be vaccine preventable. (Kessler et al. 1993, Khan et al. 1993)

The Advisory Committee on Immunization Practices currently recommends a single 0.5 mL dose of the 23-valent pneumococcal polysaccharide vaccine administered intramuscularly or subcutaneously to all dialysis patients 2 years of age or older 22, 31 (Eickhoff et al. 1961, Advisory Committee on Immunization Practices: Prevention and control of influenza: Recommendations of the Advisory Committee on Immunization Practices (ACIP). MMWR Recomm Rep 48:1-28, 1999) and the influenza vaccine administered annually, before the beginning of the influenza season, for all dialysis patients 6 months of age or older. Topical mupirocin and *S aureus* conjugate vaccine use have shown promise as alternative prevention methods in those patients for whom catheter use is unavoidable. (Johnson et al. 2002).

**FOLEY CATHETER**

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The name comes from the designer, Frederic Foley, a surgeon working in Boston, Massachusetts in the 1930s. (Foley et al. 1937). His original design was adopted by C. R. Bard, Inc. of Murray Hill, New
Jersey, who manufactured the first prototypes and named them in honor of the surgeon.

Foley catheter (F/Ch. 24) balloon blocked and outlet plug put on Foley catheters come in several sub-types: "Coudé" (French for elbowed) catheters have a 45° bend at the tip to allow easier passage through an enlarged prostate. "Councill tip" catheters have a small hole at the tip which allows them to be passed over a wire. "Three way" or "triple lumen" catheters have a third channel, which is used to infuse sterile saline or another irrigating solution. These are used primarily after surgery on the bladder or prostate, to wash away blood and blood clots.

A Foley catheter can also be used to ripen the cervix during induction of labor. When used for this purpose, the procedure is called extra-amniotic saline infusion (EASI). (Guinn et al. 2004) In this procedure, the balloon is inserted behind the cervical wall and inflated, such for example with 30 mL per hour. (Guinn et al. 2004) The remaining length of the catheter is pulled slightly taut, and taped to the inside of the woman's leg. The inflated balloon applies pressure to the cervix, as the baby's head would prior to labour, causing it to dilate. As the cervix dilates over time, the catheter is readjusted to again be slightly taut, and re-taped to maintain pressure on the cervix. When the cervix has dilated sufficiently, the catheter simply drops out

**ACUTE PERITONEAL DIALYSIS**

The best placement of the catheter within the abdominal wall was thoroughly studied by Tenckhoff, who included himself among the study subjects. The dacron cuffs are placed within the rectus muscle and in the subcutaneous tissue of the anterior abdominal wall. The cuff induces a classic inflammatory reaction characterized by formation of fibrin clots, ingrowth of granulocytes and fibroblasts and granulomata with giant cells. The resulting fibrous plug represents a curious case of beneficial bioincompatibility. It prevents bacteria from entering the subcutaneous space from the skin surface into the peritoneum. At the skin exit site, stratified squamous epithelium grows along the surface of the catheter
ending in granulation tissue near the pre-peritoneal cuff. At the peritoneal surface, simple squamous epithelium grows around the catheter, penetrating the abdominal wall and ending at the deep cuff, resulting in a smooth surface surrounding the catheter (Ash et al. 2005).

**OPTIMAL CATHETER PLACEMENT**

The midline penetration through the linea alba was commonly used in the past due to fewer vascular structures; however, it is now mostly reserved for acute catheters and as a last resource only. The preferred primary penetration sites for chronic catheters are the lateral or paramedian insertions through the rectus muscle since they are associated with better outcomes and a lower rate of complications (Helfrich et al. 1983, Wikdahl et al. 1988).

The exit site placement is critical for good outcomes. An arcuate subcutaneous tunnel and a downward exit site for the catheter was first proposed by Tenckhoff in 1968 to maintain the internal and external portions of the catheter in a caudad direction and to foster drainage from the subcutaneous tunnel and prevent infection (Tenckhoff et al. 1968). The advantages of this practice in reducing the number and severity of infections has been reported by several authors (Twardowski et al. 1985, Favazza et al. 1995). The exit site should precisely fit the catheter diameter in order to avoid extrusion of the external cuff if it is too large, or pressure necrosis if too tight. This can be easily accomplished using a commercially available perforator or by a single pass with a Parker blade # 11. The subcutaneous cuff should be 1-2 cm from the skin after healing.

**METHODS OF IMPLANTATION**

The principal methods are generally classified as surgical and percutaneous. The selection is ultimately the responsibility of the operator and is often influenced by their experience and training. The success rate mostly depends on the operator's proficiency rather than the technique.
Percutaneous placement. The earliest clinically successful percutaneous placement required the use of a large bore trochar developed by Tenckhoff.

This method is simple and can be performed at the bedside under local anesthesia. The disadvantages with this method relate to the diameter of the penetration site and that it is a blind procedure.

The percutaneous implantation has been further simplified with a modified Selding technique similar to that used for insertion of vascular catheters (Favazza et al. 1953). Although this is a blind procedure, the risk of perforation is relatively low since it uses a simple needle for penetration followed by dilatation with a blunt plastic dilator. The location of the catheter can be further improved with the use of imaging assistance, if available.

**SELDINGER TECHNIQUE DISPOSABLES**

Peritoneoscopic implantation has become increasingly popular and offers good visualization of peritoneal structures and allows early diagnosis to impediments to good function and of complications.

Laparoscopic/mini-laparoscopic implantation with or without omentopexy (Ogunc et al. 2005) is a minimally invasive approach requiring smaller incisions and usually associated with less pain and quicker return to full activities, especially in obese patients. It offers complete visualization of the catheter insertion process and permits selective proactive intervention for catheter migration, omental entrapment and obstructive adhesions. It also allows the diagnosis and treatment of previously unsuspected herniae.

**ALTERNATIVE PLACEMENT TECHNIQUES**

Moncrief-Popovich catheter and technique. Subcutaneous burial of the external segment of the catheter to prevent colonization of the catheter by skin bacteria and promote attachment of the cuff to the tissue prior to exteriorization has been described and recently tested by several experienced clinicians with encouraging results (Moncrieff et al. 1993). The initial reports by the developers claimed a reduction in the rate of peritonitis and colonization of bacterial biofilms in the catheter
segments between the two cuffs (Moncrief et al. 1993). However, a controlled randomized study failed to confirm these claims (Danielsson et al. 2002). A possible reason for the failure to reduce the incidence of infectious complications may be the inability of the body to provide an effective “seal” around the external cuff. Therefore, upon exteriorization of the catheter, the process of healing starts all over again. Prischl et al. have also reported a high incidence of seromas, subcutaneous hematomas and fibrin thrombi postoperatively with this technique (Prischl et al. 1997).

Extended Dialysis Catheters. Extended dialysis catheters have been developed to allow placement of the exit site in remote places and preferably in the presternal area (Crabtree et al. 2004). This is particularly useful for obese patients, the rare patient with a stoma and those with other sources of potential contamination in the anterior abdominal wall. The commercially available kits contain catheters of various configurations, a presternal extension tube and a titanium connector to join the aforementioned parts.

A six year non-randomized prospective study comparing swan-neck presternal catheters with swan-neck abdominal catheters showed good tolerance of the presternal catheters but not significant differences in survival or peritonitis rates (Twardowski et al. 1988). Similarly, a retrospective comparison by a single surgeon comparing a standard, double-cuff Tenckhoff catheter (n=46) or a swan-neck presternal catheter (n=14) reported a lower, but no significant difference in the rate of exit site infections (Warchol et al. 2003).

Self-Locating or Front Loading Catheters. Several European reports describing a PD catheter with either a 12 g tungsten cylinder embedded in the silicone or stainless steel weights attached to the distal end of the catheter suggest better catheter survival and less migration as compared to standard catheters (Di Paolo et al. 1996, Cavagna et al. 1999, Dantoine et al. 2002, Minguela et al. 2001). Although the data from clinical trials are limited, the results are consistently positive. Further evaluation of this simple modification to the peritoneal catheter is warranted.
While the implantation technique may offer some benefits under specific circumstances, there is no evidence that insertion technique per se or time of first use influence clinical outcomes.

**PERITONEAL DIALYSIS**

Peritoneal dialysis (PD) is a treatment for patients with severe chronic kidney disease. The process uses the patient's peritoneum in the abdomen as a membrane across which fluids and dissolved substances (electrolytes, urea, glucose, albumin and other small molecules) are exchanged from the blood. Fluid is introduced through a permanent tube in the abdomen and flushed out either every night while the patient sleeps (automatic peritoneal dialysis) or via regular exchanges throughout the day (continuous ambulatory peritoneal dialysis). PD is used as an alternative to hemodialysis though it is far less commonly used in many countries, such as the United States. It has comparable risks but is significantly less costly in most parts of the world, with the primary advantage being the ability to undertake treatment without visiting a medical facility. The primary complication of PD is infection due to the presence of a permanent tube in the abdomen.

**BEST PRACTICES**

The patient should receive ongoing monitoring to ensure adequate dialysis, and be regularly assessed for complications. Finally, the patient should be educated on the importance of infection control and an appropriate medical regimen established with their cooperation. (Wood *et al.* 2008)

The abdomen is cleaned in preparation for surgery, and a catheter is surgically inserted with one end in the abdomen and the other protruding from the skin (Haralampos, Harissis *et al.* 2006). Before each infusion the area must be cleaned, and flow into and out of the abdomen tested. A large volume of fluid is introduced to the abdomen over the next ten to fifteen minutes. (Lippincott Williams & Wilkins *et al.* 2007) The total volume is referred to as a dwell (Crowley *et al.* 2009) while the fluid itself is referred to as dialysate. The dwell can be as much as 2.5 litres, and medication can also be added to the fluid immediately before
infusion. (Lippincott Williams & Wilkins et al. 2007) The dwell remains in the abdomen and waste products diffuse across the peritoneum from the underlying blood vessels. After a variable period of time depending on the treatment (usually 4–6 hours), the fluid is removed and replaced with fresh fluid. This can occur automatically while the patient is sleeping (automated peritoneal dialysis, APD), or during the day by keeping two litres of fluid in the abdomen at all times, exchanging the fluids four to six times per day (continuous ambulatory peritoneal dialysis, CAPD). (Crowley et al. 2009, McPhee et al. 2007).

The fluid used typically contains sodium, chloride, lactate or bicarbonate and a high percentage of glucose to ensure hyperosmolarity. The amount of dialysis that occurs depends on the volume of the dwell, the regularity of the exchange and the concentration of the fluid. APD cycles between 3 and 10 dwells per night, while CAPD involves four dwells per day of 2-2.5 litres per dwell, with each remaining in the abdomen for 4–8 hours. The viscera accounts for roughly four-fifths of the total surface area of the membrane, but the parietal peritoneum is the more important of the two portions for PD.

The ability to exchange fluids between the peritoneum and blood supply can be classified as high, low or intermediate. High transporters tend to diffuse substances well (easily exchanging small molecules between blood and the dialysis fluid, with somewhat improved results frequent, short-duration dwells such as with APD) while low transporters filter fluids better (transporting fluids across the membrane into the blood more quickly with somewhat better results with long-term, high-volume dwells such) though in practice either type of transporter can generally be managed through the appropriate use of either APD or CAPD. (Daugirdas et al. 2006).

Though there are several different shapes and sizes of catheters that can be used, different insertion sites, number of cuffs in the catheter and immobilization, there is no evidence to show any advantages in terms of morbidity, mortality or number of infections, though the quality
of information is not yet sufficient to allow for firm conclusions. (Strippoli et al. 2004).

**COMPLICATIONS**

The volume of dialysate removed and weight of the patient are normally monitored; if more than 500ml of fluid are retained or a liter of fluid is lost across three consecutive treatments, the patient’s physician is generally notified. Excessive loss of fluid can result in hypovolemic shock or hypotension while excessive fluid retention can result in hypertension and edema. Also monitored is the color of the fluid removed: normally it is pink-tinged for the initial four cycles and clear or pale yellow afterwards. The dwell can also increase pressure on the diaphragm causing impaired breathing, and constipation can interfere with the ability of fluid to flow through the catheter. (Lippincott Williams & Wilkins et al. 2007).

A potentially fatal complication estimated to occur in roughly 2.5% of patients is encapsulating peritoneal sclerosis, in which the bowels become obstructed due to the growth of a thick layer of fibrin within the peritoneum. (Kawanishi et al. 2007).

The fluid used for dialysis uses glucose as a primary osmotic agent, but this may lead to peritonitis, the decline of kidney and peritoneal membrane function and other negative health outcomes. The acidity, high concentration and presence of lactate and products of the degradation of glucose in the solution (particularly the latter) may contribute to these health issues. Solutions that are neutral use bicarbonate instead of lactate and have few glucose degradation products may offer more health benefits though this has not yet been studied. (Perl et al. 2011).

**RISKS AND BENEFITS**

PD is less efficient at removing wastes from the body than hemodialysis, and the presence of the tube presents a risk of peritonitis due to the potential to introduce bacteria to the abdomen; (Crowley et al. 2009) peritonitis is best treated through the direct infusion of antibiotics into the peritoneum with no advantage for other frequently used
treatments such as routine peritoneal lavage or use of urokinase. (Wiggins, et al. 2008). The tube site can also become infected; the use of prophylactic nasal mupirocin can reduce the number of tube site infections, but does not help with peritonitis. (Strippoli et al. 2004). Infections can be as frequent as once every 15 months (0.8 episodes per patient year). Compared to hemodialysis, PD allows greater patient mobility, produces fewer swings in symptoms due to its continuous nature, and phosphate compounds are better removed, but large amounts of albumin are removed which requires constant monitoring of nutritional status. The costs and benefits of hemodialysis and PD are roughly the same - PD equipment is cheaper but the costs associated with peritonitis are higher. (McPhee et al. 2007). There is insufficient research to adequately compare the risks and benefits between CAPD and APD; a Cochrane Review of three small clinical trials found no difference in clinically important outcomes (i.e. morbidity or mortality) for patients with end stage renal disease, nor was there any advantage in preserving the functionality of the kidneys. The results suggested APD may have psychosocial advantages for younger patients and those who are employed or pursuing an education. (Rabindranath et al. 2007).

Hypertriglyceridemia and obesity are also concerns due to the large volume of glucose in the fluid, which can add as many as 1200 calories to the diet per day. (Ehrman et al. 2008). Of the three types of connection and fluid exchange systems (standard, twin-bag and y-set; the latter two involving two bags and only one connection to the catheter, the y-set uses a single y-shaped connection between the bags involving emptying, flushing out then filling the peritoneum through the same connection) the twin-bag and y-set systems were found superior to conventional systems at preventing peritonitis. (Daly et al. 2005).

**FREQUENCY**

In a 2004 worldwide survey of patients in end stage renal disease, approximately 11% were receiving PD, compared to the much more common hemodialysis. In the United Kingdom, South Korea and Mexico
PD was more common than the world average, with Mexico conducting most of its dialysis (75%) through PD, while Japan and Germany had rates lower than the world average. (Grassmann et al. 2005).

Peritoneal dialysis can be improvised in conditions such as combat surgery or disaster relief using surgical catheters and dialysate made from routinely available medical solutions to provide temporary renal replacement for patients with no other options. (Pina et al. 2010).

**INFECTIOUS COMPLICATIONS**

Catheter infections occur by means of one of three mechanisms: local insertion site infection, which travels down the catheter externally; or hub colonization followed by infection via the intralumenal route or via hematogenous seeding of the catheter.

The Institute for Healthcare Improvement recommends five steps to reduce central-line infections: hand hygiene, adherence to maximal barrier precautions, chlorhexidine skin antisepsis, selection of an optimal catheter site, and daily review of the necessity of the catheter, with prompt removal when the catheter is no longer needed. Implementation of these steps has been conclusively shown to decrease the rate of catheter-related bloodstream infection. Scheduled changing of a catheter over a guide wire or moving a catheter to a new site can increase mechanical and infectious complications, and neither is recommended. Antiseptic containing hubs and antimicrobial-impregnated catheters have been shown to decrease the rate of catheter-related bloodstream infections. Topical antibiotic ointments are ineffective, promote antibiotic-resistant bacteria, and increase fungal colonization.

**MECHANICAL COMPLICATIONS**

Mechanical complications include arterial puncture, hematoma, pneumothorax, hemothorax, arrhythmia, and improper location of the catheter, whether in an accessory vein or in the other vessels of the upper vascular system. Insertion of a catheter into the femoral vein, not shown in this video, has the highest risk of mechanical complications, but the rates of serious mechanical complications for femoral and subclavian insertion are similar. If an artery is punctured, further
attempts at that site should be abandoned, and access to an alternative site should be attempted. Internal jugular and subclavian cannulation sites are preferred because of their lower overall rate of mechanical complications. However, these sites carry a small risk of hemothorax and pneumothorax. Ultrasound guidance for internal jugular cannulation significantly reduces the number of attempts required and the risk of complications.

THROMBOTIC COMPLICATIONS

Central venous cannulation increases the risk of central venous thrombosis, with the concomitant potential risk of venous thromboembolism. Thrombosis may occur as early as the first day after cannulation. The site with the lowest risk for thrombotic complications is the subclavian vein. Prompt removal of the catheter when it is no longer needed decreases the risk of catheter-related thrombosis.

TECHNIQUE

Place the guide wire, dilator, catheter, and scalpel on the sterile drape for easy reach when needed. Have the patient turn head in the opposite direction

Using the 18 ga finder needle (largest needle in the kit) and a small syringe, enter the skin at the top of the jugular triangle. In obese patients where the landmarks are not discernable, a reasonable rule of thumb is to go three finger breadths lateral from the tracheal midline, and three finger breadths up from the clavicle.

1. Alternatively use ultrasound guidance with the portable ultrasound in a sterile sleeve to localize the vein and follow your needle into its lumen.

2. Ultrasound Guidance: In numerous studies, ultrasound guidance has been shown to increase the success of first-time catheter placement and to decrease the risk of complications. When using ultrasound guidance, enlist an assistant either to handle the probe or to remove it when it is no longer needed. The vein and artery appear circular and black on the ultrasound image; the vein is much more compressible when gentle pressure is applied to the
skin via the probe. The needle appears echogenic and can be followed into the image of the vein.
3. Palpate for the carotid impulse a make sure you are lateral to this.
4. Insert the needle at 30 degrees and aim for the ipsilateral nipple.
5. Gradually advance the needle, always gently pulling back on the plunger as you progress; a flash and easy withdrawal of dark blood, this indicates entrance into the vein.
6. If you bury the needle without blood, gradually withdraw; you may still get into the vein as you may have collapsed it on the way in.
7. Once in the vessel, steady the needle and remove the syringe, holding a thumb over it to prevent air embolism.
8. Insert j-tipped guide wire into needle; if resistance is felt do not force it.
9. Watch monitor as guide wire is advanced. Ventricular ectopy indicates placement in RV, and guide wire should be pulled back a few cm.
10. Holding guide wire, remove needle from skin.
11. Make a small nick with the number 11 blade where wire enters skin.
12. Advance dilator over guide wire with a twisting motion; there will be resistance.
13. Remove dilator, holding guide wire and having some gauze 4x4 in your hand to apply pressure to a site that will now bleed after dilation.
14. Place catheter over guide wire; it should advance easily. Hold guide wire at skin entrance and feed it back through distal port of central line (brown cap). When wire comes out, grab it at the end and finish advancing catheter.
15. Remove guide wire and flush line through all 3 ports.
16. Suture catheter in place via flange with holes. If more than a cm or 2 of catheter is exposed due to length, either suture the catheter down or use the snap-on flange provided in the kit.
17. Order a stat CXR to evaluate for line placement and complication. The tip of the catheter should be at the junction of the SVC and right atrium on chest xray. New data would suggest that this is 2cm below the superior right cardiac silhouette which is made up by the right atrial appendage. (Verhey, Gosselin, Primack, Blackburn and Kraemer et al. 2008). The Right Mediastinal Border and Central Venous Anatomy of Frontal Chest Radiograph - Direct CT Correlation. Journal of the Association of Vascular Access. 13(1), p.32.) Advancement beyond this point can lead to arrythmia and unlikely myocardial perforation.

**CHEST X-RAY WITH CATHETER IN THE RIGHT SUBCLAVIAN VEIN**

The skin is cleaned, and local anesthetic applied if required. The location of the vein is then identified by landmarks or with the use of a small ultrasound device. A hollow needle is advanced through the skin until blood is aspirated; the color of the blood and the rate of its flow help distinguish it from arterial blood (suggesting that an artery has been accidentally punctured), although this method is inaccurate. Ultrasound probably now represents the gold standard for central venous access and skills, within North American and Europe, with landmark techniques are diminishing. (O’Leary et al. 2011, Bodenham et al. 2011).

Videos are available demonstrating placement of a central venous catheter without and with ultrasound guidance.

**COMPLICATIONS**

Central line insertion may cause a number of complications. The benefit expected from their use therefore needs to outweigh the risk of those complications.

**PNEUMOTHORAX**

Pneumothorax (for central lines placed in the chest); the incidence is thought to be higher with subclavian vein catheterization. In catheterization of the internal jugular vein, the risk of pneumothorax can be minimized by the use of ultrasound guidance. For experienced clinicians, the incidence of pneumothorax is about 1.5-3.1%. Some official bodies, e.g. the National Institute for Health and Clinical
Excellence (UK), recommend the routine use of ultrasonography to minimize complications.

**CENTRAL-LINE ASSOCIATED BLOODSTREAM INFECTIONS (CLABSIs)**

All catheters can introduce bacteria into the bloodstream, but CVCs are known for occasionally causing *Staphylococcus aureus* and *Staphylococcus epidermidis* sepsis. The problem of central line-associated bloodstream infections (CLABSI) has gained increasing attention in recent years. They cause a great deal of morbidity and deaths, and increase health care costs. Historically, a small number of CVC infections were considered an acceptable risk of placing central lines. However, the seminal work by Dr. Peter Pronovost at Johns Hopkins Hospital turned that perspective on its head. Additionally, the Institute for Healthcare Improvement (IHI) has done a tremendous amount of work in improving hospitals’ focus on central line-associated bloodstream infections (CLABSI), and is working to decrease the incidence of this particular complication among US hospitals.

The National Patient Safety Goals (NPSGs) and specifically NSPG 7.04 address how to decrease infections. The NSPG 7.04 has 13 elements of performance to decrease CLABSIs.

**Disinfection of intravenous access ports before use**

National Patient Safety Goals require documentation of a checklist for CVC insertion and Disinfection of intravenous (IV) access ports before use (scrub the hub). Some literature has suggested the use of a safer vascular access route - such as intraosseous (IO) vascular access - when central lines are not absolutely necessary (such as when central lines are being placed solely for vascular access). Infection risks were initially thought to be less in jugular lines, but this only seems to be the case if the patient is obese. (Parienti et al. 2008).

If a patient with a central line develops signs of infection, blood cultures are taken from both the catheter and from a vein elsewhere in the body. If the culture from the central line grows bacteria much earlier (>2 hours) than the other site, the line is the likely source of the infection.
Quantitative blood culture is even more accurate, but this is not widely available. (Safdar et al. 2005).

Generally, antibiotics are used, and occasionally the catheter will have to be removed. In the case of bacteremia from *Staphylococcus aureus*, removing the catheter without administering antibiotics is not adequate as 38% of such patients may still develop endocarditis. (Watanakunakorn et al. 1977).

In a clinical practice guideline, the American Centers for Disease Control and Prevention recommends against routine culturing of central venous lines upon their removal. (O’Grady et al. 2002). The guideline makes a number of further recommendations to prevent line infections. (O’Grady et al. 2002).

To prevent infection, stringent cleaning of the catheter insertion site is advised. Povidone-iodine solution is often used for such cleaning, but chlorhexidine appears to be twice as effective as iodine. (Mimoz et al. 2007). Routine replacement of lines makes no difference in preventing infection. (Cobb et al. 1992).

**THROMBOSIS**

CVCs are a risk factor for forming venous thrombosisl (Rosendaal et al. 2009). Including upper extremity deep vein thrombosis. (Lee et al. 2012).

**OTHER COMPLICATIONS**

Rarely, small amounts of air are sucked into the vein as a result of the negative Intra-thoracic pressure and insertion technique. Valved insertion devices can reduce this risk. If these air bubbles obstruct blood vessels, this is known as an air embolism.

Hemorrhage (bleeding) and formation of a hematoma (bruise) is slightly more common in jugular venous lines than in others. (Parienti et al. 2008).

Arrhythmias may occur during the insertion process when the wire comes in contact with the endocardium. It typically resolved when the wire is pulled back.
Foley catheters

Foley catheters cause a variety of harms, including infection, pain and trauma. Although symptomatic urinary tract infection and asymptomatic bacteriuria are frequently discussed, genitourinary trauma receives comparatively little attention: During 6,513 surveyed Foley catheter days, urinalysis/urine culture was done on 407 (6.3%) days. This testing identified 116 possible urinary tract infection episodes (1.8% of Foley catheter days), of which only 21 (18%) involved clinical manifestations. However, the remaining 95 asymptomatic bacteriuria episodes accounted for 39 (70%) of 56 antimicrobial treated possible urinary tract infection episodes (for proportion of treated episodes with vs. without symptomatic urinary tract infection manifestations, p = 0.005). Concurrently 100 instances of catheter associated genitourinary trauma (1.5% of Foley catheter days) were recorded, of which 32 (32%) led to interventions such as prolonged catheterization or cystoscopy. Trauma prompting an intervention accounted for as great a proportion of Elimination of unnecessary Foley catheter use could prevent symptomatic urinary tract infection, unnecessary antimicrobial therapy for asymptomatic bacteriuria and Foley catheter related trauma. (Leuck et al. 2012)

As the duration of catheterisation is the principal determinant of infection with long-term indwelling catheters, it is not clear that any interventions can decrease the prevalence of bacteriuria in this setting. Catheter flushing or daily perineal care do not prevent infection and may, in fact, increase the risk of infection. Complications of infection may be prevented by giving antibacterials for bacteriuria immediately prior to any invasive urological procedure, and by avoiding catheter blockage, twisting or trauma. The major focus of future advances in prevention of catheter-acquired UTI is the development of biomaterials resistant to biofilm formation. There is substantial current research addressing this issue, but current catheter materials all remain susceptible to biofilm formation. (Nicolle et al. 2005)
There was not enough evidence to suggest whether or not any standard catheter was better than another in terms of reducing the risk of urinary tract infection in hospitalized adults catheterized short-term. Siliconized catheters may be less likely to cause urethral side effects in men; however, this result should be interpreted with some caution as the trials were small and the outcome definitions and specific catheters compared varied. (Schumm et al. 2008)

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. This article will discuss the indications for the IUC, the complications that can occur because of the IUC, and comment on the Kaiser Permanente Southern California Region’s efforts to minimize the unnecessary use of the IUC. Thoughtful and judicious use of the IUC, such as minimizing the use of urinary catheterization, either by not inserting an IUC or by removing it as soon as it is no longer needed, will most likely reduce inpatient morbidity and improve the health of the hospitalized older adult. (Lee, Malatt et al. 2011)

Numerous strategies have been developed to reduce the incidence of CAUTI but few have proven effective. Reducing the inappropriate use of catheters and development of novel technologies targeted against these increasingly multidrug-resistant pathogens may be useful in the prevention of CAUTI in our vulnerable patients.

The use of antimicrobial catheters also may be considered when the rates of CAUTI remain persistently high despite adherence to other evidence-based practices, or in patients deemed to be at high risk for CAUTI or its complications. Attention toward prevention of CAUTI will likely increase as Center for Medicare and Medicaid Services and other
third-party payers no longer reimburse for hospital-acquired UTI. (Chenoweth, Saint et al. 2011)

Catheter-associated urinary tract infection is the most common nosocomial infection, with hospitalized patients having a risk of 5% per day an indwelling catheter is in place. Use of catheters coated with silver alloy-hydrogel significantly reduces the risk of catheter-associated urinary tract infection and the burden on the NHS. (Hameed, Chinegwundoh, Thwaini et al. 2010)

Restricting catheterization to those who clinically require this invasive procedure can reduce the number of people who are exposed to the hazards of catheterization. The use of silver-coated catheters can reduce the risk of infection and encrustation. Ensuring that practice is evidence-based further reduces the risks of catheterization. (Nazarko et al 2007)

The intervention significantly decreased the duration of catheterization in two out of five departments. The frequency of late CAUTI (LCAUTI) among catheterized patients in all five departments decreased from 10.6 to 1.1 per 100 patients (P = 0.003) and the incidence of LCAUTI decreased from 12.3 to 1.8 per 1000 catheter-days (P = 0.03). Logistic regression analysis showed that duration of catheterization and iterative catheter changes were associated with LCAUTI. This study demonstrates that a simple measure can reduce the frequency of LCAUTI. (Crouzet et al. 2007)

ORGANISM IN CATHETER IN CKD

Infection is the most common cause of morbidity and the second most common cause of death in hemodialysis (HD) patients (DESchaubel et al. 1981-1997) Bacteremia accounts for more than 75% of these infectious deaths (U.S. Renal Data System: 2001). Hemodialysis vascular access is implicated as the source of bacteremias in 48 to 73% (Bloembergen et al. 1996, Kessler, et al.1993, Marr et al.1998 ) of cases with patients dependent on central venous catheters (CVC) being at highest risk (Powe et al.1999, Marr et al. 1998, Hoen et al.1998) Currently, approximately 20% of patients are dialyzed using permanent
catheters; both their placement rate and length of use has increased (DESchaubel et al. 1981-1997, U.S. Renal Data System: 2001). *Staphylococcus aureus* has previously been the primary etiologic agent implicated in causing approximately half of the bacteremic episodes (Marr et al. 1998, 10) and 70% of the vascular access site infections (11–12). However, recent studies have reported a greater percentage and variety of Gram-negative bacteria isolated in catheter-related infections (13–16). Catheter infection can occur following transmission of hand or aerosolized bacterial contaminants.

**MICRO ORGANISM MOLECULAR STUDY**

*Staphylococcus aureus* is a bacterium that is a member of the Firmicutes, and is frequently found in the human respiratory tract and on the skin. Although S. *aureus* is not always pathogenic, it is a common cause of skin infections (e.g. boils), respiratory disease (e.g. sinusitis), and food poisoning. Disease-associated strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant forms of pathogenic *S. aureus* (e.g. MRSA) is a worldwide problem in clinical medicine.

*Staphylococcus* was first identified in Aberdeen, Scotland (1880) by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint. (Ogston et al. 1984) This name was later appended to *Staphylococcus aureus* by Rosenbach who was credited by the official system of nomenclature at the time. It is estimated that 20% of the human population are long-term carriers of *S. aureus* (Kluytmans et al. 1997) which can be found as part of the normal skin flora and in anterior nares of the nasal passages. (Kluytmans et al. 1997, Cole et al. 2001) *S. aureus* is the most common species of *Staphylococcus* to cause Staph infections and is a successful pathogen due to a combination of nasal carriage and bacterial immuno-evasive strategies. (Kluytmans et al. 1997, Cole et al. 2001) *S. aureus* can cause a range of illnesses, from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-
threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence ranges from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections and is often the cause of postsurgical wound infections. Each year, some 500,000 patients in American hospitals contract a staphylococcal infection. (Bowersox et al. 1999)

**MICROBIOLOGY**

Gram stain of *S. aureus* cells which typically occur in clusters. The cell wall readily absorbs the crystal violet stain.

Yellow colonies of *S. aureus* on a blood agar plate, note regions of clearing around colonies caused by lysis of red cells in the agar (beta hemolysis).

In medical literature the bacteria is often referred to as *S. aureus* or *Staph aureus*. *Staphylococcus* should not be confused with the similarly named and medically relevant genus *Streptococcus*. *S. aureus* appears as grape-like clusters when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. (Ryan et al. 2004) *S. aureus* reproduces asexually by binary fission. The two daughter cells do not fully separate and remain attached to one another. This is why the cells are observed in clusters.

*S. aureus* is catalase-positive (meaning it can produce the enzyme catalase), so is able to convert hydrogen peroxide ($\text{H}_2\text{O}_2$) to water and oxygen. This test is sometimes used to distinguish *staphylococci* from *enterococci* and *streptococci*. Previously *S. aureus* was differentiated from other *staphylococci* by the coagulase test. However it is now known that not all *S. aureus* are coagulase positive (Ryan et al. 2004) and that incorrect species identification can impact effective treatment and control measures. (Matthews et al. 1997)

SEM micrograph of *methicillin-resistant Staphylococcus aureus*. *S. aureus* is responsible for many infections but it may also occur as a commensal. The presence of *S. aureus* does not always indicate infection.
S. aureus can survive from hours to weeks, or even months, on dry environmental surfaces, depending on strain.

S. aureus can infect tissues when the skin or mucosal barriers have been breached. This can lead to many different types of infections including furuncles and carbuncles (a collection of furuncles). In infants, S. aureus infection can cause a severe disease - staphylococcal scalded skin syndrome (SSSS). (Curran et al. 1980)

S. aureus can survive on dogs, cats, and horses, [citation needed] and can cause bumblefoot in chickens [citation needed]. S. aureus is one of the causal agents of mastitis in dairy cows. Its large polysaccharide capsule protects the organism from recognition by the cow’s immune defenses. (Goga et al. 2003)

**IMMUNOEVASIVE STRATEGIES**

Protein A

Protein A is anchored to staphylococcal peptidoglycan pentaglycine bridges (chains of five glycine residues) by the transpeptidase sortase A. (Schneewind et al. 1995) Protein A, an IgG-binding protein, binds to the Fc region of an antibody. In fact, studies involving mutation of genes coding for protein A resulted in a lowered virulence of S. aureus as measured by survival in blood, which has led to speculation that protein A-contributed virulence requires binding of antibody Fc regions. (Patel et al. 1987).

Protein A in various recombinant forms has been used for decades to bind and purify a wide range of antibodies by immunoaffinity chromatography. Transpeptidases, such as the sortases responsible for anchoring factors like Protein A to the staphylococcal peptidoglycan, are being studied in hopes of developing new antibiotics to target MRSA infections. (Zhu et al. 2008).

**STAPHYLOCOCCAL PIGMENTS**

Some strains of S. aureus are capable of producing staphyloxanthin - a golden coloured carotenoid pigment. This pigment acts as a virulence factor, primarily by being a bacterial antioxidant which helps the microbe evade the reactive oxygen species which the host
immune system uses to kill pathogens. (Clauditz et al. 2006, Liu et al. 2005)

These tests suggest the *Staphylococcus* strains use staphyloxanthin as a defence against the normal human immune system. Drugs designed to inhibit the production of staphyloxanthin may weaken the bacterium and renew its susceptibility to antibiotics. (Liu et al. 2005) In fact, because of similarities in the pathways for biosynthesis of staphyloxanthin and human cholesterol, a drug developed in the context of cholesterol-lowering therapy was shown to block *S. aureus* pigmentation and disease progression in a mouse infection model. (Liu et al. 2008)

**CATHETER-RELATED BACTEREMIA**

Several prospective studies have identified *S. aureus* as one of the leading causes of catheter-related bacteremia. More than 30% of *S. aureus* bacteremia is attributable to an infected intravascular device. It is a particular problem in certain clinical settings where central lines are frequently used such as haemodialysis adult and neonatal intensive care areas, oncology (and coronary care units) Cure rates without removal of the intravascular device are generally below 20%. Risk factors for hematogenous complications include symptom duration, hemodialysis dependence, presence of a long-term catheter or non-catheter device, failure to remove the catheter and infection with *MRSA*.

**ENDOCARDITIS**

**Rapid diagnosis and typing**

Diagnostic microbiology laboratories and reference laboratories are key for identifying outbreaks and new strains of *S. aureus*. Recent genetic advances have enabled reliable and rapid techniques for the identification and characterization of clinical isolates of *S. aureus* in real time. These tools support infection control strategies to limit bacterial spread and ensure the appropriate use of antibiotics. Real-time PCR is being increasingly employed in clinical laboratories as a technique to identifying outbreaks (Francois et al. 2008, Mackay et al. 2007).
TREATMENT AND ANTIBIOTIC RESISTANCE

The treatment of choice for *S. aureus* infection is penicillin; in most countries, though, penicillin resistance is extremely common, and first-line therapy is most commonly a penicillinase-resistant β-lactam antibiotic (for example, oxacillin or flucloxacillin). Combination therapy with gentamicin may be used to treat serious infections, such as endocarditis, (Korzeniowski *et al.* 1982, Bayer et. al 1998) but its use is controversial because of the high risk of damage to the kidneys. (Cosgrove *et al.* 2009). The duration of treatment depends on the site of infection and on severity.

Antibiotic resistance in *S. aureus* was uncommon when penicillin was first introduced in 1943. Indeed, the original petri dish on which Alexander Fleming of Imperial College London observed the antibacterial activity of the Penicillium fungus was growing a culture of *S. aureus*. By 1950, 40% of hospital *S. aureus* isolates were penicillin-resistant; and, by 1960, this had risen to 80%. (Chambers *et al.* 2001).

Aminoglycoside antibiotics, such as kanamycin, gentamicin, streptomycin, etc., were once effective against *staphylococcal* infections until strains evolved mechanisms to inhibit the aminoglycosides' action, which occurs via protonated amine and/or hydroxyl interactions with the ribosomal RNA of the bacterial 30S ribosomal subunit (Carter *et al.* 2000).

Aminoglycoside-modifying enzymes inactivate the aminoglycoside by covalently attaching either a phosphate, nucleotide, or acetyl moiety to either the amine or the alcohol key functional group (or both groups) of the antibiotic. This changes the charge or sterically hinders the antibiotic, decreasing its ribosomal binding affinity. In *S. aureus*, the best-characterized aminoglycoside-modifying enzyme is aminoglycoside adenylyltransferase 4' IA (ANT (4') IA). This enzyme has been solved by x-ray crystallography. (Sakon *et al.* 1993). The enzyme is able to attach an adenyl moiety to the 4' hydroxyl group of many aminoglycosides.

The β-lactamase-resistant penicillins (methicillin, oxacillin, cloxacillin, and flucloxacillin) were developed to treat penicillin-resistant *S. aureus*, and are still used as first-line treatment. Methicillin was the
first antibiotic in this class to be used (it was introduced in 1959), but, only two years later, the first case of MRSA was reported in England. (Jevons et al. 1961)

Despite this, MRSA generally remained an uncommon finding, even in hospital settings, until the 1990s, when there was an explosion in MRSA prevalence in hospitals, where it is now endemic. (Johnson et al. 2001).

There are number of problems with these antibiotics, such as the need for intravenous administration (there is no oral preparation available), toxicity, and the need to monitor drug levels regularly by blood tests. There are also concerns glycopeptide antibiotics do not penetrate very well into infected tissues (this is a particular concern with infections of the brain and meninges and in endocarditis). Glycopeptides must not be used to treat methicillin-sensitive S. aureus (MRSA), as outcomes are inferior. (Blot et al. 2002).

Vancomycin-resistant S. aureus (VRSA) is a strain of S. aureus that has become resistant to the glycopeptides. The first case of vancomycin-intermediate S. aureus (VISA) was reported in Japan in 1996; (Hiramatsu et al. 1997) but the first case of S. aureus truly resistant to glycopeptide antibiotics was only reported in 2002. (Chang et al. 2003). Three cases of VRSA infection had been reported in the United States as of 2005. (Menichetti et al. 2005)

CARRIAGE OF STAPHYLOCOCCUS AUREUS

The carriage of Staphylococcus aureus is an important source of nosocomial infection and community-acquired methicillin-resistant S. aureus (MRSA). Although S. aureus can be present on the skin of the host, a large proportion of its carriage is through the anterior nares of the nasal passages. (Kluymans et al. 1997) The ability of the nasal passages to harbour S. aureus results from a combination of a weakened or defective host immunity and the bacteria's ability to evade host innate immunity. (Quinn et al. 2007).
**INFECTION CONTROL**

Spread of *S. aureus* (including MRSA) generally is through human-to-human contact, although recently some veterinarians have discovered the infection can be spread through pets, (Sing *et al.* 2008) with environmental contamination thought to play a relatively unimportant part. Emphasis on basic hand washing techniques are, therefore, effective in preventing its transmission. The use of disposable aprons and gloves by staff reduces skin-to-skin contact and, therefore, further reduces the risk of transmission. Please refer to the article on infection control for further details.

Recently, there have been myriad reported cases of *S. aureus* in hospitals across America. The pathogen has had facilitated transportation in medical facilities mainly because of insufficient healthcare worker hygiene. *S. aureus* is an incredibly hardy bacterium, as was shown in a study where it survived on polyester for just under three months; (Neely *et al.* 2000) polyester is the main material used in hospital privacy curtains.

An important and previously unrecognized means of community-associated MRSA colonization and transmission is during sexual contact. (Cook *et al.* 2007).

A 2011 study (Iwase *et al.* 2010) points to this new possible way to control *S.aureus*. This study was performed from observations of the nasal microbial flora of a diverse group of people. It was discovered that there are two different strains of *S. epidermidis*, one that inhibits biofilm formation by *S. aureus*, *S. epidermidis* strain JK16 (inhibitory type), and one that does not (non-inhibitory type) *S. epidermidis* strain JK11. These findings open the way to a biological control therapy to help in the treatment of *S. aureus* infections which are becoming a growing threat due to the rise of resistance to conventional antibiotic treatments.

**ENTEROCOCCUS**

*Enterococcus* is a genus of lactic acid bacteria of the phylum Firmicutes. *Enterococci* are Gram-positive cocci that often occur in pairs (diplococci) or short chains, and are difficult to distinguish from
streptococci on physical characteristics alone. (Gilmore et al. 2002) Two species are common commensal organisms in the intestines of humans: E. faecalis (90-95%) and E. faecium (5-10%). Rare clusters of infections occur with other species, including E. casseliflavus, E. gallinarum, and E. raffinosus. (Gilmore et al. 2002).

**PHYSIOLOGY AND CLASSIFICATION**

*Enterococci* are facultative anaerobic organisms, i.e., they are capable of cellular respiration in both oxygen-rich and oxygen-poor environments. (Fischetti et al. 2000) Though they are not capable of forming spores, *enterococci* are tolerant of a wide range of environmental conditions: extreme temperature (10-45°C), pH (4.5-10.0) and high sodium chloride concentrations. (Fisher et al. 2009).

*Enterococci* typically exhibit gamma-hemolysis on sheep’s blood agar. (Ryan et al. 2004).

*Enterococci*, particularly *Enterococcus* faecalis, are a common cause of endocarditis (5 to 15 percent of community-acquired endocarditis and up to 30 percent of nosocomially-acquired endocarditis) and can be a common cause of nosocomial urinary tract infections (being recovered from up to 15 to 20 percent of UTIs in the hospital setting).

**HISTORY**

Members of the genus *Enterococcus* were classified as Group D *Streptococcus* until 1984, when genomic DNA analysis indicated a separate genus classification would be appropriate. (Schleifer et al. 1984).

Catheter-related urinary tract infection (UTI) occurs because urethral catheters inoculate organisms into the bladder and promote colonization by providing a surface for bacterial adhesion and causing mucosal irritation. (Gilmore et al. 2002) the presence of a urinary catheter is the most important risk factor for bacteriuria.

Once a catheter is placed, the daily incidence of bacteriuria is 3-10%. Between 10% and 30% of patients who undergo short-term catheterization (ie, 2-4 days) develop bacteriuria and are asymptomatic. Between 90% and 100% of patients who undergo long-term catheterization develop bacteriuria. About 80% of nosocomial UTIs are
related to urethral catheterization; only 5-10% are related to genitourinary manipulation.

The presence of potentially pathogenic bacteria and an indwelling catheter predisposes to the development of a nosocomial UTI. The bacteria may gain entry into the bladder during insertion of the catheter, during manipulation of the catheter or drainage system, around the catheter, and after removal.

Enteric pathogens (eg, *Escherichia coli*) are most commonly responsible, but *Pseudomonas* species, *Enterococcus* species, *Staphylococcus aureus*, coagulase-negative staphylococci, *Enterobacter* species, and *yeast* also are known to cause infection. Proteus and *Pseudomonas* species are the organisms most commonly associated with biofilm growth on catheters.

Risk factors for bacteriuria in patients who are catheterized include longer duration of catheterization, colonization of the drainage bag, diarrhea, diabetes, absence of antibiotics, female gender, renal insufficiency, errors in catheter care, catheterization late in the hospital course, and immunocompromised or debilitated states.

**PATHOLOGY**

Important clinical infections caused by *Enterococcus* include urinary tract infections, bacteremia, bacterial endocarditis, diverticulitis, and meningitis. (Fisher et al. 2009, Ryan et al. 2004) Sensitive strains of these bacteria can be treated with ampicillin, penicillin and vancomycin. (Pelletier et al. 1996).

From a medical standpoint, an important feature of this genus is the high level of intrinsic antibiotic resistance. Some enterococci are intrinsically resistant to β-lactam-based antibiotics (penicillins, cephalosporins, carbapenems), as well as many aminoglycosides. (Ryan et al. 2004).

In the last two decades, particularly virulent strains of *Enterococcus* that are resistant to vancomycin (*vancomycin-resistant Enterococcus, or VRE*) have emerged in nosocomial infections of hospitalized patients, especially in the US. (Fisher et al. 2009). Other
developed countries, such as the UK, have been spared this epidemic, and, in 2005, Singapore managed to halt an epidemic of VRE. VRE may be treated with quinupristin/dalfopristin (Synercid) with response rates of approximately 70%. (Tünger et al. 2004). Tigecycline has also been shown to have anti-enterococcal activity as has rifampicin. (Gilmore et al. 2002).

*Enterococcal* meningitis is a rare complication of neurosurgery. It often requires treatment with intravenous or intrathecal vancomycin, yet it is debatable as to whether its use has any impact on outcome: the removal of any neurological devices is a crucial part of the management of these infections. (Guardado et al. 2006). New epidemiological evidence has shown that enterococci are major infectious agent in chronic bacterial prostatitis. *Enterococci* are able to form biofilm in the prostate gland making their eradication difficult.

In 2004, *Enterococcus* spp. took the place of fecal coliform as the new federal standard for water quality at public salt water beaches and *E. coli* at fresh water beaches. It is believed to provide a higher correlation than fecal coliform with many of the human pathogens often found in city sewage. (Jin et al. 2004).

**Staphylococcus epidermidis**

**SPECIES**

Genus Species: *Staphylococcus epidermidis* Other Names: *Micrococcus epidermidis*, *Albococcus epidermidis*, *Staphylococcus epidermidis albus*.

**Description and significance**

*Staphylococcus epidermidis* is a gram-positive and coagulase-negative *staphylococci*. It typically lives on the human skin and mucosa and the most common infections on catheters and implants (Todar et al. 2007). *S. epidermidis* is one of five most common organisms that cause noscomial infections due to the increase in usage of biomaterials in the clinical environment. The noscomial pathogen causes infections on prosthetic valves, cerebrospinal fluid shunts, joint prosthesis vascular prostheses, valves, and in postoperative wounds and the urinary tract. It
is also the most frequent organism found in the blood of bone marrow transplant patients and on central venous catheters for patients of total parenteral nutrition).

**PATHOLOGY**

The increase use of intravascular catheters has caused a similar increase in *S. epidermidis* infections. The increase causes a problem since *S. epidermidis* is resistant to methicillin and all penicillins, penems, carbapenems, and cephalosporins which are commonly used antibiotics (Salyers et al. 2002). *S. epidermidis* has also been found to be more resistant to antibiotics than other species.

**DISEASE**

As mentioned above, *S. epidermidis* causes biofilms to grow on plastic devices placed within the body. (Otto et al. 2009). This occurs most commonly on intravenous catheters and on medical prostheses. (Hedin et al. 1993) Infection can also occur in dialysis patients or anyone with an implanted plastic device that may have been contaminated. Another disease it causes is endocarditis. This occurs most often in patients with defective heart valves. In some other cases, sepsis can occur in hospital patients.

Antibiotics are largely ineffective in clearing biofilms. The most common treatment for these infections is to remove or replace the infected implant, though in all cases, prevention is ideal. The drug of choice is often vancomycin, to which rifampin or aminoglycoside can be added. [Citation needed] Hand washing has been shown to reduce the spread of infection.

**GRAM-NEGATIVE BACTERIA**

Microscopic image of Gram-negative *Pseudomonas aeruginosa* bacteria (pink-red rods).

Gram-negative bacteria are bacteria that do not retain crystal violet dye in the Gram staining protocol. (Baron et al. 1996) In a Gram stain test, a counterstain (commonly safranin) is added after the crystal violet, coloring all Gram-negative bacteria with a red or pink color. This is because of the existence of an outer membrane preventing the
penetration of the stain. The test itself is useful in classifying two distinct
types of bacteria based on the structural differences of their bacterial cell
walls. Gram-positive bacteria will retain the crystal violet dye when
washed in a decolorizing solution. Compared with Gram-positive
bacteria, Gram-negative bacteria are more resistant against antibodies,
because of their impenetrable wall.

The pathogenic capability of Gram-negative bacteria is often
associated with certain components of Gram-negative cell envelope, in
particular, the lipopolysaccharide layer (also known as LPS or endotoxin
layer). (Baron et al. 1996) In humans, LPS triggers an innate immune
response characterized by cytokine production and immune system
activation. Inflammation is a common result of cytokine (from the Greek
cyto, cell and kinesis, movement) production, which can also produce
host toxicity.

Gram-positive- and negative bacteria are chiefly differentiated by their
cell wall structure.

CLASSIFICATION

Along with cell shape, Gram staining is a rapid diagnostic tool and
used to be used to group species at the subdivision of Bacteria.

SPECIES IDENTIFICATION HIERARCHY IN CLINICAL SETTINGS

In fact, historically the kingdom Monera was divided into four
divisions based on Gram staining: Firmacutes (+), Gracillicutes (-),
Mollicutes (0) and Mendocutes (var.). (Gibbons et al. 1978) Since 1987,
the monophyly of the Gram-negative bacteria has been disproven with
molecular studies. (Woese et al. 1987) However some authors, such as
Cavalier-Smith still treat them as a monophyletic clade and refer to the
group as subkingdom "Negibacteria". (Cavalier-Smith et al. 2006)

OUTER CELL MEMBRANE BACTERIAL CLASSIFICATION

It is important to point out that although the bacteria are
traditionally divided into two main groups, Gram-positive and Gram-
negative, based upon their Gram-stain retention property, this
classification system is ambiguous as it can refer to three distinct aspects
(staining result, cell-envelope organization, taxonomic group), which do
not necessarily coalesce for some bacterial species. (Gupta et al. 1998, Gupta et al. 2000, Desvaux et al. 2009, Sutcliffe et al. 2010) The Gram-positive and Gram-negative staining response is also not a reliable phylogenetic character as these two kinds of bacteria do not form phylogenetically coherent groups. (Gupta et al. 1998). However, Gram-staining response of bacteria is an empirical criterion; its basis lies in the marked differences in the ultrastructure and chemical composition of the two main kinds of prokaryotic cells that are found in nature. These two kinds of cells are distinguished from each other based upon the presence or absence of an outer lipid membrane, which is a reliable and fundamental characteristic of bacterial cells. (Gupta et al. 1998, Gupta et al. 1998) All Gram-positive bacteria are bounded by only a single unit lipid membrane and they generally contain a thick layer (20-80 nm) of peptidoglycan responsible for retaining the Gram-stain.

For the bacterial (prokaryotic) cells that are bounded by a single cell membrane the term Monoderm Bacteria or Monoderm Prokaryotes has been proposed. (Gupta et al. 1998, Gupta et al. 1998) In contrast to Gram-positive bacteria, all archetypical Gram-negative bacteria, are bounded by both a cytoplasmic membrane as well as an outer cell membrane and they contain only a thin layer of peptidoglycan (2-3 nm) in between these two membranes. The presence of both inner and outer cell membranes defines a new compartment in these cells, the periplasmic space or the periplasmic compartment. These bacteria/prokaryotes have been designated as Diderm Bacteria. (Gupta et al. 1998, Gupta et al. 1998) The distinction between the monoderm and diderm prokaryotes is also supported by conserved signature indels in a number of important proteins (viz. DnaK, GroEL). (Gupta et al. 1998, Gupta et al. 2000, Gupta et al. 1998, Gupta et al. 2011) Of these two structurally distinct groups of prokaryotic organisms, monoderm prokaryotes are indicated to be ancestral. Based upon a number of different observations including that the Gram-positive bacteria are the major producers of antibiotics and that Gram-negative bacteria are generally resistant to them, it has been proposed that the outer cell
membrane in Gram negative bacteria (diderms) has as a protective mechanism against antibiotic selection pressure. (Gupta et al. 1998, Gupta et al. 2000, Gupta et al. 1998, Gupta et al. 2011) Some bacteria such as *Deinococcus*, which stain Gram-positive due to the presence of a thick peptidoglycan layer, but also possess an outer cell membrane are suggested as intermediates in the transition between monoderm (Gram positive) and diderm (Gram-negative) bacteria. (Gupta et al. 1998, Gupta et al. 2011) The diderm bacteria can also be further differentiated between simple diderms lacking lipopolysaccharide, the archetypical diderm bacteria where the outer cell membrane contains lipopolysaccharide and the diderm bacteria where outer cell membrane is made up of mycolic acid. (Desvaux et al. 2009, Sutcliffe et al. 2010, Gupta et al. 2011, Marchandin et al. 2010) Additionally, a number of bacterial taxa (viz. Negativicutes, Fusobacteria, Synergistetes and Elusimicrobia) that are either part of the phylum Firmicutes or branch in its proximity are also found to possess a diderm cell structure. (Sutcliffe et al. 2010, Gupta et al. 2011, Marchandin et al. 2010) However, a conserved signature indel (CSI) in the HSP60 (GroEL) protein distinguishes all traditional phyla of Gram-negative bacteria (e.g. *Proteobacteria*, *Aquificae*, *Chlamydiae*, *Bacteroidetes*, *Chlorobi*, *Cyanobacteria*, *Fibrobacteres*, *Verrucomicrobia*, *Planctomycetes*, *Spirochetes*, *Acidobacteria*, etc.) from these other atypical diderm bacteria as well as other phyla of monoderm bacteria (e.g. *Actinobacteria*, *Firmicutes*, *Thermotogae*, *Chloroflexi*, etc.). (Gupta et al. 2011). The presence of this CSI in all sequenced species of conventional LPS-containing Gram-negative bacterial phyla provides evidence that these phyla of bacteria form a monophyletic clade and that no loss of the outer membrane from any species from this group has occurred. (Gupta et al. 2011)

Medically relevant Gram-negative cocci include three organisms, which cause a sexually transmitted disease (*Neisseria gonorrhoeae*), a meningitis (*Neisseria meningitidis*), and respiratory symptoms (*Moraxella catarrhalis*).
Medically relevant Gram-negative bacilli include a multitude of species. Some of them primarily cause respiratory problems (*Hemophilus influenzae, Klebsiella pneumoniae, Legionella pneumophila, Pseudomonas aeruginosa*), primarily urinary problems (*Escherichia coli, Proteus mirabilis, Enterobacter cloacae, Serratia marcescens*), and primarily gastrointestinal problems (*Helicobacter pylori, Salmonella enteritidis, Salmonella typhi*).

Gram-negative bacteria associated with nosocomial infections include *Acinetobacter baumannii*, which cause bacteremia, secondary meningitis, and ventilator-associated pneumonia in intensive-care units of hospital establishments.

**MEDICAL TREATMENT**

One of the several unique characteristics of Gram-negative bacteria is the structure of the outer membrane. The outer leaflet of the membrane comprises a complex lipopolysaccharide whose lipid portion acts as an endotoxin. If endotoxin enters the circulatory system, it causes a toxic reaction, with the sufferer developing a high temperature, high respiration rate, and low blood pressure. This may lead to endotoxic shock, which may be fatal.

**BACILLUS**

*Bacillus* is a genus of Gram-positive, rod-shaped bacteria and a member of the phylum Firmicutes. *Bacillus* species can be obligate aerobes or facultative anaerobes, and test positive for the enzyme catalase. Ubiquitous in nature, *Bacillus* includes both free-living and pathogenic species. Under stressful environmental conditions, the cells produce oval endospores that can stay dormant for extended periods. These characteristics originally defined the genus, but not all such species are closely related, and many have been moved to other genera.

**DESCRIPTION AND SIGNIFICANCE**

Detergent granules containing enzymes produced by *Bacillus subtilis*. From Innovation in Europe bacilli are an extremely diverse group of bacteria that include both the causative agent of anthrax (*Bacillus anthracis*) as well as several species that synthesize important antibiotics.
In addition to medical uses, *bacillus* spores, due to their extreme
tolerance to both heat and disinfectants, are used to test heat
sterilization techniques and chemical disinfectants. *Bacilli* are also used
in the detergent manufacturing industry for their ability to synthesize
important enzymes.

*Bacillus subtilis* in the spore-formation phase. From Innovation in
Europe *Bacilli* are rod-shaped, Gram-positive, sporulating, aerobes or
facultative anaerobes. Most *bacilli* are saprophytes. Each bacterium
creates only one spore, which is resistant to heat, cold, radiation,
desiccation, and disinfectants. *Bacilli* exhibit an array of physiologic
abilities that allow them to live in a wide range of habitats, including
many extreme habitats such as desert sands, hot springs, and Arctic
soils. Species in the genus *Bacillus* can be thermophilic, psychrophilic,
acidophilic, alkaliphilic, halotolerant, or halophilic and are capable at
growing at pH values, temperatures, and salt concentrations where few
other organisms can survive.

**PHYLOGENY**

The genus *Bacillus* was coined in 1835 by Christian Gottfried
Ehrenberg (who coined the genus Bacterium seven years prior) to contain
rod-shaped bacteria, later amended by Ferdinand Cohn to spore-forming,
Gram-positive/variable, rod-shaped bacteria. (Scheffers *et al.* 2012) Like
other genera associated with the early history of microbiology, such as
*Pseudomonas or Vibrio*, members of the *Bacillus* genus (266 species) are
found ubiquitously, and it is one of the genera with the largest 16S
diversity and environmental diversity.

Several studies have tried to reconstruct the phylogeny of the
genus. The *Bacillus*-specific study with the most diversity covered is by
Xu and Cote’ using 16S and the ITS region, where they divide the genus
into 10 groups, which includes the nested genera *Paenibacillus*,
*Brevibacillus*, *Geobacillus*, *Marinibacillus and Virgibacillus*. However, the
tree constructed by the living tree project, a collaboration between ARB-
Silva and LPSN where a 16S (and 23S if available) tree of all validated
species was constructed, the genus *Bacillus* contains a very large number
of nested taxa and majorly in both 16S and 23S it is paraphyletic to *Lactobacillales* (*Lactobacillus, Streptococcus, Staphylococcus, Listeria, etc*.), due to *Bacillus coahuilensis* and others. A gene concatenation study found similar results to Xu and Cote', but with a much more limited number of species in terms of groups, but used Listeria as an outgroup, so in light of the ARB tree, it may be "inside-out".

One clade, formed by *B. anthracis, B. cereus, B. mycoides, B. pseudomycoides, B. thuringiensis* and *B. weihenstephanensis* under current classification standards, should be a single species (within 97% 16S identity), but due to medical reasons, they are considered separate species, an issue also present for four species of *Shigella* and *Escherichia coli*.

**Escherichia coli**

*Escherichia coli* (abbreviated as *E. coli*) are a large and diverse group of bacteria. Most strains (or types) of *E. coli* are harmless, but a few others are pathogenic (cause disease). Some strains of *E. coli* can cause intestinal infections (including diarrhea and the serious disease hemolytic uremic syndrome), while others cause urinary tract infections, respiratory illness (including pneumonia), and other illnesses. Since *E. coli* is a natural component of the fecal content of humans and animals, it is often used as a marker for water contaminated with fecal matter.

This scanning electron micrograph (SEM) depicts a single Gram-negative *Escherichia coli* bacterium of the strain O157:H7, which is one of hundreds of strains of this bacterium. Although most strains are harmless, and live in the intestines of healthy humans and animals, this strain produces a powerful toxin, which can cause severe illness.

The presence of *E. coli* and other kinds of bacteria within the intestines of both humans and animals is necessary for health. *E. coli* and other bacteria provide a vital role in digestion of food and production and absorption of vitamins. *E. coli* in the intestine aid in the absorption of a large portion of Vitamin K and B-complex vitamins from the diet. Animals who are born and raised "germ free", that is, without any
bacteria present, have many defects and require vitamin supplements to stay alive.

**E. coli causes disease in two ways:**

- When *E. coli* from the intestine gets into other tissues or organs where it does not belong, or
- When harmful, pathogenic strains of *E. coli* get into the body

Strains of *E. coli* — and other Gram negative bacteria — are named and classified based on the type of surface they present to the immune system (aka their serogroup). For *E. coli*, two antigens are important, the O or somatic cell antigen and the H or flagellar antigen. There are more than 700 serotypes of *E. coli*. One of the most dangerous *E. coli* strains is [*Escherichia coli O157:H7 EHEC|E. coli O157:H7*]. *E. coli* are also classified based on the types of toxins they produce and how well they are able to invade human cells.

**Shiga toxin-producing *E. coli* (STEC)**

Some kinds of *E. coli* cause disease by making a toxin called Shiga toxin. This toxin is very similar to the toxin produced by a different bacteria known as *Shigella dysenteria* (that also causes bloody diarrhea and hemolytic uremic syndrome, primarily in emerging countries like Bangladesh). *E. coli* that make these toxins are called “Shiga toxin-producing” *E. coli*, or STEC. STEC are also called *verocytotox*ic *E. coli* (VTEC) or *enterohemorrhagic* *E. coli* (EHEC). These names all refer generally to the same group of bacteria. The most commonly identified STEC in North America is *E. coli* O157:H7 (often shortened to *E. coli* O157 or even just “O157”). News reports about outbreaks of “*E. coli*” infections are usually referring to *E. coli* O157.

In addition to *E. coli* O157, many other kinds (serogroups) of STEC cause disease. These other kinds are sometimes called “non-O157 STEC.” *E. coli* serogroups O26, O111, and O103 are the non-O157 serogroups that most often cause illness in people in the United States.
DIFFERENCES BETWEEN E. COLI O157 AND OTHER STEC

Most of what we know about STEC comes from outbreak investigations and studies of E. coli O157 infection, which was first identified as a pathogen in 1982. The non-O157 STEC are not nearly as well understood, partly because outbreaks due to them are rarely identified. As a whole, the non-O157 serogroup is less likely to cause severe illness than E. coli O157. However, some non-O157 STEC serogroups can cause the most severe manifestations of STEC illness.

E. COLI AND URINARY TRACT INFECTIONS

The urinary tract is the most common site of infection by E. coli. The term "uropathogenic" is used to describe E. coli when it causes UTIs. E. coli accounts for more than 90% of all uncomplicated UTIs. The recurrence rate after a first E coli infection is 44% over 12 months. The symptoms and severity of E.coli UTIs range from an uncomplicated infection to serious infections of the prostate (prostatitis), bladder, and kidneys (pyelonephritis). Uncomplicated UTIs occur more frequently in sexually active females from contamination of the area around the urethra by material from the colon.

Complicated UTIs and kidney infections are often caused by contaminated urinary catheters.

Virulence factors are structures that make it easier for E. coli to cause infection. One of these virulence factors is called 'P fimbriae', which allow the attachment of E. coli to the cells lining the urinary tract.

Serious E. coli Urinary Tract Infections can result in bacteremia, which is a transient blood infection. The presence of E. coli in the blood can cause a serious systemic reaction called endotoxic shock. E. coli is one of the most important causes of nosocomial (hospital-acquired) infections.

E. COLI RESPIRATORY INFECTIONS

E. coli is not normally found in the respiratory tract. Respiratory infections are uncommon. When they do occur the patient usually has an E. coli UTI as well. In very ill and/or immunocompromized patients, E coli may cause pneumonia.
NON-SHIGA-TOXIN PRODUCING E. COLI ENTERIC INFECTIONS

In addition to the serious STEC infection described above, E. coli can cause less serious infection of the intestinal system. Childhood and traveler’s diarrhea, are usually caused by pathogenic E. coli. These diarrheas often develop when new strains of E. coli are introduced into the body. Travelers to areas of the world where contamination of water by human or animal fecal material is common often experience episodes of severe diarrhea, while locals consuming the same products have no illness. This is because they have developed immunity to it, whereas the traveler who has just encountered the new strain has not yet done this. Often the illness is given colorful names, reflecting the country or region where travelers are affected, such as "Moctezuma’s Revenge" in Mexico, "Delhi Belly" in India, "Turkey Trot" in Asia minor, "Casablanca Crud" in North Africa, or "Thai-dal Wave" in Thailand.

SYMPTOMS

Symptoms of STEC infections

The symptoms of STEC infections vary for each person but often include severe stomach cramps, diarrhea (often bloody), and vomiting. If there is fever, it usually is not very high (less than 101˚F/less than 38.5˚C). Most people get better within 5–7 days. Some infections are very mild, but others are severe or even life-threatening.

The time between ingesting the STEC bacteria and feeling sick is called the “incubation period.” The incubation period is usually 3-4 days after the exposure, but may be as short as one day or as long as ten days. The symptoms often begin slowly with mild belly pain or non-bloody diarrhea that worsens over several days. Hemolytic uremic syndrome, if it occurs, develops an average of seven days after the first symptoms, which is usually when the diarrhea is improving.

SYMPTOMS OF TRAVELER’S DIARRHEA

Diarrhea is loose, watery stools. Most traveler's diarrhea cases begin abruptly. The illness usually results in increased frequency, volume, and weight of stool. Typically, a traveler experiences four to five loose or watery bowel movements each day. Other commonly associated
symptoms are nausea, vomiting, diarrhea, abdominal cramping, bloating, fever, urgency, and malaise. Most cases are benign and resolve in 1-2 days without treatment. Traveler’s diarrhea is rarely life-threatening; 90% of cases resolve within one week and 98% resolve within one month. (Vogt et al. 2005)

Diarrhea can cause dehydration, which means the body lacks enough fluid to function properly. Dehydration is particularly dangerous in children and older people. It must be treated promptly to avoid serious health problems.

**SYMPTOMS OF UTIS**

*E. coli*-caused UTIs symptoms include a frequent urge to urinate and a painful, burning sensation during urination. There may be fatigue, chills, or pain even when not urinating. It is common for a person with a urinary tract infection to complain that, despite the urge to urinate, only a small amount of urine is passed. The urine upon sampling may look cloudy or red if blood is present. A UTI will not cause fever if the infection is located in the bladder or urethra. A fever usually means the infection has traveled up to the kidneys to cause pyelonephritis. Other symptoms of a kidney infection include back pain below the ribs, nausea, vomiting, and chills. Pyelonephritis is a serious infection and should be treated immediately by a physician.

Some antibiotics administered in a once-a-day dose are 90% effective at preventing travelers’ diarrhea. However, antibiotics are not recommended by the CDC as prophylaxis because this can lead to the development of drug-resistant organisms. Routine antimicrobial prophylaxis also increases the traveler’s risk for adverse reactions to the medication. Finally, because antimicrobials provide no protection against either viral or parasitic pathogens, they can give travelers a false sense of security. As a result, strict adherence to preventive measures is encouraged. Bismuth subsalicylate (Pepto-Bismol) can be used as an adjunct if prophylaxis is needed. (Bentley et al. 1982).
Model of successive binary fission in *E. coli*

*E. coli* is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 microns (μm) long and 0.5 μm in diameter, with a cell volume of 0.6–0.7 (μm). (Kubitschek *et al.* 1990) It can live on a wide variety of substrates. *E. coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas Because of its long history of laboratory culture and ease of manipulation, *E. coli* also plays an important role in modern biological engineering and industrial microbiology. (Lee *et al.* 1996) The work of Stanley Norman Cohen and Herbert Boyer in *E. coli*, using plasmids and restriction enzymes to create recombinant DNA, became a foundation of biotechnology. (Russo *et al.* 2003).

*E. coli* is a very versatile host for the production of heterologous proteins, (Cornelis *et al.* 2000) and various protein expression systems have been developed which allow the production of recombinant proteins in *E. coli*. Researchers can introduce genes into the microbes using plasmids which permit high level expression of protein, and such protein may be mass produced in industrial fermentation processes. One of the first useful applications of recombinant DNA technology was the manipulation of *E. coli* to produce human insulin. (Tof *et al.* 1994)

Many proteins previously thought difficult or impossible to be expressed in *E. coli* in folded form have also been successfully expressed in *E. coli*. For example, proteins with multiple disulphide bonds may be produced in the periplasmic space or in the cytoplasm of mutants rendered sufficiently oxidizing to allow disulphide-bonds to form, (Bessette *et al.* 1999) while proteins requiring post-translational modification such as glycosylation for stability or function have been expressed using the N-linked glycosylation system of *Campylobacter jejuni* engineered into *E. coli*. (Ihssen *et al.* 2010, Wacker *et al.* 2002, Huang *et al.* 2012)
Modified *E. coli* cells have been used in vaccine development, bioremediation, and production of immobilised enzymes. (Cornelis *et al.* 2000)

**MODEL ORGANISM**

*E. coli* is frequently used as a model organism in microbiology studies. Cultivated strains (e.g. *E. coli K12*) are well-adapted to the laboratory environment, and, unlike wild type strains, have lost their ability to thrive in the intestine. Many lab strains lose their ability to form biofilms. (Fux *et al.* 2005, Vidal *et al.* 1998) These features protect wild type strains from antibodies and other chemical attacks, but require a large expenditure of energy and material resources.

In 1946, Joshua Lederberg and Edward Tatum first described the phenomenon known as bacterial conjugation using *E. coli* as a model bacterium, (Lederberg *et al.* 1946) and it remains the primary model to study conjugation. [Citation needed] *E. coli* was an integral part of the first experiments to understand phage genetics,¹ and early researchers, such as Seymour Benzer, used *E. coli* and phage T4 to understand the topography of gene structure. (Benzer *et al.* 1961) Prior to Benzer’s research, it was not known whether the gene was a linear structure, or if it had a branching pattern.

*E. coli* was one of the first organisms to have its genome sequenced; the complete genome of *E. coli K12* was published by *Science* in 1997. (Blattner *et al.* 1997).

By evaluating the possible combination of nanotechnologies with landscape ecology, complex habitat landscapes can be generated with details at the nanoscale. (Keymer *et al.* 2006) on such synthetic ecosystems, evolutionary experiments with *E. coli* have been performed to study the spatial biophysics of adaptation in an island biogeography on-chip.

Studies are also being performed into programming *E. coli* to potentially solve complicated mathematics problems, such as the Hamiltonian path problem. (Biol Eng *et al.* 2009)
CHANCES OF DEVELOPING E. COLI INFECTION

E. COLI UTIS

About 3% of all women in the United States visit a physician at least once each year for UTIs, and at least 50% of women report at least one UTI in a lifetime.

STEC INFECTIONS

For STEC infections, people of any age can become infected. Very young children and the elderly are more likely to develop severe illness and HUS than others, but even healthy older children and young adults can become seriously ill.

Experts think that there may be about 70,000 infections with E. coli O157 each year in the United States. This is only an estimate because many infected people do not seek medical care, many do not submit a stool specimen for testing, and many labs do not test for STEC.

HOW E. COLI IS SPREAD

STEC typically disappears from the feces by the time the illness is resolved, but may be shed for several weeks, even after symptoms go away. Young children tend to carry STEC longer than adults. A few people keep shedding these bacteria for several months.

COMPLICATIONS

Around 5–10% of those who are diagnosed with STEC infection develop a potentially life-threatening complication known as hemolytic uremic syndrome (HUS). Clues that a person is developing HUS include decreased frequency of urination, feeling very tired, pale skin, and unexplained bleeding from the mouth or nose. Persons with HUS should be hospitalized because their kidneys may stop working and they may develop other serious problems. Most persons with HUS recover within a few weeks, but some suffer permanent damage or die.

E. coli meningitis in neonates usually has neurological complications. The newborn usually is left with various disabilities, such as deafness or blindness.
**Bacillus licheniformis**

**Description and significance**

*Bacillus licheniformis* is a bacterium that is commonly found in soil and bird feathers. Birds that tend to stay on the ground more than the air (i.e. sparrows) and on the water (i.e. ducks) are common carriers of this bacterium; it is mostly found around the bird's chest area and back plumage.

*B. licheniformis* is part of the subtilis group along with *Bacillus subtilis* and *Bacillus pumilus*. These bacteria are commonly known to cause food poisoning and food spoilage. *B. licheniformis* also is known for contaminating dairy products. Food borne outbreaks usually involve cases of cooked meats and vegetables, raw milk, and industrially produced baby food contaminated with *B. licheniformis*. (Salkinoja-Salonen et al. 1999)

**Genome structure**

The complete nucleotide sequence of *Bacillus licheniformis* consists of the ATCC 14580 genome, which has a circular chromosome of 4,222,336 bp (base pairs) which contains 4,208 predicted protein-coding genes (average size of 873 bp), 7 rRNA operons, and 72 tRNA genes. The GC content is 46.2% and no plasmids were detected. (Rey, Ramaiya et al. 2004)

The chromosome of *B. licheniformis* has large regions that are similar to *Bacillus subtilis* and *Bacillus halodurans*. Since about 80% of the coding sequence of *B. licheniformis* contain *B. subtilis* orthologs, it is considered part of the subtilis group. But, although similar to *B. subtilis*, they differ in the amount and location of prophages, transposable elements, extracellular enzymes, and secondary metabolic pathway operons. (Rey, Ramaiya et al. 2004)

**Cell structure and metabolism**

*Bacillus licheniformis* is a rod-shaped, Gram-positive bacterium. (Veith, Herzberg et al. 2004)

It tends to form spores in soil which makes it desirable to be used for the industrial purposes such as the production of enzymes, antibiotics, and small metabolites. It produces a variety of extracellular enzymes that are associated with the cycling of nutrients in nature.

Its optimal growth temperature is 50°C, but it can also survive at much higher temperatures. Its optimal temperature for enzyme secretion
is 37°C. This bacterium can survive harsh environments by turning into spore-form; when conditions are good, it will turn back into a vegetative state.

*B. licheniformis* produces a protease that can survive at high pH levels. This protease is a desired ingredient in laundry detergent due to its ability to be used in low temperatures, which prevents shrinkage and fading colors.

**Ecology**

*Bacillus licheniformis* forms spores in soil. A pathway that leads to endospore formation is initiated when the bacterium is starved. Endospore formation is actually desired and serves as a great example of prokaryotic development and differentiation. These spores are quite tolerant of heat, cold, radiation, and other environmental stresses. Under good conditions, the spores will germinate and produce vegetative cells.

*B. licheniformis* produces a variety of extracellular enzymes that are associated with the cycling of nutrients in nature. It is an apathogenic soil organism that is mostly associated with plant and plant materials in nature. Although it is most common to isolate this bacterium from is soil, it is believed that *B. licheniformis* can actually be isolated from practically anywhere since it produces highly resistant endospores that are spread around with dust.

Ecologists are studying the effects of *B. licheniformis* on bird feathers. It is believed that this bacterium is involved in the evolution of molting and patterns of color in birds due to its feather degrading capability.

*B. licheniformis* is also known to cause food poisoning in humans; especially high in contamination rates are products such as raw milk, dairy, vegetables, processed baby foods, and cooked meats.

**Pathology**

*Bacillus licheniformis* is commonly associated with food spoilage and poisoning. It causes bread spoilage, or more specifically, a condition called "ropy bread" (Pepe, Blaiotta et al. 2003)

Contamination with this bacterium will make the bread sticky and stringy; the ropy bread will also start to develop a strong odor after contamination. Rope spores is what causes the spoilage; unfortunately these spores do not get killed during the baking process.

*B. licheniformis* can also cause food-borne gastro-enteritis, which is infection of the gut that can lead to a life threatening condition called
septicaemia. Septicaemia is blood poisoning, and is classified as having a large amount of bacteria in the blood. Dairy products are at increased risk of being contaminated with toxin-producing isolates of *B. licheniformis*. Cooked meats, raw milk, vegetables, and processed baby foods are also at risk. (Salkinoja-Salonen S.e.c. 1999)

The symptoms include stomach pains, (acute) diarrhea, and possible vomiting. These have an onset time of 2-14 hours and last no longer than 36 hours.

*B. licheniformis*, although usually associated with the gut and gastrointestinal tract, can also cause distress in other parts of the body. It can cause ophthalmitis, which is the inflammation of the eye. It can even go as far as causing abortions in pregnancies and impair sperm motility. The toxins produced by *B. licheniformis* can cause damage to cell membranes, deplete cellular ATP, and cause the acrosome to swell; it is not found to have any damaging effects on the mitochondria. (Salkinoja-Salonen et al. 1999)

**Application to Biotechnology**

Researchers are trying to recycle bird feathers by turning them into nutritious food for livestock. As mentioned, *Bacillus licheniformis* is commonly found on bird feathers; by fermentation with *B. licheniformis*, the large amounts of non-digestible proteins found in the feathers can turn into a feather meal for livestock. This is desired because it is cheap and nutritious.

*B. licheniformis* can also give more information about the evolution of molting and patterns of color in birds due to its feather degrading capability. Ecologists are looking for signs of association between the plumage feathers and *B. licheniformis* activity.

*B. licheniformis* is also an important ingredient in laundry detergent. Since it can grow in alkaline conditions, it produces a protease that can survive at high pH levels. The protease has an optimum pH at around 9 and 10, which is desirable since it can remove protein-comprised dirt in clothes. Researchers culture and isolate this protease to add it into detergents. This protease prevents shrinkage and fading colors since it allows lower temperatures to be used, which in turn lowers energy use as well.

*B. licheniformis* is used to make the antibiotic Bacitracin. Bacitracin is composed of a mixture of the cyclic polypeptides that *B. licheniformis* produces; ironically the purpose of Bacitracin is to inhibit
the growth of *B. licheniformis*. Bacitracin lyases the proplasts of *B. licheniformis* in the presence of cadmium or zinc ions. (Snoke and Cornell et al. 1965)

**Current Research**

*Bacillus licheniformis* is a spore-forming soil organism that contributes to nutrient cycling and has antifungal activity. There is current research on *B. licheniformis* (strain SB3086) and its effects as a microbial fungicide. Novozymes Biofungicide Green Releaf contains *B. licheniformis* strain SB3086 as an active main ingredient. This fungicide can be used on lawns, conifers, tree seedlings, ornamental turf and ornamental plants in outdoor, greenhouse, and nursery sites. There are concerns regarding the safety of this fungicide. Reports about *Bacillus licheniformis* having detrimental effects on insect, avian, plant, and estuarine marine species are fortunately almost non-existent. There have been reports of reproductive failure and mastitis caused by this bacterium in cattle, sheep and swine. It fortunately does not have any detrimental effects on endangered species.

Here is an increased interest in using a protease isolated from *Bacillus licheniformis* in laundry detergents. Since this bacterium grows in alkaline conditions, it produces a desirable protease that can survive at high pH levels. This protease is an active ingredient in laundry detergents, removing protein-comprised dirt in clothes. The desirable properties of this protease are its prevention of clothes shrinkage and fading colors due to its capability to be used at lower temperatures. The Research/Technology Invention Award 2006 was given to members of the BiotechGenoMik project on *B. licheniformis*; they invented a system for controlling industrial fermentation, which they named BioChip. This system uses DNA-based diagnostic tool to monitor fermentation processes such as the production of enzymes for Henkel laundry detergents.

Currently there are many electrical techniques for food processing, one such example is Ohmic heating. Ohmic heating has potential uses such as blanching, evaporation and pasteurization of food; it is a high temperature, short time, and a purely bulk heating method. There are increased concerns regarding microbial contaminations, from such bacteria as *E. coli* and *B. licheniformis*, when it comes to food processing.
Current research try to find the "death kinetics" (Pereira et al. 2006) of these bacteria. Death kinetics, in this case, involves the intensity of heat treatments and their correlation with the rate of death of a bacterium. The death kinetics for *B. licheniformis* ATCC® 14580 spores in cloudberry jam was examined under ohmic heat inactivation and conventional heat inactivation. Results of studies show that the ohmic heating has a quicker death kinetic rate, meaning shorter and less aggressive treatments can be used to kill off *B. licheniformis*.

**Feather protection through psittacofulvin**

*Bacillus licheniformis* degrades feathers of parrots, especially white feathers. Red feathers with high levels of psittacofulvin are more resistant. (Pepe et al. 2003)

**Biological laundry detergent**

*Bacillus licheniformis* is cultured in order to obtain protease for use in biological laundry detergent. The bacterium is well adapted to grow in alkaline conditions, so the protease it produces can withstand high pH levels, making it ideal for this use - the other components of detergents create an alkaline pH. The protease has a pH optimum of between 9 and 10 and is added to laundry detergents in order to digest, and hence remove, dirt made of proteins. This allows for much lower temperatures to be used, resulting in lower energy use and a reduced risk of shrinkage of garments or loss of colored dyes.

**Dental applications**

In 2012, scientists from Newcastle University studying *Bacillus licheniformis* as a possible agent to clean ships' hulls isolated an enzyme that has proven to be an unexpected tooth decay fighter as it has the ability to cut through plaque or a layer of bacteria. (Pereira et al. 2006)

**Bacillus subtilis**

*Bacillus subtilis*, known also as the hay bacillus or grass bacillus, is a Gram-positive, catalase-positive bacterium. (Madigan, Martinko et al. 2005)

A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and has the ability to form a tough, protective endospore, allowing the organism
to tolerate extreme environmental conditions. Unlike several other well-
known species, *B. subtilis* has historically been classified as an obligate
aerobe, though recent research has demonstrated that this is not strictly
correct. Nakano (Zuber et al. 1998).

Although this species is commonly found in soil, more evidence
suggests that *B. subtilis* is a normal gut commensal in humans. A 2009
study compared the density of spores found in soil (~10^6 spores per
gram) to that found in human feces (~10^4 spores per gram). The number
of spores found in the human gut is too high to be attributed solely to
consumption through food contamination. Soil simply serves as a
reservoir, suggesting that *B. subtilis* inhabits the gut and should be
considered as a normal gut commensal. (Hong, Khaneja, Tam, et al.
2009).

In 1835, the bacterium was originally named Vibrio subtilis by
Christian Gottfried Ehrenberg, (Ehrenberg et al. 1835) and renamed
*Bacillus subtilis* by Ferdinand Cohn in 1872. (Cohn et al. 1872).

In 1900s

It was used by the German medical corps in 1941 to treat
dysentery during the North African campaign after seeing local Arabic
population using it for this purpose.

Cultures of *B. subtilis* were used throughout the 1950s as an
alternative medicine due to the immunostimulatory effects of its cell
matter, which upon digestion has been found to significantly stimulate
broad spectrum immune activity including activation of specific antibody
IgM, IgG and IgA secretion (Ciprandi, Scordamaglia, Venuti, Caria, and
Canonica et al. 1986) and release of CpG dinucleotides inducing INF A/Y
producing activity of leukocytes and cytokines important in the
development of cytotoxicity towards tumor cells. (Ciprandi, Scordamaglia,
Venuti, Caria, and Canonica et al. 1986).

It was marketed throughout America and Europe from 1946 as an
immunostimulatory aid in the treatment of gut and urinary tract diseases
such as Rotavirus and Shigella, (Shylakhovenko et al. 2003) but declined
in popularity after the introduction of cheap consumer antibiotics,
despite causing less chance of allergic reaction and significantly lower toxicity to normal gut flora.

**Safety**

*B. subtilis* is only known to cause disease in severely immunocompromised patients, and can conversely be used as a probiotic in healthy individuals. (Mazza et al. 1994).

It rarely causes food poisoning. (Oggioni, Pozzi, Valensin, Galieni, Bigazzi et al. 1998).

Some *B. subtilis* strains produce the proteolytic enzyme subtilisin. *B. subtilis* spores can survive the extreme heat during cooking. Some *B. subtilis* strains are responsible for causing ropiness — a sticky, stringy consistency caused by bacterial production of long-chain polysaccharides — in spoiled bread dough. For a long time, bread ropiness was associated uniquely with *B. subtilis* species by biochemical tests. Today, molecular assays (randomly amplified polymorphic DNA PCR assay, denaturing gradient gel electrophoresis analysis, and sequencing of the V3 region of 16S ribosomal DNA) revealed greater *Bacillus* species variety in ropy breads which all seem to have a positive amylase activity and high heat.

The *Bacillus subtilis* species has a long history of safe use. It has been granted Qualified Presumption of Safety (QPS) status by the European Food Safety Authority (EFSA) (Gibson et al. 2005) and is part of the authoritative list of microorganisms with a documented history of safe use in food established by the International Dairy Federation (IDF) in collaboration with the European Food and Feed Cultures Association (EFFCA) in 2002 and updated in 2012.

**Reproduction**

*Bacillus subtilis* duplicates its single circular chromosome by initiating DNA replication at a single locus, the origin (oriC). Replication proceeds bidirectionally and two replication forks progress in the clockwise and counterclockwise directions along the chromosome halves. Chromosome replication is completed when the forks reach the terminus region, which is positioned opposite to the origin on the chromosome map, and contains several short DNA sequences (Ter sites) that promote
replication arrest. Specific proteins mediate all the steps in DNA replication. The comparison between the sets of proteins involved in chromosomal DNA replication in *B. subtilis* and in *Escherichia coli* reveals both similarities and differences. Although the basic components promoting initiation, elongation, and termination of replication are well conserved, some important differences can be found (such as one bacterium missing proteins essential in the other). These differences underline the diversity in the mechanisms and strategies that various bacterial species have adopted to carry out the duplication of their genomes (Graumann et al. 2007).

*B. subtilis* can divide symmetrically to make two daughter cells (binary fission), or asymmetrically, producing a single endospore that can remain viable for decades and is resistant to unfavourable environmental conditions such as drought, salinity, extreme pH, radiation and solvents. The endospore is formed at times of nutritional stress, allowing the organism to persist in the environment until conditions become favourable. Prior to the process of sporulation the cells might become motile by producing flagella, take up DNA from the environment, or produce antibiotics. These responses are viewed as attempts to seek out nutrients by seeking a more favourable environment, enabling the cell to make use of new beneficial genetic material or simply by killing of competition.

**Chromosomal replication**

*B. subtilis* is a model organism used to study bacterial chromosome replication. Replication of the single circular chromosome initiates at a single locus, the origin. Replication proceeds bidirectionally and two replication forks progress in clockwise and counterclockwise directions along the chromosome. Chromosome replication is completed when the forks reach the terminus region, which is positioned opposite to the origin on the chromosome map. The terminus region contains several short DNA sequences (Ter sites) that promote replication arrest. Specific proteins mediate all the steps in DNA replication. Comparison between
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**Application to Biotechnology**

*Bacillus* organisms, isolated by soil sprinkle technique, are responsible for producing antibiotics. The most antibiotic activity was seen in *Bacillus subtilis* MH-4. The most optimal activity occurs at a temperature of 37 degrees Celsius and a basic pH of 8. Glycerol is the optimal carbon source and L-glutamic acid is the optimal source of nitrogen. The antibiotic bacitracin was determined to be affective on Gram-positive bacteria only (Jamil *et al.* 2007).

Other antibiotics that *Bacillus subtilis* form are polymyxin, difficidin, subtilin, and mycobacillin. Polymyxin is affective against Gram-negative bacteria, whereas difficidin has a broader spectrum.

*Bacillus subtilis* bacteria secrete enzymes, "such as amylase, protease, pullulanase, chitinase, xylanase, lipase, among others. These enzymes are produced commercially and this enzyme production represents about 60% of the commercially produced industrial enzymes” (Morikawa *et al.* 2006)

**Uses**

*B. subtilis* has proven highly amenable to genetic manipulation, and has become widely adopted as a model organism for laboratory studies, especially of sporulation, which is a simplified example of cellular differentiation. It is also heavily flagellated, which gives *B. subtilis* the ability to move quickly in liquids. In terms of popularity as a laboratory model organism, B. subtilis is often used as the Gram-positive
equivalent of *Escherichia coli*, an extensively studied Gram-negative bacterium.

Monsanto has isolated a gene from *B. subtilis* that expresses cold shock protein B (CSPB) and spliced it into their drought-tolerant corn hybrid MON 87460, which was approved for sale in the United States in November 2011. (Noirot *et al.* 2007, Harrigan, Ridley, Miller *et al.* 2009).

Wild-type natural isolates of *B. subtilis* are difficult to work with compared to laboratory strains that have undergone domestication processes of mutagenesis and selection. These strains often have improved capabilities of transformation (uptake and integration of environmental DNA), growth, and loss of abilities needed "in the wild". And, while dozens of different strains fitting this description exist, the strain designated 168 is the most widely used.

Colonies of *B. subtilis* grown on a culture dish in a molecular biology laboratory.

As a model organism *B. subtilis* is commonly used in laboratory studies directed at discovering the fundamental properties and characteristics of Gram-positive spore-forming bacteria. In particular, the basic principles and mechanisms underlying formation of the durable endospore have been deduced from studies of spore formation in *B. subtilis*.

In addition to its role as a model organism, *B. subtilis* is used as a soil inoculant in horticulture and agriculture.

The high stability of *B. subtilis* in harsh environmental conditions makes this microorganism a perfect candidate for probiotics applications either in baked and pasteurized foods/beverages or in other galenic forms like tablets, capsules and powder. The strain *Bacillus subtilis* R0179 is well-documented for its probiotic benefices.

*B. globigii*, a closely related but phylogenetically distinct species now known as *B. atrophaeus* (Earl, Losick, Kolter *et al.* 2008). (Nakamura *et al.* 1979) was used as a biowarfare simulant during Project SHAD (aka Project 112). (Burke, Wright, Robinson, Bronk, Warren *et al.* 2004).
Subsequent genomic analysis showed that the strains used in those studies were products of deliberate enrichment for strains that exhibited abnormally high rates of sporulation.

Enzymes produced by *B. subtilis* and *B. licheniformis* are widely used as additives in laundry detergents.

**Genome**

*B. subtilis* has approximately 4,100 genes. Of these, only 192 were shown to be indispensable; another 79 were predicted to be essential as well. A vast majority of essential genes were categorized in relatively few domains of cell metabolism, with about half involved in information processing, one-fifth involved in the synthesis of cell envelope and the determination of cell shape and division, and one-tenth related to cell energetics. (Sharaf-Eldin, Elkholy, Fernández *et al.* 2008).

Several non-coding RNAs have been characterized in the *B. subtilis* genome, including Bsr RNAs. (Kobayashi, Ehrlich, Albertini, *et al.* 2003).

**Transformation**

Natural bacterial transformation involves the transfer of DNA from one bacterium to another through the surrounding medium. Transformation depends on the expression of numerous bacterial genes whose products appear to be specifically designed to carry out this process. (Saito, Kakeshita, Nakamura *et al.* 2008).

In order for a recipient bacterium to bind, take up and recombine exogenous DNA into its chromosome, it must enter a special physiological state called competence. (Chen, Dubnau *et al.* 2004).

Ordinarily (with rare exceptions) the DNA integrated into the recipient bacterial chromosome is from another bacterium of the same species, and thus the donated DNA is usually homologous to the resident chromosome.

In *B. subtilis* the length of the transferred DNA is greater than 1271 kb (more than 1 million bases). (Solomon, Grossman *et al.* 1996).

The length transferred is likely double stranded DNA and is often more than a third of the total chromosome length of 4215 kb. (Saito, Taguchi, Akamatsu *et al.* 2006)
It appears that about 7-9% of the recipient cells take up an entire chromosome. (Saito, Taguchi, Akamatsu et al. 2006).

Competence in *B. subtilis* is induced toward the end of logarithmic growth, especially under conditions of amino acid limitation. (Akamatsu, Taguchi et al. 2001).

Under these stressful conditions of semi-starvation the cells typically have just one copy of their chromosome and likely have increased DNA damage. To test whether the adaptive function of transformation is repair of DNA damages, a sequence of experiments was conducted with *B. subtilis* using UV light as the damaging agent (Anagnostopoulos, Spizizen et al. 1961), (Hoelzer, Michod et al. 1991), (Michod, Wojciechowski, Hoelzer et al. 1988) and also reviewed by Michod et al. Wojciechowski, Hoelzer, Michod et al. 1989).

These experiments led to the conclusion that competence, with uptake of DNA, is specifically induced by DNA damaging conditions, and that transformation functions as a process for recombinational repair of DNA damage. (Wojciechowski, Hoelzer, Michod et al. 1989).

There are many research studies that are currently being done on *Bacillus subtilis*. One recent research project focuses on the resistance of *Bacillus subtilis* spores to heat, radiation, and chemicals. It has been known that spores can survive hundreds, even millions, of years in a dormant state. The study investigated the important factors that contribute to spore resistance. The researcher found that the bacteria's coats were a major factor because the coat provides a barrier for the organism against toxic agents, ultraviolet radiation, and lytic enzymes. The inner membrane was also found to be important, due to its low permeability against toxic agents. DNA repair was also determined to be crucial, since it can control DNA damage due to radiation, heat, and toxins. *Bacillus subtilis* spores are also resistant to wet heat, primarily by the core's low water content. The lower the water content of the core is, the more resistant the spore is to wet heat. This research study is important in that it can lead to future studies on how the *Bacillus subtilis* spores in food and medical products can be killed effectively. Learning
about the spores resistance gives us a better understanding of which methods may or may not be useful in killing the spores (Setlow et al. 2006).

Another current research study provides evidence that the SpoIIIE DNA translocase is required for *Bacillus subtilis* forespore chromosome translocation across the septum and membrane fusion during sporulation. The researchers studied SpoIIIE mutants. They found that one mutant undergoes the translocation of DNA, but does not undergo membrane fusion normally after the engulfment. They discovered that the septum stays open in this mutation. When the sporulation septum is open, the cytoplasm is permitted to be exchanged between the daughter cells. This implies that the membrane does not fuse properly after engulfment and cytokinesis. The researchers proposed "that SpoIIIE catalyses these topologically opposite fusion events by assembling or disassembling a proteinaceous fusion pore" (Liu et al. 2006). The study demonstrated that SpoIIIE first participates in allowing a barrier of diffusion for the translocation of DNA, and then participates in the fusion of the membrane. Thus, SpoIIIE is required for the fusion of the engulfing membrane after the engulfment of the forespore (Liu et al. 2006).

A third current research project investigates *Bacillus subtilis* fermented soybean meal and its effects on enzymes in the gastrointestinal tract and intestinal morphology of piglets. The piglets were randomly given either soybean meal or fermented soybean meal. After the experiment was completed, the six piglets from each of the two treatment groups were sacrificed. The contents of the small intestine were collected, and the tissue was sampled at varying locations. The researchers found, using light microscopy, that the piglets in the treatment group that were fed fermented soybean meal had significantly taller villi at the varying locations, and had a significantly lower duodenal crypt depth in comparison to the piglets in the treatment group that were fed soybean meal. They also showed a significant increase in duodenal and jejunal protease and trypsin activities and a decrease in pancreatic trypsin activity. The findings obtained from this research demonstrate
that fermented soybean meal improves the morphology of the intestine as well as the activities of digestive enzymes (Feng et al. 2007).