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PATHOGENIC COCCI

GRAM-NEGATIVE INTESTINAL PATHOGENS

Manual for practical lessons

Poltava - 2006

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Staphylococcus

Clinical Manifestations

Staphylococci can cause many forms of infection. (1) *S aureus* causes superficial skin lesions (boils, styes) and localized abscesses in other sites. (2) *S aureus* causes deep-seated infections, such as osteomyelitis and endocarditis and more serious skin infections (furunculosis). (3) *S aureus* is a major cause of hospital acquired (noso-comial) infection of surgical wounds and, with *S epidermidis*, causes infections associated with indwelling medical devices. (4) *S aureus* causes food poisoning by releasing enterotoxins into food. (5) *S aureus* causes toxic shock syndrome by release of superantigens into the blood stream. (6) *S saprophiticus* causes urinary tract infections, especially in girls. (7) Other species of staphylococci (*S lugdunensis, S haemo-lyticus, S warneri, S schleiferi, S intermedius*) are infrequent pathogens.

Structure

Staphylococci are Gram-positive cocci 1 µm in diameter. They form clumps.

Classification

S aureus and *S intermedius* are coagulase positive. All other staphylococci are coagulase negative. They are salt tolerant and often hemolytic. Identification requires biotype analysis.

Natural Habitat

S aureus colonizes the nasal passage and axillae. *S epidermidis* is a common human skin commensal. Other species of staphylococci are infrequent human commensals. Some are commensals of other animals.

Pathogenesis

S aureus expresses many potential virulence factors. (1) Surface proteins that promote colonization of host tissues. (2) Factors that probably inhibit phagocytosis (capsule, immunoglobulin binding protein A). (3) Toxins that damage host tissues and cause disease symptoms. Coagulase-negative staphylococci are normally less virulent and express fewer virulence factors. *S epidermidis* readily colonizes implanted devices.

Host Defenses

Phagocytosis is the major mechanism for combatting staphylococcal infection. Antibodies are produced which neutralize toxins and promote opsonization. The capsule and protein A may interfere with phagocytosis. Biofilm growth on implants is impervious to phagocytosis.

Treatment

Infections acquired outside hospitals can usually be treated with penicillinaseresistant β -lactams. Hospital acquired infection is often caused by antibiotic resistant strains and can only be treated with vancomycin.

Antibiotic Resistance

Multiple antibiotic resistance is increasingly common in *S aureus* and *S epidermidis*. Methicillin resistance is indicative of multiple resistance. Methicillin-resistant *S aureus* (MRSA) causes outbreaks in hospitals and can be epidemic.

Epidemiology

Epidemiological tracing of *S aureus* is traditionally performed by phage typing, but has limitations. Molecular typing methods are being tested experimentally.

Diagnosis

Diagnosis is based on performing tests with colonies. Tests for clumping factor, coagulase, hemolysins and thermostable deoxyribonuclease are routinely used to identify *S aureus*. Commercial latex agglutination tests are available. Identification of *S epidermidis* is confirmed by commercial biotyping kits.

Control

Patients and staff carrying epidemic strains, particularly MRSA, should be isolated. Patients may be given disinfectant baths or treated with a topical antibiotic to eradicate carriage of MRSA. Infection control programs are used in most hospitals.

INTRODUCTION

Bacteria in the genus Staphylococcus are pathogens of man and other mammals. Traditionally they were divided into two groups on the basis of their ability to clot blood plasma (the coagulase reaction). The coagulase-positive staphylococci constitute the most pathogenic species *S aureus*. The coagulase-negative staphylococci (CNS) are now known to comprise over 30 other species. The CNS are common commensals of skin, although some species can cause infections. It is now obvious that the division of staphylococci into coagulase positive and negative is artificial and indeed, misleading in some cases. Coagulase is a marker for *S aureus* but there is no direct evidence that it is a virulence factor. Also, some natural isolates of *S aureus* are defective in coagulase. Nevertheless, the term is still in widespread use among clinical microbiologists.

S aureus expresses a variety of extracellular proteins and polysaccharides, some of which are correlated with virulence. Virulence results from the combined effect of many factors expressed during infection. Antibodies will neutralize staphylococcal toxins and enzymes, but vaccines are not available. Both antibiotic treatment and surgical drainage are often necessary to cure abscesses, large boils and wound infections. Staphylococci are common causes of infections associated with indwelling medical devices. These are difficult to treat with antibiotics alone and often require removal of the device. Some strains that infect hospitalized patients are resistant to most of the antibiotics used to treat infections, vancomycin being the only remaining drug to which resistance has not developed.

Taxonomy

DNA-ribosomal RNA (rRNA) hybridization and comparative oligonucleotide analysis of 16S rRNA has demonstrated that staphylococci form a coherent group at the genus level. This group occurs within the broad Bacillus-Lactobacillus-Streptococcus cluster defining Gram-positive bacteria with a low G + C content of DNA.

At least 30 species of staphylococci have been recognized by biochemical analysis and in particular by DNA-DNA hybridization. Eleven of these can be isolated from humans as commensals. *S aureus* (nares) and *S epidermidis* (nares, skin) are common commensals and also have the greatest pathogenic potential. *S saprophyticus* (skin, occasionally) is also a common cause of urinary tract infection. *S haemolyticus, S simulans, S cohnii, S warneri* and *S lugdunensis* can also cause infections in man.

Identification of Staphylococci in the Clinical laboratory

Structure

Staphylococci are Gram-positive cocci about $0.5 - 1.0 \ \mu m$ in diameter. They grow in clusters, pairs and occasionally in short chains. The clusters arise because staphylococci divide in two planes. The configuration of the cocci helps to distinguish micrococci and staphylococci from streptococci, which usually grow in chains. Observations must be made on cultures grown in broth, because streptococci grown on solid medium may appear as clumps. Several fields should be examined before deciding whether clumps or chains are present.

Catalase Test

The catalase test is important in distinguishing streptococci (catalase-negative) staphylococci which are catalase positive. The test is performed by flooding an agar slant or broth culture with several drops of 3% hydrogen peroxide. Catalase-positive cultures bubble at once. The test should not be done on blood agar because blood itself will produce bubbles.

Isolation and Identification

The presence of staphylococci in a lesion might first be suspected after examination of a direct Gram stain. However, small numbers of bacteria in blood preclude microscopic examination and require culturing first.

The organism is isolated by streaking material from the clinical specimen (or from a blood culture) onto solid media such as blood agar, tryptic soy agar or heart infusion agar. Specimens likely to be contaminated with other microorganisms can be plated on mannitol salt agar containing 7.5% sodium chloride, which allows the halo-tolerant staphylococci to grow. Ideally a Gram stain of the colony should be performed and tests made for catalase and coagulase production, allowing the coagulase-positive *S aureus* to be identified quickly. Another very useful test for *S aureus* is the production of thermostable deoxyribonuclease. *S aureus* can be confirmed by testing colonies for agglutination with latex particles coated with immunoglobulin G and fibrinogen which bind protein A and the clumping factor, respectively, on the bacterial cell surface. These are available from commercial suppliers (e.g., Staphaurex). The most recent latex test (Pastaurex) incorporates monoclonal antibodies to serotype 5 and 8 capsular polysaccharide in order to reduce the number of false

negatives. (Some recent clinical isolates of *S aureus* lack production of coagulase and/or clumping factor, which can make identification difficult.)

The association of *S epidermidis* (and to a lesser extent of other coagulase-negative staphylococci) with nosocomial infections associated with indwelling devices means that isolation of these bacteria from blood is likely to be important and not due to chance contamination, particularly if successive blood cultures are positive. Nowa-days, identification of S *epidermidis* and other species of *Staphylococcus* is performed using commercial biotype identification kits, such as API Staph Ident, API Staph-Trac, Vitek GPI Card and Microscan Pos Combo. These comprise preformed strips containing test substrates.

Epidemiology of *Staphylococcus Aureus* Infections

Because *S aureus* is a major cause of nosocomial and community-acquired infections, it is necessary to determine the relatedness of isolates collected during the investigation of an outbreak. Typing systems must be reproducible, discriminatory, and easy to interpret and to use. The traditional method for typing *S aureus* is phage-typing. This method is based on a phenotypic marker with poor reproducibility. Also, it does not type many isolates (20% in a recent survey at the Center for Disease Control and Prevention), and it requires maintenance of a large number of phage stocks and propagating strains and consequently can be performed only by specialist reference laboratories.

Many molecular typing methods have been applied to the epidemiological analysis of *S aureus*, in particular, of methicillin-resistant strains (MRSA). Plasmid analysis has been used extensively with success, but suffers the disadvantage that plasmids can easily be lost and acquired and are thus inherently unreliable. Methods designed to recognize restriction fragment length polymorphisms (RFLP) using a variety of gene probes, including rRNA genes (ribotyping), have had limited success in the epidemiology of MRSA. In this technique the choice of restriction enzyme used to cleave the genomic DNA, as well as the probes, is crucial. Random primer PCR offers potential for discriminating between strains but a suitable primer has yet to be identified for *S aureus*. The method currently regarded as the most reliable is pulsed field gel electrophoresis, where genomic DNA is cut with a restriction enzyme that generates large fragments of 50-700 kb.

Clinical Manifestations of *S Aureus*

S aureus is notorious for causing boils, furuncles, styes, impetigo and other superficial skin infections in humans (Figure 12-1). It may also cause more serious infections, particularly in persons debilitated by chronic illness, traumatic injury, burns or immunosuppression. These infections include pneumonia, deep abscesses, osteomyelitis, endocarditis, phlebitis, mastitis and meningitis, and are often associated with hospitalized patients rather than healthy individuals in the community. *S aureus* and *S epidermidis* are common causes of infections associated with indwelling devices such as joint prostheses, cardiovascular devices and artificial heart valves (Fig. 12-2).



FIGURE 12-1 Pathogenesis of staphylococcal infections



FIGURE 12-2 Infections associated with indwelling devices

Pathogenesis of S Aureus Infections

S aureus expresses many cell surface-associated and extracellular proteins that are potential virulence factors. For the majority of diseases caused by this organism, pathogenesis is multifactorial. Thus it is difficult to determine precisely the role of

any given factor. This also reflects the inadequacies of many animal models for staphylococcal diseases.

However, there are correlations between strains isolated from particular diseases and expression of particular factors, which suggests their importance in pathogenesis. With some toxins, symptoms of a human disease can be reproduced in animals with pure proteins. The application of molecular biology has led to recent advances in the understanding of pathogenesis of staphylococcal diseases. Genes encoding potential virulence factors have been cloned and sequenced and proteins purified. This has facilitated studies at the molecular level on their modes of action, both in in vitro and in model systems. In addition, genes encoding putative virulence factors have been inactivated, and the virulence of the mutants compared to the wild-type strain in animal models. Any diminution in virulence implicates the missing factor. If virulence is restored when the gene is returned to the mutant then "Molecular Koch's Postulates" have been fulfilled. Several virulence factors of *S aureus* have been confirmed by this approach.

Adherence

In order to initiate infection the pathogen must gain access to the host and attach to host cells or tissues.

S aureus Adheres to Host Proteins

S aureus cells express on their surface proteins that promote attachment to host proteins such as laminin and fibronectin that form part of the extracellular matrix (Figure 12-3). Fibronectin is present on epithelial and endothelial surfaces as well as being a component of blood clots. In addition, most strains express a fibrinogen/fibrin binding protein (the clumping factor) which promotes attachment to blood clots and traumatized tissue. Most strains of *S aureus* express fibronectin and fibrinogen-binding proteins.



FIGURE 12-3 Summary of virulence factors of Staphylococcus aureus

The receptor which promotes attachment to collagen is particularly associated with strains that cause osteomyelitis and septic arthritis. Interaction with collagen may al-

so be important in promoting bacterial attachment to damaged tissue where the underlying layers have been exposed.

Evidence that these staphylococcal matrix-binding proteins are virulence factors has come from studying defective mutants in vitro adherence assays and in experimental infections. Mutants defective in binding to fibronectin and to fibrinogen have reduced virulence in a rat model for endocarditis, suggesting that bacterial attachment to the sterile vegetations caused by damaging the endothelial surface of the heart valve is promoted by fibronectin and fibrinogen. Similarly, mutants lacking the collagen-binding protein have reduced virulence in a mouse model for septic arthritis. Furthermore, the soluble ligand-binding domain of the fibrinogen, fibronectin and collagen-binding proteins expressed by recombinant methods strongly blocks interactions of bacterial cells with the corresponding host protein.

Role of Adherence in Infections Associated with Medical Devices

Infections associated with indwelling medical devices ranging from simple intravenous catheters to prosthetic joints and replacement heart valves can be caused by *S aureus* and *S epidermidis* (Figure 12-2). Very shortly after biomaterial is implanted in the human body it becomes coated with a complex mixture of host proteins and platelets. In one model system involving short-term contact between biomaterial and blood, fibrinogen was shown to be the dominant component and was primarily responsible for adherence of *S aureus* in subsequent in vitro assays. In contrast, with material that has been in the body for longer periods (e.g., human intravenous catheters) the fibrinogen is degraded and no longer promotes bacterial attachment. Instead, fibronectin, which remains intact, becomes the predominant ligand promoting attachment.

Adherence to Endothelial Cells

S aureus can adhere to the surface of cultured human endothelial cells and become internalized by a phagocytosis-like process. It is not clear if attachment involves a novel receptor or a known surface protein of *S aureus*. Some researchers think that *S aureus* can initiate endocarditis by attaching to the undamaged endothelium. Others feel that trauma of even a very minor nature is required to promote attachment of bacteria.

Avoidance of Host Defenses

S aureus expresses a number of factors that have the potential to interfere with host defense mechanisms. However, strong evidence for a role in virulence of these factors is lacking.

Capsular Polysaccharide

The majority of clinical isolates of *S aureus* express a surface polysaccharide of either serotype 5 or 8. This has been called a microcapsule because it can be visualized only by electron microscopy after antibody labeling, unlike the copious capsules of other bacteria which are visualized by light microscopy. *S aureus* isolated from infections expresses high levels of polysaccharide but rapidly loses it upon laboratory subculture. The function of the capsule is not clear. It may impede phagocytosis, but in in vitro tests this was only demonstrated in the absence of complement. Conversely, comparing wild-type and a capsule defective mutant strain in an endocarditis model suggested that polysaccharide expression actually impeded colonization of damaged heart valves, perhaps by masking adhesins.

Protein A

Protein A is a surface protein of *S aureus* which binds immunoglobulin G molecules by the Fc region (Fig. 12-3). In serum, bacteria will bind IgG molecules the wrong way round by this non-immune mechanism. In principle this will disrupt opsonization and phagocytosis. Indeed mutants of *S aureus* lacking protein A are more efficiently phagocytozed in vitro, and studies with mutants in infection models suggest that protein A enhances virulence.

Leukocidin

S aureus can express a toxin that specifically acts on polymorphonuclear leukocytes. Phagocytosis is an important defense against staphylococcal infection so leukocidin should be a virulence factor. This toxin is discussed in more detail in the next section.

Damage to the Host

S aureus can express several different types of protein toxins which are probably responsible for symptoms during infections. Some damage the membranes of erythrocytes, causing hemolysis; but it is unlikely that hemolysis is relevant in vivo. The leukocidin causes membrane damage to leukocytes and is not hemolytic. Systemic release of α -toxin causes septic shock, while enterotoxins and TSST-1 cause toxic shock.

Membrane Damaging Toxins

(a) a-toxin

The best characterized and most potent membrane-damaging toxin of *S aureus* is α -toxin. It is expressed as a monomer that binds to the membrane of susceptible cells. Subunits then oligomerize to form hexameric rings with a central pore through which cellular contents leak.

Susceptible cells have a specific receptor for a-toxin which allows low concentrations of toxin to bind, causing small pores through which monovalent cations can pass. At higher concentrations, the toxin reacts non-specifically with membrane lipids, causing larger pores through which divalent cations and small molecules can pass. However, it is doubtful if this is relevant under normal physiological conditions.

In humans, platelets and monocytes are particularly sensitive to α -toxin. They carry high affinity sites which allow toxin to bind at concentrations that are physiologically relevant. A complex series of secondary reactions ensue, causing release of eicosanoids and cytokines which trigger production of inflammatory mediators. These events cause the symptoms of septic shock that occur during severe infections caused by *S aureus*.

The notion that α -toxin is a major virulence factor of *S aureus* is supported by studies with the purified toxin in animals and in organ culture. Also, mutants lacking α -toxin are less virulent in a variety of animal infection models.

(b) **B-toxin**

 β -toxin is a sphingomyelinase which damages membranes rich in this lipid. The classical test for β -toxin is lysis of sheep erythrocytes. The majority of human isolates of *S aureus* do not express β -toxin. A lysogenic bacteriophage is inserted into the gene that encodes the toxin. This phenomenon is called negative phage conversion. Some of the phages that inactivate the β -toxin gene carry the determinant for an enterotoxin and staphylokinase (see below).

In contrast the majority of isolates from bovine mastitis express β -toxin, suggesting that the toxin is important in the pathogenesis of mastitis. This is supported by the fact that β -toxin-deficient mutants have reduced virulence in a mouse model for mastitis.

(c) **\delta-toxin**

The δ -toxin is a very small peptide toxin produced by most strains of *S aureus*. It is also produced by *S epidermidis* and *S lugdunensis*. The role of δ -toxin in disease is unknown.

(d) γ -toxin and leukocidin

The γ -toxin and the leukocidins are two-component protein toxins that damage membranes of susceptible cells. The proteins are expressed separately but act together to damage membranes. There is no evidence that they form multimers prior to insertion into membranes. The γ -toxin locus expresses three proteins. The B and C components form a leukotoxin with poor hemolytic activity, whereas the A and B components are hemolytic and weakly leukotoxic.

The classical Panton and Valentine (PV) leukocidin is distinct from the leukotoxin expressed by the γ -toxin locus. It has potent leukotoxicity and, in contrast to γ -toxin, is non-hemolytic. Only a small fraction of *S aureus* isolates (2% in one survey) express the PV leukocidin, whereas 90% of those isolated from severe dermonecrotic lesions express this toxin. This suggests that PV leukocidin is an important factor in necrotizing skin infections.

PV-leukocidin causes dermonecrosis when injected subcutaneously in rabbits. Furthermore, at a concentration below that causing membrane damage, the toxin releases inflammatory mediators from human neutrophils, leading to degranulation. This could account for the histology of dermonecrotic infections (vasodilation, infiltration and central necrosis).

Superantigens: enterotoxins and toxic shock syndrome toxin

S aureus can express two different types of toxin with superantigen activity, enterotoxins, of which there are six serotypes (A, B, C, D, E and G) and toxic shock syndrome toxin (TSST-1). Enterotoxins cause diarrhea and vomiting when ingested and are responsible for staphylococcal food poisoning. When expressed systemically, enterotoxins can cause toxic shock syndrome (TSS) - indeed enterotoxins B and C cause 50% of non-menstrual TSS. TSST-1 is very weakly related to enterotoxins and does not have emetic activity. TSST-1 is responsible for 75% of TSS, including all menstrual cases. TSS can occur as a sequel to any staphylococcal infection if an enterotoxin or TSST-1 is released systemically and the host lacks appropriate neutralizing antibodies. Tampon-associated TSS is not a true infection, being caused by growth of *S aureus* in a tampon and absorption of the toxin into the blood stream. TSS came to prominence with the introduction of super-absorbent tampons; and although the number of such cases has decreased dramatically, they still occur despite withdrawal of certain types of tampons from the market.

Superantigens stimulate T cells non-specifically without normal antigenic recognition (Figure 12-4). Up to one in five T cells may be activated, whereas only 1 in 10,000 are stimulated during antigen presentation. Cytokines are released in large amounts, causing the symptoms of TSS. Superantigens bind directly to class II major histocompatibility complexes of antigen-presenting cells outside the conventional antigen-binding grove. This complex recognizes only the Vb element of the T cell receptor. Thus any T cell with the appropriate Vb element can be stimulated, whereas normally antigen specificity is also required in binding.



FIGURE 12-4 Superantigens and the non-specific stimulation of T cells Epidermolytic (exfoliative) toxin (ET)

This toxin causes the scalded skin syndrome in neonates, with widespread blistering and loss of the epidermis. There are two antigenically distinct forms of the toxin, ETA and ETB. There is evidence that these toxins have protease activity. Both toxins have a sequence similarity with the *S aureus* serine protease, and the three most important amino acids in the active site of the protease are conserved. Furthermore, changing the active site of serine to a glycine completely eliminated toxin activity. However, ETs do not have discernible proteolytic activity but they do have esterase activity. It is not clear how the latter causes epidermal splitting. It is possible that the toxins target a very specific protein which is involved in maintaining the integrity of the epidermis.

Other Extracellular Proteins

Coagulase

Coagulase is not an enzyme. It is an extracellular protein which binds to prothrombin in the host to form a complex called staphylothrombin. The protease activity characteristic of thrombin is activated in the complex, resulting in the conversion of fibrinogen to fibrin. This is the basis of the tube coagulase test, in which a clot is formed in plasma after incubation with the S aureus broth-culture supernatant. Coagulase is a traditional marker for identifying *S aureus* in the clinical microbiology laboratory. However, there is no evidence that it is a virulence factor, although it is reasonable to speculate that the bacteria could protect themselves from host defenses by causing localized clotting. Notably, coagulase deficient mutants have been tested in several infection models but no differences from the parent strain were observed. There is some confusion in the literature concerning coagulase and clumping factor, the fibrinogen-binding determinant on the *S aureus* cell surface. This is partly due to loose terminology, with the clumping factor sometimes being referred to as bound coagulase. Also, although coagulase is regarded as an extracellular protein, a small fraction is tightly bound on the bacterial cell surface where it can react with prothrombin. Finally, it has recently been shown that the coagulase can bind fibrinogen as well as thrombin, at least when it is extracellular. Genetic studies have shown unequivocally that coagulase and clumping factor are distinct entities. Specific mutants lacking coagulase retain clumping factor activity, while clumping factor mutants express coagulase normally.

Staphylokinase

Many strains of *S aureus* express a plasminogen activator called staphylokinase. The genetic determinant is associated with lysogenic bacteriophages. A complex formed between staphylokinase and plasminogen activates plasmin-like proteolytic activity which causes dissolution of fibrin clots. The mechanism is identical to streptokinase, which is used in medicine to treat patients suffering from coronary thrombosis. As with coagulase there is no evidence that staphylokinase is a virulence factor, although it seems reasonable to imagine that localized fibrinolysis might aid in bacterial spreading.

Enzymes

S aureus can express proteases, a lipase, a deoxyribonuclease (DNase) and a fatty acid modifying enzyme (FAME). The first three probably provide nutrients for the bacteria, and it is unlikely that they have anything but a minor role in pathogenesis. However, the FAME enzyme may be important in abscesses, where it could modify anti-bacterial lipids and prolong bacterial survival. The thermostable DNase is an important diagnostic test for identification of *S aureus*.

Coagulase Negative Staphylococci

Staphylococci other than *S aureus* can cause infections in man. *S epidermidis* is the most important coagulase-negative staphylococcus (CNS) species and is the major cause of infections associated with prosthetic devices and catheters. CNS also cause peritonitis in patients receiving continuous ambulatory peritoneal dialysis and endocarditis in those with prosthetic valves. These infections are not usually nosocomially acquired. Other species such as *S haemolyticus*, *S warneri*, *S hominis*, *S capitis*, *S intermedius*, *S schleiferi* and *S simulans* are infrequent pathogens. *S lugdunesis* is a newly recognized species. It is probably more pathogenic than are other CNS species, with cases of endocarditis and other infections being reported. It is likely that the incidence of infections caused by these organisms is underestimated because of difficulties in identification. Diagnosis of CNS infections is difficult. Infections are often indolent and chronic with few obvious symptoms. This is due to the smaller array of virulence factors and toxins compared to those in the case of S aureus. S epidermidis is a skin commensal and is one of the most common contaminants of samples sent to the diagnostic laboratory, while S lugdunensis is often confused with S aureus. Precise identification of CNS species requires the use of expensive test kits, such as the API-Staph. In contrast to S aureus, little is known about mechanisms of pathogenesis of S epidermidis infections. Adherence is obviously a crucial step in the initiation of foreign body infections. Much research has been done on the interaction between S epider*midis* and plastic material used in implants, and a polysaccharide adhesion (PS/A) has been identified. Mutants lacking PS/A are less virulent in an animal model for foreign body infection, and immunization with purified PS/A is protective. Bacteriaplastic interactions are probably important in colonization of catheters through the point of entry. However, host proteins are quickly deposited on implants. S epidermidis does not bind to fibrinogen but most isolates bind fibronectin, albeit less avidly than *S aureus*. However, it is not known if a protein analogous to the fibronectin binding protein of *S aureus* is involved.

A characteristic of clinical isolates of *S epidermidis* is the production of "slime." This is a controversial topic. Some feel that slime is an in vitro manifestation of the ability to form a biofilm in vivo, for example on the surface of a prosthetic device, and is thus a virulence marker. In vitro, slime is formed during growth in broth as a biofilm on the surface of the growth vessel. The composition of this slime is probably influenced by the growth medium. One study with defined medium showed that the slime was predominantly secreted teichoic acid, a polymer normally found in the cell wall of staphylococci. Some polysaccharides in slime from bacteria grown on solid medium are derived from the agar.

Resistance of Staphylococci to Antimicrobial Drugs

Hospital strains of *S aureus* are often resistant to many different antibiotics. Indeed strains resistant to all clinically useful drugs, apart from the glycopeptides vancomycin and teicoplanin, have been described. The term MRSA refers to methicillin resistance and most methicillin-resistant strains are also multiply resistant. Plasmid-associated vancomycin resistance has been detected in some enterococci and the resistance determinant has been transferred from enterococci to *S aureus* in the laboratory and may occur naturally. *S epidermidis* nosocomial isolates are also often resistant to several antibiotics including methicillin. In addition, *S aureus* expresses resistance to antiseptics and disinfectants, such as quaternary ammonium compounds, which may aid its survival in the hospital environment.

Since the beginning of the antibiotic era *S aureus* has responded to the introduction of new drugs by rapidly acquiring resistance by a variety of genetic mechanisms including (1) acquisition of extrachromosomal plasmids or additional genetic information in the chromosome via transposons or other types of DNA insertion and (2) by mutations in chromosomal genes (Table 12-1).

Many plasmid-encoded determinants have recently become inserted into the chromosome at a site associated with the methicillin resistance determinant. There may be an advantage to the organism having resistance determinants in the chromosome because they will be more stable. There are essentially four mechanisms of resistance to antibiotics in bacteria: (1) enzymatic inactivation of the drug, (2) alterations to the drug target to prevent binding, (3) accelerated drug efflux to prevent toxic concentrations accumulating in the cell, and (4) a by-pass mechanism whereby an alternative drug-resistant version of the target is expressed.

Future Prospects

Antimicrobial Drugs

Ever since the first use of penicillin, *S aureus* has shown a remarkable ability to adapt. Resistance has developed to new drugs within a short time of their introduction. Some strains are now resistant to most conventional antibiotics. It is worrisome that there do not seem to be any new antibiotics on the horizon. Any recent developments have been modifications to existing drugs.

The original strategy used by the pharmaceutical industry to find antimicrobial drugs was to screen natural products and synthetic chemicals for antimicrobial activity. The mechanism of action was then investigated.

New approaches are being adopted to find the next generation of antimicrobials. Potential targets such as enzymes involved in an essential function (e.g., in cell division) are identified based on knowledge of bacterial physiology and metabolism. Screening methods are then developed to identify inhibitors of a specific target molecule. In addition, with detailed molecular knowledge of the target molecule, specific inhibitors can be designed.

Vaccines and New Approaches to Combatting Nosocomial Infections

No vaccine is currently available to combat staphylococcal infections. There may now be a case for considering methods to prevent disease, particularly in hospitalized patients.

Hyperimmune serum from human volunteer donors or humanized monoclonal antibodies directed towards surface components (e.g., capsular polysaccharide or surface protein adhesions) could both prevent bacterial adherence and also promote phagocytosis of bacterial cells. Indeed a prototype vaccine based on capsular polysaccharide from *S aureus* has been administered to volunteers to raise hyperimmune serum, which could be given to patients in hospital before surgery. A vaccine based on fibronectin binding protein induces protective immunity against mastitis in cattle and might also be used as a vaccine in humans.

When the molecular basis of the interactions between the bacterial surface proteins and the host matrix protein ligands are known it might be possible to design compounds that block the interactions and thus prevent bacterial colonization. These could be administered systemically or topically.

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Streptococcus

General Concepts

Streptococcus pyogenes, other Streptococci, and Enterococcus

Clinical Manifestations

Acute *Streptococcus pyogenes* infections may take the form of pharyngitis, scarlet fever (rash), impetigo, cellulitis, or erysipelas. Invasive infections can result in necrotizing fasciitis, myositis and streptococcal toxic shock syndrome. Patients may also develop immune-mediated sequelae such as acute rheumatic fever and acute glomerulonephritis. *S agalactiae* may cause meningitis, neonatal sepsis, and pneumonia in neonates; adults may experience vaginitis, puerperal fever, urinary tract infection, skin infection, and endocarditis. Viridans streptococci can cause endocarditis, and Enterococcus is associated with urinary tract and biliary tract infections. Anaerobic streptococci participate in mixed infections of the abdomen, pelvis, brain, and lungs.

Structure

Streptococci are Gram-positive, nonmotile, nonsporeforming, catalase-negative cocci that occur in pairs or chains. Older cultures may lose their Gram-positive character. Most streptococci are facultative anaerobes, and some are obligate (strict) anaerobes.

Most require enriched media (blood agar). Group A streptococci have a hyaluronic acid capsule.

Classification and Antigenic Types

Streptococci are classified on the basis of colony morphology, hemolysis, biochemical reactions, and (most definitively) serologic specificity. They are divided into three groups by the type of hemolysis on blood agar: b-hemolytic (clear, complete lysis of red cells), a hemolytic (incomplete, green hemolysis), and g hemolytic (no hemolysis). Serologic grouping is based on antigenic differences in cell wall carbohydrates (groups A to V), in cell wall pili-associated protein, and in the polysaccharide capsule in group B streptococci.

Pathogenesis

Streptococci are members of the normal flora. Virulence factors of group A streptococci include (1) M protein and lipoteichoic acid for attachment; (2) a hyaluronic acid capsule that inhibits phagocytosis; (3) other extracellular products, such as pyrogenic (erythrogenic) toxin, which causes the rash of scarlet fever; and (4) streptokinase, streptodornase (DNase B), and streptolysins. Some strains are nephritogenic. Immune-mediated sequelae do not reflect dissemination of bacteria. Nongroup A strains have no defined virulence factors.

Host Defenses

Antibody to M protein gives type-specific immunity to group A streptococci. Antibody to erythrogenic toxin prevents the rash of scarlet fever. Immune mechanisms are important in the pathogenesis of acute rheumatic fever. Maternal IgG protects the neonate against group B streptococci.

Epidemiology

Group A β-hemolytic streptococci are spread by respiratory secretions and fomites. The incidence of both respiratory and skin infections peaks in childhood. Infection can be transmitted by asymptomatic carriers. Acute rheumatic fever was previously common among the poor; susceptibility may be partly genetic. Group B streptococci are common in the normal vaginal flora and occasionally cause invasive neonatal infection.

Diagnosis

Diagnosis is based on cultures from clinical specimens. Serologic methods can detect group A or B antigen; definitive antigen identification is by the precipitin test. Bacitracin sensitivity presumptively differentiates group A from other b-hemolytic streptococci (B, C, G); group B streptococci typically show hippurate hydrolysis; group D is differentiated from other viridans streptococci by bile solubility and optochin sensitivity. Acute glomerulonephritis and acute rheumatic fever are identified by antistreptococcal antibody titers. In addition, acute rheumatic fever is diagnosed by clinical criteria.

Control

Prompt penicillin treatment of streptococcal pharyngitis reduces the antigenic stimulus and therefore prevents glomerulonephritis and acute rheumatic fever. Vancomycin resistance among the enterococci is an emerging microbial threat. Vaccines are under development.

Streptococcus pneumoniae

Clinical Manifestations

S pneumoniae causes pneumonia, meningitis, and sometimes occult bacteremia.

Structure

Pneumococci are lancet-shaped, catalase-negative, capsule-forming, α -hemolytic cocci or diplococci. Autolysis is enhanced by adding bile salts.

Classification and Antigenic Types

There are more than 85 antigenic types of *S pneumoniae*, which are determined by capsule antigens. There is no Lancefield group antigen.

Pathogenesis

S pneumoniae is a normal member of the respiratory tract flora; invasion results in pneumonia. The best defined virulence factor is the polysaccharide capsule, which protects the bacterium against phagocytosis.

Host Defenses

Protection against infection depends on a normal mucociliary barrier and intact phagocytic and T-independent immune responses. Type-specific anti-capsule anti-body is protective.

Epidemiology

Pneumococcal pneumonia is most common in elderly, debilitated, or immunosuppressed individuals. The disease often sets in after a preceding viral infection damages the respiratory ciliated epithelium; incidence therefore peaks in the winter.

Diagnosis

Diagnosis is based on a sputum Gram stain and culture; blood or cerebrospinal fluid may also be cultured. Capsular antigen can be detected serologically. Pneumococci are distinguished from viridans streptococci by the quellung (capsular swelling) reaction, bile solubility, and optochin inhibition.

Control

Treatment is usually with penicillin. However, strains resistant to penicillin and multiple antibiotics are rapidly emerging. A vaccine is available.

INTRODUCTION

The genus *Streptococcus*, a heterogeneous group of Gram-positive bacteria, has broad significance in medicine and industry. Various streptococci are important ecologically as part of the normal microbial flora of animals and humans; some can also cause diseases that range from subacute to acute or even chronic. Among the significant human diseases attributable to streptococci are scarlet fever, rheumatic heart disease, glomerulonephritis, and pneumococcal pneumonia. Streptococci are essential in industrial and dairy processes and as indicators of pollution.

The nomenclature for streptococci, especially the nomenclature in medical use, has been based largely on serogroup identification of cell wall components rather than on species names. For several decades, interest has focused on two major species that cause severe infections: *S pyogenes* (group A streptococci) and *S pneumoniae* (pneumococci). In 1984, two members were assigned a new genus - the group D enterococcal species (which account for 98% of human enterococcal infections) be-

came *Enterococcus faecalis* (the majority of human clinical isolates) and *E faecium* (associated with a remarkable capacity for antibiotic resistance).

In recent years, increasing attention has been given to other streptococcal species, partly because innovations in serogrouping methods have led to advances in understanding the pathogenetic and epidemiologic significance of these species. A variety of cell-associated and extracellular products are produced by streptococci, but their cause-effect relationship with pathogenesis has not been defined. Some of the other medically important streptococci are *S agalactiae* (group B), an etiologic agent of neonatal disease; *E faecalis* (group D), a major cause of endocarditis, and the viridans streptococci. Particularly for the viridans streptococci, taxonomy and nomenclature are not yet fully reliable or consistent. Important members of the viridans streptococci, normal commensals, include *S mutans* and *S sanguis* (involved in dental caries), *S mitis* (associated with bacteremia, meningitis, periodontal disease and pneumonia), and "*S milleri*" (associated with suppurative infections in children and adults). There remains persistent taxonomic confusion regarding "*S milleri*." These and other streptococci of medical importance are listed in Table 13-1 by serogroup designation, normal ecologic niche, and associated disease.

Clinical Manifestations

In humans, diseases associated with the streptococci occur chiefly in the respiratory tract, bloodstream, or as skin infections. Human disease is most commonly associated with Group A streptococci. Acute group A streptococcal disease is most often a respiratory infection (pharyngitis or tonsillitis) or a skin infection (pyoderma). Also medically significant are the late immunologic sequelae, not directly attributable to dissemination of bacteria, of group A infections (rheumatic fever following respiratory infection and glomerulonephritis following respiratory or skin infection) which remain a major worldwide health concern. Much effort is being directed toward clarifying the risk and mechanisms of these sequelae and identifying rheumatogenic and nephritogenic strains. *S pneumoniae* remains a primary cause of serious focal and systemic infections, the first most common cause of community acquired pneumonia in the United States and of fatal bacterial pneumonia in developing countries. Hemorrhagic shock in association with *S pneumoniae* sepsis in previously healthy children has been reported recently in the United States. Of major biologic importance is a renewed interest in safe and effective streptococcal vaccines.

Structure

Both *S pyogenes* and *S pneumoniae* are Gram-positive cocci, nonmotile, and nonsporulating; they usually require complex culture media. *S pyogenes* characteristically is a round-to-ovoid coccus 0.6-1.0 μ m in diameter (Fig. 13-1). They divide in one plane and thus occur in pairs, or (especially in liquid media or clinical material) in chains of varying lengths. S *pneumoniae* appears as a 0.5-1.25 μ m diplococcus, typically described as lancet-shaped but sometimes difficult to distinguish morphologically from other streptococci. Streptococcal cultures older than the logarithmic phase, which is the most active growth period of a culture, may lose their Grampositive staining characteristics.



FIGURE 13-1 Morphology of the streptococci in comparison with staphylococci. Streptococci divide in a single plane and tend not to separate, causing chain formation. Capsules are antiphagocytic.

Unlike *Staphylococcus* (Chapter 12), all streptococci lack the enzyme catalase. Most are facultative anaerobes but some are obligate anaerobes. Streptococci often have a mucoid or smooth colonial morphology, and *S pneumoniae* colonies exhibit a central depression caused by rapid partial autolysis. As *S pneumoniae* colonies age, viability is lost during fermentative growth in the absence of catalase and peroxidase because of the accumulation of peroxide. Some group B and D streptococci produce pigment. Recently, nutritionally deficient streptococci (also known as wall-deficient, L form, thiol-requiring, satelliting, or pyridoxal-dependent) have been recovered from a variety of clinical sources, including blood, abscesses, and oral and urethral ulcers. These variants demonstrate bizarre pleomorphism microscopically and do not grow on routine subculture.

<u>Classification, Antigenic Types and Extracellular Growth Products</u> The type of hemolytic reaction displayed on blood agar has long been used to classify the streptococci. β -Hemolysis is associated with complete lysis of red cells surrounding the colony, whereas α -hemolysis is a partial or "greening" hemolysis associated with reduction of red cell hemoglobin. Nonhemolytic colonies have been termed γ -hemolytic. Hemolysis is affected by the species and age of red cells as well as by other properties of the base medium. Use of the hemolytic reaction in classification is not completely satisfactory. Some group A streptococci appear nonhemolytic; group B can manifest α -, β -, or even γ -hemolysis; most *S pneumoniae* are α -hemolytic but can cause β -hemolysis during anaerobic incubation. The viridans group, although linked by the property of α -hemolysis, is actually an extremely diverse group of organisms that does not usually react with Lancefield grouping sera. The taxonomy and biochemical and genetic relationships of these organisms continue to be.

Antigenic Types

The cell wall structure of group A streptococci is among the most studied of any bacteria (Fig. 13-2). The cell wall is composed of repeating units of Nacetylglucosamine and N-acetylmuramic acid, the standard peptidoglycan. For decades, the definitive identification of streptococci has rested on the serologic reactivity of cell wall polysaccharide antigens originally delineated by Rebecca Lancefield. Eighteen group-specific antigens were established. The group A polysaccharide is a polymer of N-acetylglucosamine and rhamnose. Some group antigens are shared by more than one species; no Lancefield group antigen has been identified for *S pneumoniae* or for some other α - or γ -streptococci. With advances in serologic methods, other streptococci have been shown to possess several established group antigens.



FIGURE 13-2 Cell surface structure of *S pyogenes* **and extracellular substances.** The cell wall also consists of several structural proteins (Figure 13-2). In group A streptococci, the R and T proteins may serve as epidemiologic markers, but the M proteins are clearly virulence factors associated with resistance to phagocytosis. More than 50 types of *S pyogenes* M proteins have been identified on the basis of antigenic specificity. Both the M proteins and lipoteichoic acid are supported externally to the cell wall on fimbriae, and the lipoteichoic acid, in particular, appears to mediate bacterial attachment to host epithelial cells. M protein, peptidoglycan, N-acetylglucosamine, and group-specific carbohydrate portions of the cell wall have antigenic epitopes similar in size and charge to those of mammalian muscle and connective tissue. Recently emerging strains of increased virulence are distinctly mucoid, rich in M protein and highly encapsulated.

The capsule of *S pyogenes* is composed of hyaluronic acid, which is chemically similar to that of host connective tissue and is therefore nonantigenic. In contrast, the antigenically reactive and chemically distinct capsular polysaccharide of *S pneumoniae* allows the single species to be separated into more than 80 serotypes. The antiphagocytic *S pneumoniae* capsule is the most clearly understood virulence factor of these organisms; type 3 *S pneumoniae*, which produces copious quantities of capsular material, are the most virulent. Unencapsulated *S pneumoniae* are avirulent. The polysaccharide capsule in *S agalactiae* allows differentiation into types Ia, Ib, Ic, II and III.

Finally, the cytoplasmic membrane of *S pyogenes* has antigens similar to those of human cardiac, skeletal, and smooth muscle, heart valve fibroblasts, and neuronal tissues, resulting in a molecular mimicry.

Extracellular Growth Products

The importance of the interaction of streptococcal products with mammalian blood and tissue components is becoming widely recognized. The soluble extracellular growth products or toxins of the streptococci, especially of *S pyogenes* (see Fig. 13-2), have been studied intensely. Streptolysin S is an oxygen-stable cytolysin; Streptolysin O is a reversibly oxygen-labile cytolysin. Both are leukotoxic, as is NADase. Hyaluronidase (spreading factor) can digest host connective tissue hyaluronic acid as well as the organism's own capsule. Streptokinases participate in fibrin lysis. Streptodornases A-D possess deoxyribonuclease activity; B and D possess ribonuclease activity as well. Protease activity similar to that in Staph aureus has been shown in strains causing soft tissue necrosis or toxic shock syndrome. This large repertoire of products may be important in the pathogenesis of *S pyogenes* by enhancing virulence; however, antibodies to these products appear not to protect the host even though they have diagnostic importance.

Three pyrogenic exotoxins of *S pyogenes* (SPEs) are recognized: types A, B, C. These toxins act as superantigens by a mechanism similar to those described for staphylococci, not requiring processing by antigen presenting cells. Rather, they stimulate T cells by binding class II MHC molecules directly and nonspecifically. With superantigens about 20% of T cells may be stimulated (vs 1/10,000 T cells stimulated by conventional antigens) resulting in massive detrimental cytokine release. When *S pyogenes* is lysogenized by certain bacteriophages, the SPEs A or C are produced; nonlysogenized strains are atoxic. SPE B is encoded by the bacterial chromosome. Re-emergence in the late 1980's of these exotoxin-producing strains has been associated with a toxic shock-like syndrome similar in pathogenesis and manifestation to staphylococcal toxic shock syndrome (Ch.12) and other forms of invasive disease associated with severe tissue destruction. SPE's have also been identified from non group A streptococci (groups B, C, F. G) in association with the toxic shock-like syndrome.

Virulence factors in the other streptococcal species, including the enterococci, are less well identified. In group B streptococci, carbohydrate surface antigens associat-

ed with antiphagocytosis have been identified, as has neuraminidase, which may play a role in pathogenesis. Among the viridans streptococci, production of the exopolysaccharide (glycocalyx) is associated with the ability to adhere to the cardiac valves and to form vegetations on the valve leaflets.

Pathogenesis

Streptococcus pyogenes and Streptococcus pneumoniae

Streptococci vary widely in pathogenic potential. Despite the remarkable array of cell-associated and extracellular products previously described (Fig.13- 2), no clear scheme of pathogenesis has been worked out. *S pneumoniae* and, to a lesser extent, *S pyogenes* are part of the normal human nasopharyngeal flora. Their numbers are usually limited by competition from the nasopharyngeal microbial ecosystem and by nonspecific host defense mechanisms, but failure of these mechanisms can result in disease. More often disease results from the acquisition of a new strain following alteration of the normal flora. *S pyogenes* causes inflammatory purulent lesions at the portal of entry, often the upper respiratory tract or the skin. Some strains of strepto-cocci show a predilection for the respiratory tract; others, for the skin. Generally, streptococcal isolates from the pharynx and respiratory tract do not cause skin infections.

Invasion of other portions of the upper or lower respiratory tracts results in infections of the middle ear (otitis media), sinuses (sinusitis), or lungs (pneumonia). In addition, meningitis can occur by direct extension of infection from the middle ear or sinuses to the meninges or by way of bloodstream invasion from the pulmonary focus. Bacteremia can also result in infection of bones (osteomyelitis) or joints (arthritis).

S pyogenes (a group A streptococcus) is the leading cause of uncomplicated bacterial pharyngitis and tonsillitis (Fig. 13-3). Indeed, only group A streptococci are sought routinely in cases of pharyngitis, although groups B, C, and G are sometimes identified. S pyogenes infections can also result in sinusitis, otitis, mastoiditis, pneumonia with empyema, joint or bone infections, necrotizing fasciitis or myositis, and, more infrequently, in meningitis or endocarditis. S pyogenes infections of the skin can be superficial (impetigo) or deep (cellulitis). Although scarlet fever was formerly a severe complication of streptococcal infection, because of antibiotic therapy it is now little more than streptococcal pharyngitis accompanied by rash. Similarly, erysipelas, a form of cellulitis accompanied by fever and systemic toxicity, is less common today. There has, however, been an apparent recent increase in variety, severity and sequelae of S pyogenes infections. Because cases of streptococcal disease are not reported to national disease clearinghouses in the US, absolute numbers are not available. However, the recent resurgence of severe invasive infections has prompted descriptions of "flesh eating bacteria" in the news media. There has been no major change in susceptibility of S pyogenes to commonly used antibiotics but rather in the strain variations described above (antigenic types and extracellular growth products). However, a complete explanation for the decline and resurgence is not yet available.



FIGURE 13-3 Pathogenesis of *S pyogenes* infections.

The capsule of *S pneumoniae* renders it resistant to phagocytosis. The ability to evade this important host defense mechanism allows S pneumoniae to survive, multiply, and spread to various organs (Fig.13-4). The cell wall of S pneumoniae contains teichoic acid. The inflammatory response induced by Gram-positive cell walls differs from that induced by the endotoxin of Gram-negative organisms, but does include recruitment of polymorphonuclear neutrophils, changes in permeability and perfusion, cytokine release, and stimulation of platelet-activating factor. The role of other S pneumoniae moieties in virulence is less clear: protein A, pneumolysin, and peptide permeases. S pneumoniae is the leading cause of bacterial pneumonia beyond the neonatal period. Pleural effusion is the most common and empyema (pus in the pleural space) one of the most serious complications of S pneumoniae. This organism is also the most common cause of sinusitis, acute bacterial otitis media, and conjunctivitis beyond early childhood. Dissemination from a respiratory focus results in serious disease: outpatient bacteremia in children, meningitis, occasionally acute septic arthritis and bone infections in patients with sickle cell disease and, more rarely, peritonitis (especially in patients with nephrotic syndrome) or endocarditis.



FIGURE 13-4 Pathogenesis of *S pneumoniae* infections. Postinfectious Sequelae

Infection with *S pyogenes* (but not *S pneumoniae*) can give rise to serious nonsuppurative sequelae: acute rheumatic fever and acute glomerulonephritis. These sequelae begin 1-3 weeks after the acute illness, a latent period consistent with an immune-mediated rather than pathogen-disseminated etiology. Whether all *S pyogenes* strains are rheumatogenic is still controversial; however, clearly not all are nephritogenic. These differences in pathogenic potential are not yet understood.

Acute rheumatic fever is a sequela only of pharyngeal infections, but acute glomerulonephritis can follow infections of the pharynx or the skin. Although there is no adequate explanation for the precise pathogenesis of acute rheumatic fever or for its failure to occur after streptococcal pyoderma, an abnormal or enhanced immune response seems essential. Also, persistence of the organism, due perhaps in part to the greater avidity with which the organism adheres to host pharyngeal cells, is associated with an increased likelihood of rheumatic fever. Acute glomerulonephritis results from deposition of antigen-antibody-complement complexes on the basement membrane of kidney glomeruli. The antigen may be streptococcal in origin or it may be a host tissue species with antigenic determinants similar to those of streptococcal antigen (cross-reactive epitopes for endocardium, sarcolemma, vascular smooth muscle). In the United States, the incidence of acute rheumatic fever had decreased dramatically. Although several areas reported a resurgence in cases in the late 1980's, subsequently, a slow, steady decline continued. Acute rheumatic fever can result in permanent damage to the heart valves. Less than 1% of sporadic streptococcal pharyngitis infections result in acute rheumatic fever; however, recurrences are common, and life-long antibiotic prophylaxis is recommended following a single case. The incidence of acute glomerulonephritis in the United States is more variable, perhaps due to cycling of nephritogenic strains, but appears to be decreasing; recurrences are uncommon, and prophylaxis following an initial attack is unnecessary.

Other Streptococcal Species

Lancefield Group Streptococci

Streptococcal groups B, C, and G initially were recognized as animal pathogens (see Table 13-1) and as part of the normal human flora. Recently, the pathogenic potential for humans of some of these non-group-A streptococci has been clarified. Group B streptococci, a major cause of bovine mastitis, are a leading cause of neonatal septicemia and meningitis, accounting for a significant changing clinical spectrum of diseases in both pregnant women and their infants. Mortality rates in full-term infants range from 2-8% but in pre-term infants are approximately 30%. Early-onset neonatal disease (associated with sepsis, meningitis and pneumonia at 6d life) is thought to be transmitted vertically from the mother; late-onset (from 7d to 3 mos age) meningitis is acquired horizontally, in some instances as a nosocomial infection. Group B organisms also have been associated with pneumonia in elderly patients. They are part of the normal oral and vaginal flora and have also been isolated in adult urinary tract infection, chorioamnionitis and endometritis, skin and soft tissue infection, osteomyelitis, meningitis, bacteremia without focus, and endocarditis. Infection in patients with HIV can occur at any age.

Streptococci of groups C and G are associated with mild, as well as severe human disease. None of these groups has been implicated in acute rheumatic fever or acute glomerulonephritis. Group D streptococci are important etiologic agents of urinary tract infections and infections associated with biliary tract procedures, as well as cases of disseminated infection, bacteremia, and endocarditis. Streptococcus bovis bacteremia has been recognized more often in cases of bowel disease.

Group F streptococci are associated with abscess formation and purulent disease. Group R streptococci, well-documented causes of meningitis and septicemia in pigs, also pose a serious health hazard to workers in the pork industry.

Viridans Streptococci

The biochemically and antigenically diverse group of organisms classified as viridans streptococci, as well as other non-groupable streptococci of the oral and gastrointestinal cavities and urogenital tract, include important etiologic agents of bacterial endocarditis. Dental manipulation and dental disease with the associated transient bacteremia are the most common predisposing factors in bacterial endocarditis, especially if heart valves have been damaged by previous rheumatic fever or by congenital cyanotic heart disease. *S mutans* and *S sanguis* are odontopathogens responsible for the formation of dental plaque, the dense adhesive microbial mass that colonizes teeth and is linked to caries and other human oral disease (see Ch. 99). *S mutans* is the more cariogenic of the two species, and its virulence is directly related to its ability to synthesize glucan from fermentable carbohydrates as well as to modify glucan in promoting increased adhesiveness.

Anaerobes

Like their aerobic counterparts, anaerobic streptococci are part of the normal flora, particularly of the mouth and intestinal tract; they are also part of the normal flora of the upper respiratory and genital tracts and the skin. These anaerobic organisms are linked to a wide variety of serious mixed infections of the female genital tract as well as to brain, pulmonary, and abdominal abscesses.

Host Defenses

The streptococci are part of the endogenous microbial flora of the nasopharynx. Disease may result from circumvention of the normal specific or nonspecific host defense mechanisms. More often, both *S pyogenes* and *S pneumoniae* are exogenous secondary invaders following viral disease or disturbances in the normal bacterial flora.

In the normal host, nonspecific defense mechanisms prevent organisms from penetrating beyond the superficial epithelium of the upper respiratory tract. These mechanisms include mucociliary movement and the cough, sneeze and epiglottal reflexes. The host phagocytic system is a second line of defense against pathogens.

Organisms can be opsonized by activation of the classical or alternate complement pathway or by specific immunoglobulin binding.

The capsules of both S pyogenes and S pneumoniae allow the organisms to evade opsonization. The hyaluronic acid outer surface of *S pyogenes* is only weakly antigenic; however, protective immunity results from the development of type-specific antibody to the M protein of the fimbriae, which protrude from the cell wall through the capsular structure. This antibody, which follows respiratory and skin infections, is persistent. Presumably, IgA in the respiratory secretions and serum IgG are the important protective antibody classes. S pyogenes is rapidly killed following phagocytosis enhanced by specific antibody. Prompt, effective antibiotic treatment of streptococcal infections may preclude development of this persistent antibody. Evidence has shown that antibody to the erythrogenic toxin involved in scarlet fever is also long lasting. This is the basis of the Dick test, an in vivo skin test, rarely used today, which measures host antitoxin. The capsular polysaccharides of S pneumoniae are highly antigenic and type-specific. Type-specific anticapsular antibodies to these Tindependent antigens result in effective opsonization and host recovery. In untreated S pneumoniae infections, recovery clearly is due to opsonizing antibody. Even when adequate and appropriate antibiotic therapy is given, opsonizing antibody probably contributes significantly to recovery from pneumococcal disease. The normal host is somewhat resistant to S pneumoniae disease, but compromised hosts of several types are highly susceptible to serious infections: alcoholics, the semicomatose, very young, and very old individuals, patients who have undergone splenectomy, and patients with underlying diseases (specifically, chronic cardiac, pulmonary, or renal disease; sickle cell anemia; leukopenia; multiple myeloma; cirrhosis; and diabetes). Cross-reactive antigens, especially of S pyogenes and various mammalian tissues, help explain the autoimmune responses that develop following some infections. The level of humoral response to infection with S pyogenes is greater in patients with rheumatic fever than in patients with uncomplicated pharyngitis. In addition, cellmediated immunity may play a significant role in acute rheumatic fever.

Neonatal susceptibility to group B streptococci may result from immature neonatal phagocytic function, humoral immunity, or cell-mediated immunity, or from lack of passively acquired maternal antibody.

Evidence from the Rhesus monkey animal model in dental research shows that IgG may be a more important antibody class than IgA or IgM in protection against caries. Part of the reason may be that IgG is the antibody isotype most efficient at enhancing phagocytosis of *S mutans*. Cell-mediated immunity appears to participate in the protective host response against caries.

<u>Epidemiology</u>

The streptococci are widely distributed in nature and frequently form part of the normal human flora (see Table 29-1). Approximately 5-15% of humans carry *S pyogenes* or *S agalactiae* in the nasopharynx. *S pneumoniae* infects humans exclusively, and no reservoir is found in nature. The carrier rate of *S pneumoniae* in the normal human nasopharynx is 20-40%.

All ages, races, and sexes are susceptible to streptococcal disease. Because *S pneumoniae* is a particularly labile organism, sensitive to heat, cold, and drying, horizontal transmission requires close person-to-person contact. Infection is more likely at the extremes of life (<2 yr, > 65 yr), when host resistance is reduced, as described in the preceding section, or after the introduction of a more virulent strain. In the United States, pneumococcal disease is most prevalent during winter, coinciding with increased rates of acquisition but not necessarily of carriage. Alaskan natives have higher rates of invasive pneumococcal disease than do other American populations. The reason for this is unclear.

The incidence of respiratory disease attributed to *S pyogenes* peaks at about 6 years of age, and then again at 13 years of age, and is most common during late winter and early spring in temperate climates. Skin infections are more common among preschool-age children, and are most prevalent in late summer and early fall in temperate climates (when hot, humid weather prevails), and at all times in tropical climates. *S pyogenes* is spread by respiratory droplets or by contact with fomites used by the index individual, either patient or carrier. Skin infections often follow minor skin irritation, such as insect bites. There are occasional reports of streptococcal disease traced to rectal carriers, and of food-borne and vector-born outbreaks. In children, invasive disease with *S pyogenes* may follow varicella, or be associated with burns or malignancy; in adults with surgical or nonsurgical wounds or underlying medical problems, i.e., diabetes, cirrhosis, underlying peripheral vascular disease, or malignancy.

The world prevalence of the serious late sequelae of *S pyogenes* infections (acute rheumatic fever and acute glomerulonephritis) has shifted from temperate to tropical climates. In particular, acute rheumatic fever had ceased to be a major health concern in the US., despite no concomitant decline in group A streptococcal pharyngitis. These diseases previously affected persons with a low standard of living and limited access to medical care. Since 1985, there have been scattered outbreaks of acute

rheumatic fever in some regions of the United States. Temporal and geographic clustering provides further evidence for "rheumatogenic" strains. Whether ethnic or racially determined factors affect this shift is not known.

Other streptococcal groups show striking epidemiologic features. An increasing prevalence of non-group-A as compared to group A streptococci in throats has been reported. Studies of the vaginal flora among women of child-bearing age show a *S agalactiae* carrier rate of 15-40%. Vertical transmission of the organisms to neonates of vaginally infected mothers ranges from 40-73%, but the incidence of neonates with disease (in contrast to colonized, healthy neonates) is low, 1-2%. *S suis* has been linked to meningitis among meat handlers. Isolation of *S milleri* or *S bovis* from the bloodstream should raise suspicion of immunosuppression or underlying disease visceral abscess formation or other bowel disease (including colon carcinoma).

In the United States, enterococci are the second most common nosocomial pathogenes associated with both endogenous colonization and patient-to-patient spread. A wide variety of infections results, especially urinary tract and surgical wound infections, with a marked propensity for antibiotic resistance. The widespread usage of newer cephalosporins, which have poor activity against enterococci, allows "break through" of enterococci as clinically significant isolates, the development of resistance in areas of heavy antibiotic use, and a selective advantage to these organisms.

<u>Diagnosis</u>

Clinical

It is not usually possible to diagnose streptococcal pharyngitis or tonsillitis on clinical grounds alone. Accurate differentiation from viral pharyngitis is difficult even for the experienced clinician, and therefore the use of bacteriologic methods is essential. However, distinguishing acute streptococcal pharyngitis from the carrier state may be difficult. When documented streptococcal pharyngitis is accompanied by an erythematous punctiform rash (Fig.13-4), the diagnosis of scarlet fever can be made. With streptococcal toxic shock syndrome, unlike staphylococcal toxic shock syndrome where the organism is elusive, there is often a focal infection or bacteremia. Criteria for diagnosis of streptococcal toxic shock syndrome include hypotension and shock, isolation of *S pyogenes*, as well as 2 or more of the following: ARDS, renal impairment, liver abnormality, coagulopathy, rash with desquamating soft tissue necrosis. The invasive, potentially fatal *S pyogenes* infections require early recognition, definitive diagnosis, and early aggressive treatment.

Rheumatic fever is a late sequela of pharyngitis and is marked by fever, polyarthritis, and carditis. A combination of clinical and laboratory criteria (Table 13-2) is used in the diagnosis of acute rheumatic fever. Since the original Jones criteria were published in 1944, these have been modified (1955), revised (1965, 1984) and updated (1992). The other late sequela, acute glomerulonephritis, is preceded by pharyngitis or pyoderma; is characterized by fever, blood in the urine (hematuria), and edema; and is sometimes accompanied by hypertension and elevated blood urea nitrogen (azotemia). Pneumococcal pneumonia is a life-threatening disease, often characterized by edema and rapid lobar consolidation.

Specimens For Direct Examination And Culture

S pyogenes is usually isolated from throat cultures. In cases of cellulitis or erysipelas thought to be caused by *S pyogenes*, aspirates obtained from the advancing edge of the lesion may be diagnostic. *S pneumoniae* is usually isolated from sputum or blood. Precise streptococcal identification is based on the Gram stain and on biochemical properties, as well as on serologic characteristics when group antigens are present.

Identification

Hemolysis should not be used as a stringent identification criterion. Bacitracin susceptibility is a widely used screening method for presumptive identification of *S pyogenes*; however, some *S pyogenes* are resistant to bacitracin (up to 10%) and some group C and G streptococci (about 3-5%) are susceptible to bacitracin. Some of the group B streptococci also may be bacitracin sensitive, but are presumptively identified by their properties of hippurate hydrolysis and CAMP positivity. *S pneumoniae* can be separated from other a-hemolytic streptococci on the basis of sensitivity to surfactants, such as bile or optochin (ethylhydrocupreine hydrochloride). These agents activate autolytic enzymes in the organisms that hydrolyze peptidoglycan.

In many instances, presumptive identification is not carried further. Serologic grouping has not been performed as often as it might be because of the lack of available methods and the practical constraints of time and cost; however, only serologic methods, provide definitive identification of the streptococci. The Lancefield capillary precipitation test is the classical serologic method. *S pneumoniae*, which lacks a demonstrable group antigen by the Lancefield test, is conventionally identified by the quellung or capsular swelling test that employs type-specific anticapsular antibody. Inspection of Gram-stained sputum remains a reliable predictor for initial antibiotic therapy in community-acquired pneumonia.

New methods for serogrouping that show sensitivity and specificity now are being explored. Organisms from throat swabs, incubated for only a few hours in broth, can be examined for the presence of S pyogenes using the direct fluorescent antibody or enzyme-linked immunosorbent technique. Additional rapid antigen detection systems for the group carbohydrate have become increasingly popular. However, the sensitivity (70-90%) of these currently available rapid tests for group A streptococcal carbohydrate does not allow exclusion of streptococcal pharyngitis without conventional throat culture (sensitivity of a single throat culture is 90-99%). A third generation assay, the optical immunoassay, is currently being evaluated. S pneumoniae can be identified rapidly by counterimmuno-electrophoresis, a modification of the gel precipitin method. The coagglutination test is a more sensitive modification of the conventional direct bacterial agglutination test. The Fc portion of group-specific antibody binds to the protein A of dead staphylococci, leaving the Fab portion free to react with specific streptococcal antigen. The attachment of antibody to other carrier particles in suspension (for example, latex) also is used. The fact that whole streptococcal cells can be used in recently developed methods circumvents the difficulties involved in extracting components that retain appropriate antigenic reactivity. These

newer serogrouping methods should make it more practical to identify not only bhemolytic isolates from the blood or normally sterile sites, but also a-and nonhemolytic strains. It has become increasingly important to identify more of these strains to avoid simply misclassifying them as contaminants. Such information will expand our understanding of the importance of non-group-A streptococci.

Serologic Titers

Antibodies to some of the extracellular growth products of the streptococci are not protective but can be used in diagnosis. The antistreptolysin O (ASO) titer which peak 2-4 wks after acute infection and anti-NADase titers (which peaks 6-8 weeks after acute infection) are more commonly elevated after pharyngeal infections than after skin infections. In contrast, antihyaluronidase is elevated after skin infections, and anti-DNase B rises after both pharyngeal and skin infections. Titers observed during late sequelae (acute rheumatic fever and acute glomerulonephritis) reflect the site of primary infection. Although it is not as well known as the ASO test, the anti-DNase B test appears superior because high-titer antibody is detected following skin and pharyngeal infections and during the late sequelae. Those titers should be interpreted in terms of the age of the patient and geographic locale.

Although not used in diagnosis, bacteriocin production and phage typing of streptococci are employed in research and epidemiologic studies.

<u>Control</u>

Antibiotic Treatment

Penicillin remains the drug of choice for S pyogenes. It is safe, inexpensive, and of narrow spectrum, and there is no direct or indirect evidence of loss of efficacy. Prior to the 1990's, S pneumoniae was also uniformly sensitive to penicillin but a recent abrupt shift in the usefulness of penicillin has occurred. The group D enterococci are resistant to penicillins, including penicillinase-resistant penicillins such as methicillin, nafcillin, dicloxacillin, and oxacillin, and are becoming increasingly resistant to many other antibiotics. Group B streptococci are often resistant to tetracycline but remain sensitive to the clinically achievable blood levels of penicillin, even though they have penicillin minimal inhibitory concentrations (MIC) considerably higher than those of *S pyogenes*. Although the duration of penicillin therapy varies with the degree of invasiveness, streptococcal pharyngitis is generally adequately treated with 10 days of antibiotic therapy, and pneumococcal pneumonia with 7-14 days. If penicillin allergy occurs, an alternative drug for treating pharyngitis is erythromycin, although sporadic erythromycin and tetracycline resistance has been reported, leaving clindamycin or the newer macrolides as possible treatments. The most important goal of therapy in acute streptococcal pharyngitis is still to prevent rheumatic fever. However, therapy also hastens clinical recovery, avoids suppurative complications and renders the patient non-infectious for others. In addition to antibiotics, the patient with S pyogenes myositis or necrotizing fasciitis requires surgical debridement. Lifelong prophylaxis against recurrences of rheumatic fever is achieved with longacting penicillin or erythromycin. Sulfonamides will not eradicate the streptococcus and thus are not acceptable therapy for streptococcal pharyngitis, but sulfadiazine is effective for preventing recurrent attacks of rheumatic fever. Additional prophylactic

coverage before some dental and surgical procedures is necessary in the presence of rheumatic heart disease or prosthetic heart valves. Although streptococcal pharyngitis is usually a benign, self-limited disease, therapy is important to prevent rheumatic fever. There is no convincing evidence that antibiotic therapy prevents glomerulonephritis. Disconcertingly, some patients in recent outbreaks of acute rheumatic fever do not give a history of preceding pharyngitis.

Methods of treating the asymptomatic pharyngeal carrier of *S pyogenes* remain controversial. Recent evidence suggests that up to 20% of children and young adults are carriers, the carrier state involves no risk to the carrier or to others, and it is frequently difficult to eradicate despite the exquisite sensitivity of the organism to penicillin in vitro. A similar failure of antibiotic therapy to eradicate nasopharyngeal carriage or to prevent reinfection with *S pneumoniae* also occurs.

Although antibiotic resistance in *S pneumoniae* is common in many parts of the world, in the United States such strains previously had a geographically limited focus. Recent widespread emergence of *S pneumoniae* resistant to penicillin and other antibiotics has become a microbial threat in the United States as well. Even cefotaxime and ceftriaxone resistance has been documented. Isolates must be carefully screened for susceptibility by oxacillin disc testing, with definitive MIC determination by the E test (A B Biodisk NA, Piscataway, NJ), a convenient and reliable method for detection of resistance to penicillin and extended spectrum cephalosporins.

It is inappropriate to universally treat of pregnant women who are carriers of group B streptococci, or their colonized neonates, for several reasons: the high carrier rate; cost; the associated high risk of penicillin hypersensitivity; the potential increase in infections with penicillin-resistant organisms; the difficulty in altering colonization of women (even when their sexual partners were also treated); and the low risk of neonatal disease. The controversy continues despite recent recommendations for universal screening of preg nant women and selective intrapartum chemoprophylaxis for screen-positive mothers with preterm labor, premature or prolonged rupture of membranes, fever in labor, multiple births or previous infants with group B strepto-coccal disease.

Clearly, penicillin has reduced the severe morbidity and mortality associated with *S pneumoniae*. The emergence of resistance has now forced re-evaluation of empiric therapy. Clinicians must report clusters of *S pneumoniae* infection and be aware of local patterns of resistance. Penicillin susceptible organisms show MICs 0.06 mg/ml, intermediate strains 0.1-1.0 mg/ml and high level resistant strains 2 mg/ml. For nonmeningeal infection by intermediate strains, parenteral penicillin at high dose can probably be used since the mechanism of resistance involves alteration in penicillin binding proteins (PBP) and saturation. For meningeal infection with intermediate strains or any infection by high level resistant strains only ceftriaxone and cefotaxime retain sufficient activity. Resistance even to these extended spectrum cephalosporins was first reported for the US in 1991. At this writing only vancomycin remains uniformly effective but as discussed below, its use incurs potential for selec-

tion of vancomycin resistant enterococci (VRE) or risk of transferring vancomycin resistance from enterococci to *S pneumoniae*.

Currently, no single agent is reliably bactericidal against enterococci. Serious infections with group D enterococci often require a classic synergistic regime combining penicillin or ampicillin with an aminoglycoside, designed to weaken the cell wall with the β -lactam and facilitate entry of the bacteriocidal aminoglycoside. Other β lactam drugs with good activity against enterococci include piperacillin and imipenem. An alternate drug of choice is vancomycin, but vancomycin-resistant strains of enterococci have been isolated. Nosocomial acquisition of these resistant organisms is of grave concern.

This antibiotic resistance among the streptococci/enterococci is an increasing problem. Studies show that in vitro exchange of resistant DNA can occur in conjugation via plasmids and transposons, or in transduction with bacteriophages. The mechanisms involved in the in vivo genetic exchange are not clearly defined. Evidence is accumulating that other streptococci may be the important donors of resistance markers. Transposon transfer is thought to be the most likely mechanism in S pneumoniae, although point mutations also occur. In the setting of heavy ß-lactam use, selective pressure is important in emergence of resistant strains. The first penicillinresistant S pneumoniae were reported in 1967 in Australia and in 1974 in North America. In New Guinea, where the first penicillin-resistant strains were reported in 1971, one-third of S pneumoniae isolates from patients with severe pneumococcal disease were resistant by 1978. In Hungary in 1992, 69% of S pneumoniae isolates were penicillin resistant. This resistance is not ß-lactamase mediated but due to alteration in PBP which results in decreased binding of penicillin by the organism, rendering the drug less effective and requiring higher concentrations for saturation. Some strains resistant to erythromycin or tetracycline also have been reported, as well as some multiply resistant strains. In South Africa, outbreaks of infection with strains of S pneumoniae resistant to B-lactam antibiotics (penicillins and cephalosporins) as well as to tetracycline, chloramphenicol, erythromycin, streptomycin, clindamycin, sulfonamides, and rifampin were reported in 1977. Although antibiotic resistance among S pneumoniae was infrequent in the United States, a major shift occurred from 1988 to 1990, resulting in the present situation of 15-25% of S pneumoniae intermediately or completely resistant to penicillin. Communities with "low prevalence" have 5-10% resistance. Single or multiply resistant strains are transmitted person to person, especially in settings of frequent salivary exchange, antibiotic use and hand-to-hand transmission (as in day care centers) or of crowding (corrections facilities, homeless shelters, nursing homes, military training groups). Control of the problem of emerging, antibiotic-resistant S pneumoniae is multifactorial: 1) surveillance for clusters of invasive disease, resistance and prevalent serotypes; 2) education of physicians and the public about antibiotic use (decrease unnecessary antibiotic use for obviously viral infections and decrease antibiotic prophylaxis for otitis by use of intermittent or expectant dosing or of non ß-lactam based prophylaxissulfa. Use topical treatment for impetigo, and short course therapies and narrow spectrum antibiotics); 3) adherence to infection control strategies in day care centers;

4) aggressive promotion of the current 23-valent *S pneumoniae* vaccine and support of efforts to design a new vaccine effective in those <2 years of age, analogous to the eminently successful Haemophilus influenzae type B vaccine (see Ch. 30) where bacterial polysaccharide is conjugated to protein to elicit a T cell-dependent response.

Among the enterococci, resistance to a wide variety of common antibiotics has emerged, with some strains resistant to all currently available antibiotics. There is no clinically proven treatment effective against enterococci multiply resistant to lactams, aminoglycosides, and vancomycin. The emergence of such organisms poses a stunning management dilemma. Resistance among the enterococci can be either intrinsic or acquired (by de novo genetic mutation or acquisition of DNA from resistant organisms). Enterococcal resistance to lactams is also mediated by altered PBP as in pneumococci, allowing cell wall synthesis even in the presence of antibiotic, or much less commonly by β -lactamase. Resistance to aminoglycosides is mediated by decreased uptake or aminoglycoside modifying enzymes, and to vancomycin by decreased cell wall affinity for glycopeptide antibiotics. Further research into the mechanisms of resistance and new class(es) of antibiotics is essential.

A final concern about emerging resistance among the enterococci is the potential for genetic transfer of resistance genes to more virulent pathogens: Staph aureus, S pneumoniae and even Gram-negative organisms. So significant is this threat of emerging enterococcal resistance that the Centers for Disease Control and Prevention has issued a document addressing national guidelines. These include recommendations for 1) education of physicians and the public about the impact of vancomycin resistant enterococci (VRE), 2) vigilant surveillance for and detection of VRE, 3) strict enforcement of infection control strategies in hospitals, and 4) prudent vancomycin use or monotherapeutic use of extended spectrum cephalosporins. In a recent study of vancomycin use in US hospitals, use was about equally divided for treatment of a specific isolate, for prophylaxis, and for empiric coverage. The recommendations discourage vancomycin use for routine surgical prophylaxis, empiric prophylaxis in the patient with febrile neutropenia, the low birth weight infant or patients with vascular or peritoneal catheters, treatment of a single blood culture positive for coagulase-negative staphylococci, primary treatment of antibiotic-associated colitis, attempted eradication of colonization by methicillin-resistant Staph aureus (MRSA), or selective decontamination of the gastrointestinal tract.

Vaccination

As chemotherapeutic management becomes more difficult because of the threat of resistance, prevention becomes more important. With the introduction of antibiotics, previously successful pneumococcal vaccines fell into disuse. However, although prompt treatment with antibiotics has reduced the serious consequences of *S pneumoniae* infections (pre-antibiotic mortality rate of 30%), the disease incidence remains unchanged, and attention has been redirected to vaccines for *S pneumoniae* as well as for other streptococci. Pneumococcal vaccines (containing the pneumococcal polysaccharides of the most prevalent serotypes) have been licensed in several countries, including the United States. Initial use shows them to be useful and safe, but

they remain under-utilized. The spectre of multidrug resistant *S pneumoniae* may provide a new incentive for their use. In 1983, the United States Food and Drug Administration licensed a vaccine containing 23 serotypes, representing coverage against nearly 89% of the pneumococcal isolates submitted to the CDC in the 1987-1988 National Surveillance Study. The population target of pneumococcal vaccines includes those at high risk for serious pneumococcal disease: the elderly (65 and older) and children (2 years of age and older) with sickle cell anemia, with an immunocompromised state (lymphoma, asplenia, myeloma, acquired immunodeficiency syndrome), with nephrotic syndrome, or with chronic cardiopulmonary disease. Vaccines for the other streptococci remain experimental.

Vaccine production for the streptococci presents several formidable problems. For both *S pyogenes* and *S pneumoniae*, a large number of serotypes must be included in effective vaccines since successful selection of a common epitope remains elusive. Continuing surveillance to determine prevalent serotypes is necessary to insure that the vaccine formulations remain appropriate. For *S pyogenes*, it is critical to determine rheumatogenic and nephritogenic strains to limit the required multivalency of the vaccines. Alternatively a newly described conserved portion of M protein is a distant goal. Toxicity has been associated with M protein preparations, but lack of immunogenicity in highly purified preparations of antigens is still a problem. With streptococcal vaccines, the potential risk of antigenic cross-reactivity with cardiac tissue and an associated increased risk of acute rheumatic fever must be appreciated.

In group B neonatal disease chemoprophylaxis does not appear as practical as vaccine control. Passive immunity in group B streptococcal neonatal infection appears protective. Polyvalent hyperimmune gamma globulin and human monoclonal IgM antibody which reacts with multiple serotypes are undergoing efficacy studies. Active immunization of pregnant women with undegraded sialic acid-containing polysaccharide group B antigens is another important aspect of control.

The streptococci are ubiquitous, and their significance in medicine is remarkable. Exciting advances are being made in diagnosis and in understanding the mechanisms of pathogenesis, as well as in control of these well-known organisms. Problems with antibiotic resistance must preclude complacency in dealing with these common pathogens.

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Neisseria

General Concepts

Neisseria gonorrhoeae

Clinical Manifestations

Symptomatic or asymptomatic localized infections include urethritis, cervicitis, proctitis, pharyngitis, and conjunctivitis. Disseminated infections occur either by extension to adjacent organs (pelvic inflammatory disease, epididymitis) or by bac-
teremic spread (skin lesions, tenosynovitis, septic arthritis, endocarditis, and meningitis).

Structure

Cells are Gram-negative cocci, usually seen in pairs with the adjacent sides flattened. <u>Classification and Antigenic Types</u>

N gonorrhoeae strains have been typed on the basis of their growth requirements (auxotyping) or by antigenic differences in the porin protein (serotyping). More recently, restriction fragment length polymorphisms in genes encoding ribosomal RNA (ribotyping) and the separation of large DNA fragments by pulsed- field gel electrophoresis have been used to type isolates.

Pathogenesis

Gonorrhea is usually acquired by sexual contact. Gonococci adhere to columnar epithelial cells, penetrate them, and multiply on the basement membrane. Adherence is facilitated through pili and opa proteins. Gonococcal lipopolysaccharide stimulates the production of tumor necrosis factor, which causes cell damage. Gonococci may disseminate via the bloodstream. Strains that cause disseminated infections are usually resistant to serum and complement.

Host Defenses

Infection stimulates inflammation and local immunity; however, it is not known whether the secretory immune response is protective. Serum antibodies also appear. Individuals with genetic defects in late-acting complement components are at increased risk for disseminated infections. Protection, if it exists, may be strain specific.

Epidemiology

Gonorrhea is a sexually transmitted disease of worldwide importance. The highest attack rate in both men and women occurs between 15 and 29 years of age. Host-related factors such as the number of sexual partners, contraceptive practices, sexual preference, and population mobility contribute to the incidence of gonorrhea.

<u>Diagnosis</u>

Gonorrhea cannot be diagnosed solely on clinical grounds. For men, a Gram-stained smear of urethral exudate showing intracellular Gram-negative diplococci is diagnostic. For women, and for men when a direct smear is not definitive, culturing on selective medium is often required. N gonorrhoeae must be differentiated from other Neisseria species. Where appropriate, isolates should be examined for antibiotic resistance. A non-amplified DNA probe test is commercially available. This test does not require viable organisms and is useful where maintenance of viability during specimen transport is a problem; however, it is not as sensitive as culture. Serologic tests are not recommended for uncomplicated infections.

<u>Control</u>

Recommended treatment for uncomplicated infections is a third-generation cephalosporin or a fluoroquinolone plus an antibiotic (e.g., doxycycline) effective against possible coinfection with Chlamydia trachomatis. Sex partner(s) should be referred and treated. No effective vaccine yet exists. Condoms are effective in preventing gonorrhea.

Neisseria meningitidis

Clinical Manifestations

Infection with N meningitidis has two presentations, meningococcemia, characterized by skin lesions, and acute bacterial meningitis. Fulminant disease (with or without meningitis) is characterized by multisystem involvement and high mortality.

Structure

Cell morphology is identical to that of N gonorrhoeae. The antiphagocytic polysaccharide capsule is a prominent feature.

Classification and Antigenic Types

N meningitidis is grouped, on the basis of capsular polysaccharides, into 12 serogroups, some of which are subdivided according to the presence of outer membrane protein and lipopolysaccharide antigens.

Pathogenesis

Infection is by aspiration of infective particles, which attach to epithelial cells of the nasopharyngeal and oropharyngeal mucosa, cross the mucosal barrier, and enter the bloodstream. If not cleared blood-borne bacteria may enter the central nervous system and cause meningitis.

Host Defenses

Meningococci establish systemic infections only in individuals who lack serum bacterial antibodies directed against the capsular or noncapsular antigens of the invading strain, or in patients deficient in the late-acting complement components.

Epidemiology

Asymptomatic carriage of meningococci in the nasopharynx provides a reservoir for infection but also enhances host immunity. Attack rates peak in infants 3 months to 1 year old. Meningococcal meningitis occurs both sporadically (mainly groups B and C meningococci) and in epidemics (mainly group A meningococci), with the highest incidence during late winter and early spring.

Diagnosis

Symptoms are suggestive; diagnosis is confirmed by identifying N meningitidis in specimens of blood, cerebrospinal fluid, and nasopharyngeal secretions collected before antibiotic administration.

Control

Penicillin is the drug of choice. Household contacts require chemoprophylaxis with rifampin. Groups A, C, AC, and ACYW135 capsular polysaccharide vaccines are available. In children under 1 year old, antibody levels decline rapidly after immunization. Routine vaccination is not recommended.

Other Genera and Species

Moraxella is an oxidase-positive bacterium, sometimes mistaken for Neisseria, that may be isolated from eye infections and respiratory tract infections. M catarrhalis causes lower respiratory infection in adults with chronic lung disease and is a common cause of otitis media, sinusitis, and conjunctivitis in children. Kingella and Eikenella species are short bacilli or coccoid bacteria that act as opportunistic pathogens. They are sometimes secondary invaders of damaged tissues.

INTRODUCTION

The family Neisseriaceae comprises the genera Neisseria, Moraxella, Kingella, and Acinetobacter. The only significant human pathogens are N gonorrhoeae, the agent of gonorrhea, and N. meningitidis, an agent of acute bacterial meningitidis. N gonor-rhoeae infections have a high prevalence and low mortality, whereas N meningitidis infections have a low prevalence and high mortality.

Gonococcal infections are acquired by sexual contact and usually affect mucous membranes of the urethra in men and the endocervix in women, although the infection may disseminate to a variety of tissues. The pathogenic mechanism involves the attachment of the gonococci to nonciliated epithelial cells via pili (fimbriae) and the production of cytotoxic factors (endotoxin). Similarly, the lipopolysaccharide of meningococci is highly toxic, but an additional virulence factor is the antiphagocytic capsule. Both pathogens produce proteases that cleave and inactivate human immunoglobulin A1 (IgA1), a major mucosal immunoglobulin of humans. Many normal individuals harbor meningococci, whereas gonococci are present only if sexual contact with an infected person has occurred. Epidemics of meningococcal meningitis occur sporadically. Gonococcal infections occur frequently and affect large numbers of sexually active people. Other species in this genus are primarily parasites on mucosal surfaces of humans and other animals. Human disease caused by these organisms usually is associated with opportunistic infections in compromised patients.

Nesseria gonorrhoeae

Clinical Manifestations

Gonorrheal infection is generally limited to superficial mucosal surfaces lined with columnar epithelium. The areas most frequently involved are the cervix, urethra, rectum, pharynx, and conjunctiva (Fig. 14-1). Squamous epithelium, which lines the adult vagina, is not susceptible to infection by the gonococcus. However, the prepubertal vaginal epithelium, which has not been keratinized under the influence of estrogen, may be infected. Hence, gonorrhea in young girls may present as vulvovaginitis. Mucosal infections are usually characterized by a marked local neutrophilic response (purulent discharge).

The most common symptom of uncomplicated gonorrhea is a discharge that may range from a scanty, clear, or cloudy fluid to one that is copious and purulent. Dysuria is often present. Men with asymptomatic urethritis are an important reservoir for transmission. In addition, such men and those who ignore their symptoms are at increased risk for developing complications.

Endocervical infection is the most common form of uncomplicated gonorrhea in women. Such infections are usually characterized by vaginal discharge and sometimes by dysuria (because of coexistent urethritis). The cervical os may be erythematous and friable, with a purulent exudate. About 50 percent of women with cervical infections are asymptomatic. Local complications include abscesses in Bartholin's and Skene's glands.



FIGURE 14-1 Clinical manifestations of N gonorrhoeae infection.

Rectal infections with N gonorrhoeae occur in about one-third of women with cervical infection. They most often result from autoinoculation with cervical discharge and are rarely symptomatic. Rectal infections in homosexual men usually result from anal intercourse and are more often symptomatic. The symptoms and signs of gonococcal proctitis range from mild burning on defecation to itching to severe tenesmus and from mucopurulent discharge to frank blood in the stools.

Pharyngeal infections are diagnosed most often in women and homosexual men with a history of fellatio. Such infections may be a focal source of gonococcemia.

Ocular infections can have serious consequences (corneal scarring or perforation); prompt diagnosis and treatment are therefore important. Ocular infections (ophthalmia neonatorum) occur most commonly in newborns who are exposed to infected secretions in the birth canal. Keratoconjunctivitis is occasionally seen in adults as a result of autoinoculation.

Disseminated gonococcal infections result from gonococcal bacteremia. Asymptomatic infections of the pharynx, urethra, or cervix often serve as focal sources for bacteremia. The most common form of disseminated gonococcal infection is the dermatitis-arthritis syndrome. It is characterized by fever, chills, skin lesions, and arthralgias (usually involving the hands, feet, and elbows), which are due to periarticular inflammation of the tendon sheaths. Occasionally, a patient develops a septic joint with effusion. Skin lesions may be macular, pustular, centrally necrotic, or hemorrhagic. Rarely, disseminated gonococcal infection causes endocarditis or meningitis. Gonococci may ascend from the endocervical canal through the endometrium to the fallopian tubes and ultimately to the pelvic peritoneum, resulting in endometritis, salpingitis, and finally, peritonitis. Women usually present with pelvic and abdominal pain, fever, chills, and cervical motion tenderness. This complex of signs and symptoms is referred to as pelvic inflammatory disease (PID). This disease may also be caused by other sexually transmitted organisms (e.g., Chlamydia trachomatis) as well as by non-sexually transmitted bacteria that are part of the normal vaginal flora. Complications of pelvic inflammatory disease include tubo-ovarian abscesses, pelvic peritonitis, or Fitz-Hugh and Curtis syndrome, which is an inflammation of Glisson's capsule of the liver. As many as 15 percent of women with uncomplicated cervical infections may develop pelvic inflammatory disease. The disease may have serious consequences, including an increased probability of infertility and ectopic pregnancy.

Structure

Neisseria species are Gram-negative cocci, 0.6 to $1.0 \ \mu m$ in diameter. The organisms are usually seen in pairs with the adjacent sides flattened. Pili, hairlike filamentous appendages extend several micrometers from the cell surface and have a role in adherence. The outer membrane is composed of proteins, phospholipids, and lipopoly-saccharide (LPS). Features that distinguish gonococcal LPS from enteric LPS are the highly branched basal oligosaccharide structure and the absence of repeating O-antigen subunits. For these reasons gonococcal LPS, as well as that of other mucosal pathogens, is referred to as lipooligosaccharide (LOS). Gonococci characteristically release outer membrane fragments (blebs) during growth. These blebs contain LOS and may have a role in pathogenesis.

Classification and Antigenic Types

The gonococcus is an obligate human pathogen. It is one of two Neisseria species that cause significant human infections. The genus also includes several nonpathogenic species (Table 14-1), which may be part of the normal flora and therefore can be confused with N gonorrhoeae. Gonococcal strains can be characterized according to their nutritional requirements (auxotyping). A panel of monoclonal antibodies specific for epitopes on protein I have also been used to type strains. Strains exhibiting specific reaction patterns are termed serovars. A combined auxotype-serovar classification provides greater resolution among gonococcal isolates and is useful in epidemiologic investigations.

Pathogenesis

Our knowledge of the molecular basis of gonococcal pathogenesis is incomplete (Fig. 14-2). Attachment of gonococci to mucosal cells is mediated in part by pili, although nonspecific factors such as surface charge and hydrophobicity may be important. Pili undergo both phase and antigenic variation. Opa proteins (protein II), which are located in the outer membrane, are also involved in attachment to host cells. Gonococci attach only to microvilli of nonciliated columnar epithelial cells; attachment to ciliated cells is not observed.

Lumen (urethral, vaginal, anal, and pharyngeal)



FIGURE 14-2 Pathogenesis of uncomplicated gonorrhea. Gonococci can invade columnar epithelial cells, although they do not invade ciliated columnar epithelium of the genitourinary tract.

Much of our knowledge of gonococcal invasion comes from studies with tissue culture cells and human fallopian tube organ culture. After gonococci attach to the nonciliated epithelial cells of the fallopian tube, they are surrounded by the microvilli, which draw them to the surface of the mucosal cell. The gonococci appear to enter the epithelial cells by a process called parasite-directed endocytosis. This process seems to be initiated by microbial factors because it does not occur unless the gonococci are viable and because it involves host cells that are not normally phagocytic. An unidentified factor in serum enhances engulfment of gonococci. The process is inhibited by drugs that block the actions of the microtubule (demecolcine) and microfilament (cytochalasin B) systems. During endocytosis the membrane of the mucosal cell retracts, pinching off a membrane-bound vacuole that contains gonococci; this vacuole is rapidly transported to the base of the cell, where gonococci are released by exocytosis into the subepithelial tissue. Gonococci are not destroyed within the phagocytic vacuole; it is not clear whether they replicate in the vacuoles.

The major porin protein of the gonococcal outer membrane, Por (protein I), has been proposed as a candidate invasin (a substance that helps mediate invasion into a host cell). The insertion of Por into neutrophils treated with the chemotactic peptide, fMLP and leukotriene B4, inhibits degranulation but not the generation of the superoxide anion. The significance of these observations with respect to the pathogenesis of gonorrhea remains to be determined. Each gonococcal strain expresses only one type of Por; however, the Por of different strains may exhibit antigenic differences. Gonococci can produce one or several outer membrane proteins called Opa proteins (proteins II). These proteins are subject to phase variation and are usually found on cells from colonies possessing an opaque phenotype (O+). At any one time, a gonococcus may express zero, one, or several different Opa proteins, though each strain has 10 or more genes for different Opas. Trypsin-like proteases present in cervical mucus may help select for protease-resistant transparent (O-) colony phenotypes. O+ colony phenotypes (protease sensitive) predominate in cultures taken during the middle portion of the menstrual cycle. Cervical proteases increase during the second half of the cycle, resulting in an increase in the O- phenotype. The O- colony types can be isolated from tubal as well as endocervical cultures; O+ colony phenotypes have been isolated more often from endocervical cultures than from tubal cultures.

Rmp (Protein III) is an outer membrane protein found in all strains of N gonorrhoeae. It does not undergo phase variation and is found in a complex with Por and LOS. It shares partial homology with the Omp A protein of Escherichia coli. Antibodies to Rmp, induced either by a neisserial infection or by colonization with E coli, block bactericidal antibodies directed against Por and LOS. Rmp antibodies may facilitate infection with N gonorrhoeae.

LOS has a profound effect on the virulence and pathogenesis of N gonorrhoeae. Gonococci can express several antigenic types of LOS and can alter the type of LOS they express by an as yet unknown mechanism. Gonococcal LOS produces mucosal damage in fallopian tube organ cultures and brings about the release of enzymes, such as proteases and phospholipases, that may be important in pathogenesis. More recent evidence suggests that gonococcal LOS stimulates the production of tumor necrosis factor (TNF) in fallopian tube organ cultures; inhibition of tumor necrosis factor with specific antiserum prevents tissue damage. Thus, gonococcal LOS appears to have an indirect role in mediating tissue damage. Gonococcal LOS is also involved in the resistance of N gonorrhoeae to the bactericidal activity of normal human serum. Oligosaccharides containing epitopes defined by specific monoclonal antibodies are associated with a serum-resistant phenotype.

Gonococci can utilize host-derived cytidine monophospho-N-acetylneuraminic acid (CMP-NANA) in vivo to sialylate the oligosaccharide component of its LOS, converting a serum-sensitive organism to a serum-resistant one. When such organisms are grown in vitro without CMP-NANA, their resistance to killing by normal human serum is rapidly lost. Organisms with non-sialylated LOS are more invasive than those with sialylated LOS. There is antigenic similarity between neisserial LOS and antigens present on human erthyrocytes. This similarity to self may preclude an effective immune response to these LPS antigens.

Gonococci are highly autolytic and release peptidoglycan fragments during growth. These fragments, released by bacterial and/or host peptidoglycan hydrolases, are toxic for fallopian tube mucosa and may contribute to the intense inflammatory reactions characteristic of gonococcal disease.

N gonorrhoeae is highly efficient at utilizing transferrin-bound iron for in vitro growth; many strains can also utilize lactoferrin-bound iron. Gonococci (and menin-gococci) bind only human transferrin and lactoferrin. This specificity is thought to be

the reason these organisms are exclusively human pathogens. Nevertheless, the role of transferrin- and lactoferrin-bound iron in in vivo growth is unknown. Gonococci express several new proteins when grown under iron-restricted conditions similar to the conditions occurring in the host. Some of these proteins function as receptors for transferrin, lactoferrin, heme, and hemoglobin; others function in the transport of iron into the cell. Gonococci cannot grow anaerobically unless low concentrations of the alternative electron acceptor nitrite are present. Under these conditions they produce novel proteins. These proteins are apparently produced during an infection because antibodies against them are present in the serum specimens of patients with uncomplicated gonorrhea, disseminated gonococcal infection, or pelvic inflammatory disease. These data suggest that some gonococci in the host are growing under anaerobic conditions. Further studies will determine the relevance of these proteins to pathogenesis.

Strains of N gonorrhoeae (and N meningitidis) produce two distinct extracellular IgA1 proteases, which cleave the heavy chain of human immunoglobulin A1 (IgA1) at different points within the hinge region. Type 1 protease cleaves a prolyl-seryl peptide bond and type 2 protease cleaves a prolyl-threonyl bond in the hinge region of the heavy chain. This region is missing in human IgA2, and so this isotype is not susceptible to cleavage. Each gonococcal or meningococcal isolate elaborates only one of these two enzymes. Split products of IgA1 have been found in the genital secretions of women with gonorrhea, suggesting that the gonococcal IgA1 protease is present and active during genital infection. Fab fragments of IgA1 may bind to the gonococcal cell surface and block the Fc-mediated functions of intact immunoglobulins.

Host Defenses

Not everyone exposed to N gonorrhoeae acquires the disease. This may be due to variations in the size or virulence of the inoculum, to nonspecific resistance, or to specific immunity. A 50 percent infective dose (ID50) of about 1,000 organisms has been established, based on the experimental urethral inoculation of male volunteers. There is no reliable ID50 for women, although it is assumed to be similar.

Nonspecific factors have been implicated in natural resistance to gonococcal infection. In women, changes in the genital pH and hormones may increase resistance to infection at certain times of the menstrual cycle. Urinary solutes exhibit bactericidal and bacteriostatic activity of N gonorrhoeae. Factors in urine that seem to be important are pH, osmolarity, and the concentration of urea. The variability in the susceptibility of gonococcal strains to the bactericidal and bacteriostatic properties of urine is thought to be one of the reasons some men do not develop a gonococcal infection when exposed.

Most uninfected individuals have serum antibodies that react with gonococcal antigens. These antibodies probably result from colonization or infection with various Gram-negative bacteria that possess cross-reactive antigens. These "natural" antibodies differ, both qualitatively and quantitatively, from person to person, but may be important in an individual's natural resistance or susceptibility to infection. Infection with N gonorrhoeae stimulates both mucosal and systemic antibodies to a variety of gonococcal antigens. Mucosal antibodies are primarily IgA and IgG. In genital secretions, antibodies have been identified that react with Por, Opa and LOS, and some of the iron-regulated proteins. Vaccine trials have suggested that antipilus antibodies inhibit the pilus-mediated attachment of the homologous gonococcal strain. Complement is present in endocervical secretions, but in a much lower concentration than in blood. However, there is little evidence to support a role for a complement-mediated bactericidal defense mechanism on the genital mucosa. In general, the IgA response is brief and declines rapidly after treatment; IgG levels decline more slowly.

More information is available about the function of systemic humoral immune mechanisms in gonococcal infection. Gonococcal antigens such as pili, Por, Opa, Rmp, and LOS elicit a serum antibody response during an infection. Antipilus antibody levels tend to be higher in women than in men and are related to the number of previous infections. The predominant IgG subclass that reacts with a variety of gonococcal antigens is IgG3, followed by IgG1 and IgG4. IgG2 is minimal, suggesting that polysaccharides are not important in the immune response to gonococcal infection. Anti-Por antibodies may be bactericidal for the gonococcus. IgG that reacts with Rmp blocks the bactericidal activity of antibodies directed against Por and LOS. Genital infection with N gonorrhoeae stimulates a serum antibody response against the LOS of the infecting strain. Disseminated gonococcal infection results in higher levels of anti-LOS antibody than do genital infections.

Strains that cause uncomplicated genital infections usually are killed by normal human serum and are termed serum sensitive. This bactericidal activity is mediated by IgM and IgG that recognize sites on the LOS. Strains that cause disseminated infections are not killed by most normal human serum and are referred to as serum resistant. Resistance is mediated, in part, by IgA that blocks the IgG-mediated bactericidal activity of the serum. Serum specimens from convalescent patients with disseminating infections contain bactericidal IgG to the LOS of the infecting strain.

Individuals with inherited complement deficiencies have a markedly increased risk of acquiring systemic neisserial infections and are subject to recurring episodes of systemic gonococcal and meningococcal infections, indicating that the complement system is important in host defense.Gonococci activate complement by both the classic and alternative pathways. Complement activation by gonococci leads to the formation of the C5b-9 complex (membrane attack complex) on the outer membrane. In normal human serum, similar numbers of C5b-9 complexes are deposited on serumsensitive and serum-resistant organisms, but the membrane attack complex is not functional on serum-resistant organisms. Other complement-mediated functions, such as opsonophagocytosis and chemotaxis, are more efficient with serum-sensitive than with serum-resistant gonococci. This may be a significant factor in the pathogenesis of disseminated gonococcal infection and probably contributes to the relative lack of genital symptoms observed with this disease.

Normal human serum contains opsonic anti-Por IgG. Antibodies to various surfaceexposed antigens are also present in cervical and urethral secretions of patients with gonorrhea and probably contribute to the opsonophagocytosis of the organism. Opa is important in gonococcus-neutrophil interactions. Gonococci expressing certain Opas interact with neutrophils in the absence of antibodies. Once phagocytosed, gonococci are killed by both oxygen-dependent and oxygen-independent mechanisms. The survival of gonococci within neutrophils has been the subject of considerable controversy, with no clear-cut answer yet available. The opsonization and phagocytosis of gonococci are comparatively more important in mucosal infections than in protection from systemic gonococcal (and meningococcal) infections.

Epidemiology

The only natural host for N gonorrhoeae is the human. Gonorrhea has all but disappeared in Scandinavia and several other European countries. In the United States, gonorrhea remains the most frequently reported infectious disease. Between 1977 and 1993, the number of reported cases decreased 56 percent, from 1 million to 439,673 cases per year. The Centers for Disease Control (CDC) estimates that there are two unreported cases for every reported case of gonorrhea. Gonorrhea is transmitted almost exclusively by sexual contact. The highest rates occur in women between the ages of 15 and 19 years and in men 20 and 24 years of age. Persons who have multiple sex partners are at highest risk. Rates of gonorrhea are higher in males and in minority and inner-city populations.

Gonorrhea is usually contracted from a sex partner who is either asymptomatic or has only minimal symptoms. It is estimated that the efficiency of transmission after one exposure is about 35 percent from an infected woman to an uninfected man and 50 to 60 percent from an infected man to an uninfected woman. More than 90 percent of men with urethral gonorrhea will develop symptoms within 5 days; fewer than 50 percent of women with anogenital gonorrhea will do so. Women with asymptomatic infections are at higher risk of developing pelvic inflammatory disease and disseminated gonococcal infection.

Diagnosis

Gonococcal infection produces several common clinical syndromes that have multiple causes or that mimic other conditions. Laboratory tests are often required to differentiate among the etiologic agents causing urethritis or cervicitis. The etiologic diagnosis of salpingitis and pelvic peritonitis is quite difficult because mixed infections are common and laparoscopy is required to obtain appropriate cultures. Gonococcal perihepatitis may mimic acute cholecystitis. All of the above syndromes are also caused by C trachomatis, a sexually transmitted bacterium that causes more infections in the United States than N gonorrhoeae. The gonococcal arthritis-dermatitis syndrome, must be, differentiated from meningococcemia and Reiter syndrome, in particular, and from other causes of septic arthritis.

Customarily, the laboratory diagnosis of gonorrhea is made presumptively and then confirmed; the latter process involves identifying characteristics that distinguish N gonorrhoeae from other Neisseria spp. that may be present in the specimen. Non-pathogenic Neisseria are normal inhabitants of the oropharynx and nasopharynx and occasionally are isolated from other sites infected by N gonorrhoeae. A presumptive diagnosis of gonorrhea may be made from Gram-stained smears of urethral, cervical,

and rectal specimens if Gram-negative diplococci are observed within leukocytes; it is equivocal if only extracellular Gram-negative diplococci are seen and negative if no Gram-negative diplococci are seen. Gram stain diagnosis has a sensitivity and specificity of >95 percent in men with symptomatic urethritis. The specificity of Gram stain diagnosis in women is also high if the cervix is wiped clean to remove cervical secretions before collecting the specimen; however, the sensitivity is only about 50 percent. The sensitivity and specificity of the Gram stain for rectal specimens are lower than with cervical specimens.

Specimens for the laboratory diagnosis of gonorrhea should be collected before treating the patient. Ideally, specimens should be inoculated onto appropriate media and incubated immediately after collection at 35 to 36.5°C in a CO2-enriched atmosphere, which can be obtained by using a candle extinction jar or a CO2 incubator. Urethral specimens are normally obtained from heterosexual men; urethral, rectal, and pharyngeal specimens are normally obtained from homosexual men; and cervical and rectal specimens are normally obtained from women. Specimens are collected with cotton, polyester, or calcium alginate swabs. When appropriate, specimens may also be obtained from the urethra and from Bartholin's and Skene's glands of infected women. Blood cultures should be performed for patients with suspected disseminated infection. Synovial fluid cultures should be performed for patients with septic arthritis.

Urethral, cervical, and pharyngeal specimens are inoculated onto selective medium such as modified Thayer-Martin, Martin-Lewis, or NYC medium. These are complex media that contain antimicrobial and antifungal agents to inhibit the growth of unwanted organisms. Rectal specimens should be inoculated onto modified Thayer-Martin medium which contains trimethoprim lactate to inhibit the growth and swarming of Proteus species. Specimens collected from normally sterile sites such as blood, synovial fluid, and conjunctivae may be inoculated onto a nonselective medium such as chocolate agar.

The combination of oxidase-positive colonies and Gram-negative diplococci provides a presumptive identification of N gonorrhoeae. Fluorescent-antibody staining, coagglutination, specific biochemical tests, and DNA probes may be used for confirmation. DNA probes have also been used to detect gonococci in urethral and cervical specimens. A commercial test based on this approach is available. Serologic tests for uncomplicated gonorrhea have not proved satisfactory.

<u>Control</u>

There is no effective vaccine to prevent gonorrhea. Candidate vaccines consisting of pilus protein or Por are of little benefit. The development of an effective vaccine has been hampered by the lack of a suitable animal model and the fact that an effective immune response has never been demonstrated. Condoms are effective in preventing the transmission of gonorrhea.

Contact tracing to identify source contacts (i.e., those who infected the index patient) has been useful in identifying asymptomatic individuals or those with ignored symptoms. Contact tracing has also been used to identify contacts who were exposed to the index patient and who may have become infected.

The evolution of antimicrobial resistance in N gonorrhoeae may ultimately affect the control of gonorrhea. Strains with multiple chromosomal resistance to penicillin, tet-racycline, erythromycin, and cefoxitin have been identified in the United States and most other parts of the world. Sporadic high-level resistance to spectinomycin and fluoroquinolones have been reported.

Penicillinase-producing strains of N gonorrhoeae were first described in 1976. Five related ß-lactamase plasmids of different sizes have been identified in these strains. The strains cause more than one-half of all gonococcal infections in parts of Africa and Asia. Their prevalence has increased dramatically in the United States since 1984 and has affected nearly every major metropolitan area.

Plasmid-mediated high-level resistance of N gonorrhoeae to tetracycline was first described in 1986 and has now been reported in most parts of the world. This resistance is due to the presence of the streptococcal tetM determinant on a gonococcal conjugative plasmid.

The current CDC Treatment Guidelines recommend treatment of all gonococcal infections with antibiotic regimens effective against resistant strains. The recommended antimicrobial agents are ceftriaxone, cefixime, ciprofloxacin, or oflaxacin. Since a significant proportion of patients with gonorrhea are also infected with C trachomatis, doxycycline or erythromycin has been added to treat this concomitant infection.

Neisseria meningitidis

Clinical Presentation

N meningitidis infection results from the bloodborne dissemination (meningococcemia) of the meningococcus, usually following an asymptomatic or mildly symptomatic nasopharyngeal carrier state or a mild rhinopharyngitis (Fig. 14-3). The mildest form is a transient bacteremic illness characterized by a fever and malaise; symptoms resolve spontaneously in 1 to 2 days. Acute meningococcemia is more serious and is often complicated by meningitis. The manifestations of meningococcal meningitis are similar to acute bacterial meningitis caused by organisms such as Streptococcus pneumoniae, Haemophilus influenzae, and E coli. The manifestations result from both infection and increased intracranial pressure. Chills, fever, malaise, and headache are the usual manifestations of infection; headache, vomiting, and rarely, papilledema may result from increased intracranial pressure. Signs of meningeal inflammation are also present. The onset of meningococcal meningitis may be abrupt or insidious.

Infants with meningococcal meningitis rarely display signs of meningeal irritation. Irritability and refusal to take food are typical; vomiting occurs early in the disease and may lead to dehydration. Fever is typically absent in children younger than 2 months of age. Hypothermia is more common in neonates. As the disease progresses, apneic episodes, seizures, disturbances in motor tone, and coma may develop.

In older children and adults, specific symptoms and signs are usually present, with fever and altered mental status the most consistent findings. Headache is an early, prominent complaint and is usually very severe. Nausea, vomiting, and photophobia are also common symptoms.



FIGURE 14-3 Clinical manifestations of N meningitidis infection.

Neurologic signs are common; approximately one-third of patients have convulsions or coma when first seen by a physician. Signs of meningeal irritation such as cervical rigidity (Brudzinski sign), thoracolumbar rigidity, hamstring spasm (Kernig sign), and exaggerated reflexes are common.

Petechiae (minute hemorrhagic spots in the skin) or purpura (hemorrhages into the skin) occurs from the first to the third day of illness in 30 to 60 percent of patients with meningococcal disease, with or without meningitis. The lesions may be more prominent in areas of the skin subjected to pressure, such as the axillary folds, the belt line, or the back.

Fulminant meningococcemia (Waterhouse-Friderichsen syndrome) occurs in 5 to 15 percent of patients with meningococcal disease and has a high mortality rate. It begins abruptly with sudden high fever, chills, myalgias, weakness, nausea, vomiting, and headache. Apprehension, restlessness, and frequently, delirium occur within the next few hours. Widespread purpuric and ecchymotic skin lesions appear suddenly. Typically, no signs of meningitis are present. Pulmonary insufficiency develops within a few hours, and many patients die within 24 hours of being hospitalized despite appropriate antibiotic therapy and intensive care.

Structure

The only distinguishing structural feature between N meningitidis and N gonorrhoeae is the presence of a polysaccharide capsule in the former. The capsule is antiphagocytic and is an important virulence factor.

Classification and Antigenic Types

Meningococcal capsular polysaccharides provide the basis for grouping these organisms. Twelve serogroups have been identified (A, B, C, H, I, K, L, X, Y, Z, 29E, and W135). The most important serogroups associated with disease in humans are A, B, C, Y, and W135. The chemical composition of these capsular polysaccharides, where known, is listed in Table 14-2. The prominent outer membrane proteins of N meningitidis have been designated class 1 through class 5. The class 2 and 3 proteins function as porins and are analogous to gonococcal Por. The class 4 and 5 proteins are analogous to gonococcal Rmp and Opa, respectively. Serogroup B and C meningococci have been further subdivided on the basis of serotype determinants located on the class 2 and 3 proteins. A few serotypes are associated with most cases of meningococcal disease, whereas other serotypes within the same serogroup rarely caused disease. All known group A strains have the same protein serotype antigens in the outer membrane. Another serotyping system is based on the antigenic diversity of meningococcal LOS. The LOS types are independent of the protein serotypes, although certain combinations frequently occur together.

Pathogenesis

The human nasopharynx is the only known reservoir of N meningitidis. Meningococci are spread via respiratory droplets, and transmission requires aspiration of infective particles. Meningococci attach to the nonciliated columnar epithelial cells of the nasopharynx. Attachment is mediated by pili and possibly by other outer membrane components. Invasion of the mucosal cells occurs by a mechanism similar to that observed with gonococci. However, once internalized, meningococci remain in an apical location within the epithelial cell; the route by which they gain access to the subepithelial space remains unclear. Trimers of class 2 and 3 proteins have the ability to translocate from intact cells and insert into eukaryotic cell membranes to form voltage-dependent channels. This process may be important in invasion.

Purified meningococcal LOS is highly toxic and is as lethal for mice as the LOS from E coli or Salmonella typhimurium; however, meningococcal LOS is 5 to 10 times more effective than enteric LPS in eliciting a dermal Shwartzman reaction in rabbits. Meningococcal LPS suppresses leukotriene B4 synthesis in human polymorphonuclear leukocytes. The loss of leukotriene B4 deprives the leukocytes of a strong chemokinetic and chemotactic factor.

The events after bloodstream invasion are unclear. Relatively little information is known about how the meningococcus enters the central nervous system.

Host Defenses

The integrity of the pharyngeal and respiratory epithelium may be important in protection from invasive disease. Chronic irritation of the mucosa due to dust or low humidity, or damage to the mucosa resulting from a concurrent viral or mycoplasmal upper respiratory infection, may be predisposing factors for invasive disease.

The presence of serum bactericidal IgG and IgM is probably the most important host factor in preventing invasive disease. These antibodies are directed against both capsular and noncapsular surface antigens. The antibodies are produced in response to colonization with carrier strains of N meningitidis, N lactamica, or other nonpathogenic Neisseria species. Protective antibodies are also stimulated by cross-reacting antigens on other bacterial species. The role of bactericidal antibodies in prevention

of invasive disease explains why high attack rates are seen in infants from 6 to 9 months old, the age at which maternally acquired antibodies are being lost.

The immunity conferred by specific antibody may not be absolute. Illness has been documented in individuals with levels of antibodies considered to be protective. It has been postulated that the activity of the bactericidal antibodies might be blocked by IgA, induced by other meningococcal strains, or by cross-reacting antigens on enteric or other respiratory bacteria. Since IgA does not bind complement, it may block binding sites for the bactericidal IgG and IgM. Persons with complement deficiencies (C5, C6, C7, or C8) may develop meningococcemia despite protective antibody. This underscores the importance of the complement system in protection from meningococcal disease.

Epidemiology

The meningococcus usually inhabits the human nasopharynx without causing detectable disease. This carrier state may last for a few days to months and is important because it not only provides a reservoir for meningococcal infection but also enhances host immunity. Between 5 and 30 percent of normal individuals are carriers at any given time, yet few develop meningococcal disease. Even during epidemics of meningococcal meningitis in military recruits, when the carrier rate may reach 95 percent, the incidence of systemic disease is less than 1 percent. Meningococcal carriage rates are highest in older children and young adults, but the attack rates are higher in children, peaking at 5 years of age (group B) and 4 to 14 years of age (group C). The low incidence of disseminated disease following colonization suggests that host rather than bacterial factors play an important determining role.

Meningococcal meningitis occurs sporadically and in epidemics, with the highest incidence during late winter and early spring. Most epidemics are caused by group A strains, but small outbreaks have occurred with group B and C strains. Sporadic cases generally are caused by group B, C, and Y strains. Whenever group A strains become prevalent in the population, the incidence of meningitis increases markedly.

Diagnosis

The most characteristic manifestation of meningococcemia is the skin rash, which is essential for its recognition. Petechiae are the most common type of skin lesion. Illdefined pink macules and maculopapular lesions also occur. Lesions are sparsely distributed over the body. They tend to occur in crops and on any part of the body; however, the face is usually spared and involvement of the palms and soles is less common. The skin rash may progress from a few ill-defined lesions to a widespread eruption within a few hours.

Acute bacterial meningitis has characteristic signs and symptoms. Except in epidemic situations, it is difficult to identify the causative agent without laboratory tests.

In cases of suspected meningococcal disease, specimens of blood, cerebrospinal fluid, and nasopharyngeal secretions should be collected before administration of any antimicrobial agents and examined for the presence of N meningitidis. Success in isolation is reduced by prior therapy; however, the microscopic diagnosis is not significantly affected. The cerebrospinal fluid should be concentrated by centrifugation and a portion of the sediment cultured on chocolate or blood agar. The plates should be incubated in a candle jar or CO2 incubator. The presence of oxidase-positive colonies and Gram-negative diplococci provides a presumptive identification of N meningitidis. Production of acid from glucose and maltose but not sucrose, lactose, or fructose may be used for confirmation (Table 14-1). The serologic group may be determined by a slide agglutination test, using first polyvalent and then monovalent antisera.

Nasopharyngeal specimens must be obtained from the posterior nasopharyngeal wall behind the soft palate and then should be inoculated onto a selective medium such as Thayer-Martin medium and processed as above.

Blood specimens are inoculated in 10- to 15-ml aliquots onto each of three blood bottles to give a final concentration of 10% (vol/vol). Evacuated bottles should be vented. Some strains of N meningitidis are inhibited by the sodium polyanethol-sulfonate contained in blood medium. Toxicity may be overcome by the addition of gelatin. Sodium amylosulfate is not toxic for the meningococcus. Blood cultures are subcultured blindly onto chocolate or blood agar for confirmation.

Gram-stained smears of cerebrospinal fluid may be diagnostic; however, finding neisseriae in these smears is often more difficult than finding the strains that cause pneumococcal meningitis. Quellung tests may be of value.

<u>Control</u>

Group A, C, Y, and W135 capsular polysaccharide vaccines are available and can be used to control outbreaks due to the meningococcal serogroups covered by the vaccine. The A, C, AC, and ACYW135 polysaccharide formulations are currently licensed in the United States. The polysaccharide vaccines are ineffective in young children, and the duration of protection is limited in children vaccinated at 1 to 4 years of age. Routine vaccination of the civilian population in industrialized countries is not currently recommended because the risk of infection is low and most endemic disease occurs in young children. The group B capsular polysaccharide is a homopolymer of sialic acid and is not immunogenic in humans. A group B meningococcal vaccine consisting of outer membrane protein antigens has recently been developed but is not licensed in the United States.

Meningococcal disease arises from association with infected individuals, as evidenced by the 500- to 800-fold greater attack rate among household contacts than among the general population. Because such household members are at high risk, they require chemoprophylaxis. Sulfonamides were the chemoprophylactic agent of choice until the emergence of sulfonamide-resistant meningococci. At present, approximately 25 percent of clinical isolates of N meningitidis in the United States are resistant to sulfonamides; rifampin is therefore the chemoprophylactic agent of choice.

Penicillin is the drug of choice to treat meningococcemia and meningococcal meningitis. Although penicillin does not penetrate the normal blood-brain barrier, it readily penetrates the blood-brain barrier when the meninges are acutely inflamed. Either chloramphenicol or a third-generation cephalosporin such as cefotaxime or ceftriaxone is used in persons allergic to penicillins.

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Salmonella

General Concepts

Clinical Manifestations

Salmonellosis ranges clinically from the common Salmonella gastroenteritis (diarrhea, abdominal cramps, and fever) to enteric fevers (including typhoid fever) which are life-threatening febrile systemic illness requiring prompt antibiotic therapy. Focal infections and an asymptomatic carrier state occur. The most common form of salmonellosis is a self-limited, uncomplicated gastroenteritis.

Structure, Classification, and Antigenic Types

Salmonella species are Gram-negative, flagellated facultatively anaerobic bacilli characterized by O, H, and Vi antigens. There are over 1800 known serovars which current classification considers to be separate species.

Pathogenesis

Pathogenic salmonellae ingested in food survive passage through the gastric acid barrier and invade the mucosa of the small and large intestine and produce toxins. Invasion of epithelial cells stimulates the release of proinflammatory cytokines which induce an inflammatory reaction. The acute inflammatory response causes diarrhea and may lead to ulceration and destruction of the mucosa. The bacteria can disseminate from the intestines to cause systemic disease.

Host Defenses

Both nonspecific and specific host defenses are active. Non-specific defenses consist of gastric acidity, intestinal mucus, intestinal motility (peristalsis), lactoferrin, and lysozyme. Specific defenses consist of mucosal and systemic antibodies and genetic resistance to invasion. Various factors affect susceptibility.

Epidemiology

Non-typhoidal salmonellosis is a worldwide disease of humans and animals. Animals are the main reservoir, and the disease is usually food borne, although it can be spread from person to person. The salmonellae that cause Typhoid fever and other enteric fevers spread mainly from person-to-person via the fecal-oral route and have no significant animal reservoirs. Asymptomatic human carriers ("typhoid Marys") may spread the disease.

Diagnosis

Salmonellosis should be considered in any acute diarrheal or febrile illness without obvious cause. The diagnosis is confirmed by isolating the organisms from clinical specimens (stool or blood).

<u>Control</u>

Effective vaccines exist for typhoid fever but not for non-typhoidal salmonellosis. Those diseases are controlled by hygienic slaughtering practices and thorough cooking and refrigeration of food.

INTRODUCTION

Salmonellae are ubiquitous human and animal pathogens, and salmonellosis, a disease that affects an estimated 2 million Americans each year, is common throughout the world. Salmonellosis in humans usually takes the form of a self-limiting food poisoning (gastroenteritis), but occasionally manifests as a serious systemic infection (enteric fever) which requires prompt antibiotic treatment. In addition, salmonellosis causes substantial losses of livestock.

Clinical Manifestations

Some infectious disease texts recognize three clinical forms of salmonellosis: (1) gastroenteritis, (2) septicemia, and (3) enteric fevers. This chapter focuses on the two extremes of the clinical spectrumgastroenteritis and enteric fever. The septicemic form of salmonella infection can be an intermediate stage of infection in which the patient is not experiencing intestinal symptoms and the bacteria cannot be isolated from fecal specimens. The severity of the infection and whether it remains localized in the intestine or disseminates to the bloodstream may depend on the resistance of the patient and the virulence of the Salmonella isolate.

The incubation period for Salmonella gastroenteritis (food poisoning) depends on the dose of bacteria. Symptoms usually begin 6 to 48 hours after ingestion of contaminated food or water and usually take the form of nausea, vomiting, diarrhea, and abdominal pain. Myalgia and headache are common; however, the cardinal manifestation is diarrhea. Fever (38°C to 39°C) and chills are also common. At least two-thirds of patients complain of abdominal cramps. The duration of fever and diarrhea varies, but is usually 2 to 7 days.

Enteric fevers are severe systemic forms of salmonellosis. The best studied enteric fever is typhoid fever, the form caused by S typhi, but any species of Salmonella may cause this type of disease. The symptoms begin after an incubation period of 10 to 14 days. Enteric fevers may be preceded by gastroenteritis, which usually resolves before the onset of systemic disease. The symptoms of enteric fevers are nonspecific and include fever, anorexia, headache, myalgias, and constipation. Enteric fevers are severe infections and may be fatal if antibiotics are not promptly administered.

Structure, Classification, and Antigenic Types

Salmonellae are Gram-negative, flagellated, facultatively anaerobic bacilli possessing three major antigens: H or flagellar antigen; O or somatic antigen; and Vi antigen (possessed by only a few serovars). H antigen may occur in either or both of two forms, called phase 1 and phase 2. The organisms tend to change from one phase to the other. O antigens occur on the surface of the outer membrane and are determined by specific sugar sequences on the cell surface. Vi antigen is a superficial antigen overlying the O antigen; it is present in a few serovars, the most important being S typhi.

Antigenic analysis of salmonellae by using specific antisera offers clinical and epidemiological advantages. Determination of antigenic structure permits one to identify the organisms clinically and assign them to one of nine serogroups (A-I), each containing many serovars. H antigen also provides a useful epidemiologic tool with which to determine the source of infection and its mode of spread.

As with other Gram-negative bacilli, the cell envelope of salmonellae contains a complex lipopolysaccharide (LPS) structure that is liberated on lysis of the cell and, to some extent, during culture. The lipopolysaccharide moiety may function as an endotoxin, and may be important in determining virulence of the organisms. This macromolecular endotoxin complex consists of three components, an outer Opolysaccharide coat, a middle portion (the R core), and an inner lipid A coat. Lipopolysaccharide structure is important for several reasons. First, the nature of the repeating sugar units in the outer O-polysaccharide chains is responsible for O antigen specificity; it may also help determine the virulence of the organism. Salmonellae lacking the complete sequence of O-sugar repeat units are called rough because of the rough appearance of the colonies; they are usually avirulent or less virulent than the smooth strains which possess a full complement of O-sugar repeat units. Second, antibodies directed against the R core (common enterobacterial antigen) may protect against infection by a wide variety of Gram-negative bacteria sharing a common core structure or may moderate their lethal effects. Third, the endotoxin component of the cell wall may play an important role in the pathogenesis of many clinical manifestations of Gram-negative infections. Endotoxins evoke fever, activate the serum complement, kinin, and clotting systems, depress myocardial function, and alter lymphocyte function. Circulating endotoxin may be responsible in part for many of the manifestations of septic shock that can occur in systemic infections.

Pathogenesis

Salmonellosis includes several syndromes (gastroenteritis, enteric fevers, septicemia, focal infections, and an asymptomatic carrier state) (Fig. 1). Particular serovars show a strong propensity to produce a particular syndrome (S typhi, S paratyphi-A, and S schottmuelleri produce enteric fever; S choleraesuis produces septicemia or focal infections; S typhimurium and S enteritidis produce gastroenteritis); however, on occasion, any serotype can produce any of the syndromes. In general, more serious infections occur in infants, in adults over the age of 50, and in subjects with debilitating illnesses.

Most non-typhoidal salmonellae enter the body when contaminated food is ingested (Fig. 2). Person-to-person spread of salmonellae also occurs. To be fully pathogenic, salmonellae must possess a variety of attributes called virulence factors. These include (1) the ability to invade cells, (2) a complete lipopolysaccharide coat, (3) the ability to replicate intracellularly, and (4) possibly the elaboration of toxin(s). After



Figure 21-1 Pathogenesis of salmonellosis.



Figure 21-2 Scheme of the Pathogenesis of Salmonella enterocolitis and diarrhea.

ingestion, the organisms colonize the ileum and colon, invade the intestinal epithelium, and proliferate within the epithelium and lymphoid follicles. The mechanism by which salmonellae invade the epithelium is partially understood and involves an initial binding to specific receptors on the epithelial cell surface followed by invasion. Invasion occurs by the organism inducing the enterocyte membrane to undergo "ruffling" and thereby to stimulate pinocytosis of the organisms (Fig. 3). Invasion is dependent on rearrangement of the cell cytoskeleton and probably involves increases in cellular inositol phosphate and calcium. Attachment and invasion are under distinct genetic control and involve multiple genes in both chromosomes and plasmids.



Figure 21-3 Invasion of intestinal mucosa by Salmonella.

After invading the epithelium, the organisms multiply intracellularly and then spread to mesenteric lymph nodes and throughout the body via the systemic circulation; they are taken up by the reticuloendothelial cells. The reticuloendothelial system confines and controls spread of the organism. However, depending on the serotype and the effectiveness of the host defenses against that serotype, some organisms may infect the liver, spleen, gallbladder, bones, meninges, and other organs (Fig. 1). Fortunately, most serovars are killed promptly in extraintestinal sites, and the most common human Salmonella infection, gastroenteritis, remains confined to the intestine.

After invading the intestine, most salmonellae induce an acute inflammatory response, which can cause ulceration. They may elaborate cytotoxins that inhibit protein synthesis. Whether these cytotoxins contribute to the inflammatory response or to ulceration is not known. However, invasion of the mucosa causes the epithelial cells to synthesize and release various proinflammatory cytokines, including: IL-1, IL-6, IL-8, TNF-2, IFN-U, MCP-1, and GM-CSF. These evoke an acute inflammatory response and may also be responsible for damage to the intestine. Because of the intestinal inflammatory reaction, symptoms of inflammation such as fever, chills, abdominal pain, leukocytosis, and diarrhea are common. The stools may contain polymorphonuclear leukocytes, blood, and mucus.

Much is now known about the mechanisms of Salmonella gastroenteritis and diarrhea. Figures 2 and 3 summarize the pathogenesis of Salmonella enterocolitis and diarrhea. Only strains that penetrate the intestinal mucosa are associated with the appearance of an acute inflammatory reaction and diarrhea (Fig. 4); the diarrhea is due to secretion of fluid and electrolytes by the small and large intestines. The mechanisms of secretion are unclear, but the secretion is not merely a manifestation of tissue destruction and ulceration. Salmonella penetrate the intestinal epithelial cells but, unlike Shigella and invasive E. coli, do not escape the phagosome. Thus, the extent of intercellular spread and ulceration of the epithelium is minimal. Salmonella escape from the basal side of epithelial cells into the lamina propria. Systemic spread of the organisms can occur, giving rise to enteric fever. Invasion of the intestinal mucosa is followed by activation of mucosal adenylate cyclase; the resultant increase in cyclic AMP induces secretion. The mechanism by which adenylate cyclase is stimulated is not understood; it may involve local production of prostaglandins or other components of the inflammatory reaction. In addition, Salmonella strains elaborate one or more enterotoxin-like substances which may stimulate intestinal secretion. However, the precise role of these toxins in the pathogenesis of Salmonella enterocolitis and diarrhea has not been established.

Host Defenses

Various host defenses are important in resisting intestinal colonization and invasion by Salmonella (Table 2). Normal gastric acidity (pH < 3.5) is lethal to salmonellae. In healthy individuals, the number of ingested salmonellae is reduced in the stomach, so that fewer or no organisms enter the intestine. Normal small intestinal motility also protects the bowel by sweeping ingested salmonellae through quickly. The normal intestinal microflora protects against salmonellae, probably through anaerobes, which liberate short-chain fatty acids that are thought to be toxic to salmonellae. Alteration of the anaerobic intestinal flora by antibiotics renders the host more susceptible to salmonellosis. Secretory or mucosal antibodies also protect the intestine against salmonellae. Animal strains genetically resistant to intestinal invasion by salmonellae have been described. When these host defenses are absent or blunted, the host becomes more susceptible to salmonellosis; factors that render the host more susceptible to salmonellosis are listed in Table 3. For example, in AIDS, Salmonella infection is common, frequently persistent and bacteremic, and often resistant to even prolonged antibiotic treatment. Relapses are common. The role of host defenses in salmonellosis is extremely important, and much remains to be learned.

Epidemiology

Contaminated food is the major mode of transmission for non-typhoidal salmonellae because salmonellosis is a zoonosis and has an enormous animal reservoir. The most common animal reservoirs are chickens, turkeys, pigs, and cows; dozens of other domestic and wild animals also harbor these organisms. Because of the ability of salmonellae to survive in meats and animal products that are not thoroughly cooked, animal products are the main vehicle of transmission. The magnitude of the problem is demonstrated by the following recent yields of salmonellae: 41% of turkeys examined in California, 50% of chickens cultured in Massachusetts, and 21% of commercial frozen egg whites examined in Spokane, WA.

The epidemiology of typhoid fever and other enteric fevers primarily involves person-to-person spread because these organisms lack a significant animal reservoir. Contamination with human feces is the major mode of spread, and the usual vehicle is contaminated water. Occasionally, contaminated food (usually handled by an individual who harbors S typhi) may be the vehicle. Plasmid DNA fingerprinting and bacteria phage lysotyping of Salmonella isolates are powerful epidemiologic tools for studying outbreaks of salmonellosis and tracing the spread of the organisms in the environment.

In typhoid fever and non-typhoidal salmonellosis, two other factors have epidemiologic significance. First, an asymptomatic human carrier state exists for the agents of either form of the disease. Approximately 3% of persons infected with S typhi and 0.1% of those infected with non-typhoidal salmonellae become chronic carriers. The carrier state may last from many weeks to years. Thus, human as well as animal reservoirs exist. Interestingly, children rarely become chronic typhoid carriers. Second, use of antibiotics in animal feeds and indiscriminant use of antibiotics in humans increase antibiotic resistance in salmonellae by promoting transfer of R factors.

Salmonellosis is a major public health problem because of its large and varied animal reservoir, the existence of human and animal carrier states, and the lack of a concerted nationwide program to control salmonellae.

Diagnosis

The diagnosis of salmonellosis requires bacteriologic isolation of the organisms from appropriate clinical specimens. Laboratory identification of the genus Salmonella is done by biochemical tests; the serologic type is confirmed by serologic testing. Feces, blood, or other specimens should be plated on several nonselective and selective agar media (blood, MacConkey, eosin-methylene blue, bismuth sulfite, Salmonella-Shigella, and brilliant green agars) as well as intoenrichment broth such as selenite or tetrathionate. Any growth in enrichment broth is subsequently subcultured onto the various agars. The biochemical reactions of suspicious colonies are then determined on triple sugar iron agar and lysine-iron agar, and a presumptive identification is made. Biochemical identification of salmonellae has been simplified by systems that permit the rapid testing of 10-20 different biochemical parameters simultaneously. The presumptive biochemical identification of Salmonella then can be confirmed by antigenic analysis of O and H antigens using polyvalent and specific antisera. Fortunately, approximately 95% of all clinical isolates can be identified with the available group A-E typing antisera. Salmonella isolates then should be sent to a central or reference laboratory for more comprehensive serologic testing and confirmation.

<u>Control</u>

Salmonellae are difficult to eradicate from the environment. However, because the major reservoir for human infection is poultry and livestock, reducing the number of

salmonellae harbored in these animals would significantly reduce human exposure. In Denmark, for example, all animal feeds are treated to kill salmonellae before distribution, resulting in a marked reduction in salmonellosis. Other helpful measures include changing animal slaughtering practices to reduce cross-contamination of animal carcasses; protecting processed foods from contamination; providing training in hygienic practices for all food-handling personnel in slaughterhouses, food processing plants, and restaurants; cooking and refrigerating foods adequately in food processing plants, restaurants, and homes; and expanding of governmental enteric disease surveillance programs.

Recently, The U.S. Department of Agriculture has approved the radiation of poultry to reduce contamination by pathogenic bacteria, e.g. salmonella and campylobacter. Unfortunately, radiation pasteurization has not yet been widely accepted in the U.S. Adoption and implementation of this technology would greatly reduce the magnitude of the salmonella problem.

Vaccines are available for typhoid fever and are partially effective, especially in children. No vaccines are available for non-typhoidal salmonellosis. Continued research in this area and increased understanding of the mechanisms of immunity to enteric infections are of great importance.

General salmonellosis treatment measures include replacing fluid loss by oral and intravenous routes, and controlling pain, nausea, and vomiting. Specific therapy consists of antibiotic administration. Typhoid fever and enteric fevers should be treated with antibiotics. Antibiotic therapy of non-typhoidal salmonellosis should be reserved for the septicemic, enteric fever, and focal infection syndromes. Antibiotics are not recommended for uncomplicated Salmonella gastroenteritis because they do not shorten the illness and they significantly prolong the fecal excretion of the organisms and increase the number of antibiotic-resistant strains.

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Shigella

General Concepts

Clinical Manifestations

Symptoms of shigellosis include abdominal pain, tenesmus, watery diarrhea, and/or dysentery (multiple scanty, bloody, mucoid stools). Other signs may include abdominal tenderness, fever, vomiting, dehydration, and convulsions.

Structure, Classification, and Antigenic Types

Shigellae are Gram-negative, nonmotile, facultatively anaerobic, non-spore-forming rods. Shigella are differentiated from the closely related Escherichia coli on the basis of pathogenicity, physiology (failure to ferment lactose or decarboxylate lysine) and serology. The genus is divided into four serogroups with multiple serotypes: A (S dysenteriae, 12 serotypes); B (S flexneri, 6 serotypes); C (S boydii, 18 serotypes); and D (S sonnei, 1 serotype).

Pathogenesis

Infection is initiated by ingestion of shigellae (usually via fecal-oral contamination). An early symptom, diarrhea (possibly elicited by enterotoxins and/or cytotoxin), may occur as the organisms pass through the small intestine. The hallmarks of shigellosis are bacterial invasion of the colonic epithelium and inflammatory colitis. These are interdependent processes amplified by local release of cytokines and by the infiltration of inflammatory elements. Colitis in the rectosigmoid mucosa, with concomitant malabsorption, results in the characteristic sign of bacillary dysentery: scanty,. unformed stools tinged with blood and mucus.

Host Defenses

Inflammation, copious mucus secretion, and regeneration of the damaged colonic epithelium limit the spread of colitis and promote spontaneous recovery. Serotypespecific immunity is induced by a primary infection, suggesting a protective role of antibody recognizing the lipopolysaccharide (LPS) somatic antigen. Other Shigella antigens include enterotoxins, cytotoxin, and plasmid-encoded proteins that induce bacterial invasion of the epithelium. The protective role of immune responses against these antigens is unclear.

Epidemiology

Shigellosis is endemic in developing countries were sanitation is poor. Typically 10 to 20 percent of enteric disease, and 50% of the bloody diarrhea or dysentery of young children, can be characterized as shigellosis, and the prevalence of these infections decreases significantly after five years of life. In developed countries, single-source, food or water-borne outbreaks occur sporadically, and pockets of endemic shigellosis can be found in institutions and in remote areas with substandard sanitary facilities.

Diagnosis

Shigellosis can be correctly diagnosed in most patients on the basis of fresh blood in the stool. Neutrophils in fecal smears is also a strongly suggestive sign. Nonetheless, watery, mucoid diarrhea may be the only symptom of many S sonnei infections, and any clinical diagnosis should be confirmed by cultivation of the etiologic agent from stools.

<u>Control</u>

Prevention of fecal-oral transmission is the most effective control strategy. Severe dysentery is treated with ampicillin, trimethoprim-sulfamethoxazole, or, in patients over 17 years old, a 4-fluorquinolone such as ciprofloxacin. Vaccines are not currently available, but some promising candidates are being developed.

INTRODUCTION

Gram-negative, facultative anaerobes of the genus Shigella are the principal agents of bacillary dysentery. This disease differs from profuse watery diarrhea, as is commonly seen in choleraic diarrhea or in enterotoxigenic Escherichia coli diarrhea, in that the dysenteric stool is scant and contains blood, mucus, and inflammatory cells. In some individuals suffering from shigellosis, however, moderate volume diarrhea is a prodrome or the sole manifestation of the infection. Bacillary dysentery constitutes a significant proportion of acute intestinal disease in the children of developing countries, and this infection is a major contributor to stunted growth of these children. Shigellosis also presents a significant risk to travelers from developed countries when visiting in endemic areas, and sporadic food or water-borne outbreaks occur in developed countries.

The pathogenic mechanism of shigellosis is complex, involving a possible enterotoxic and/or cytotoxic diarrheal prodrome, cytokine-mediated inflammation of the colon, and necrosis of the colonic epithelium. The underlying physiological insult that initiates this inflammatory cascade is the invasion of Shigella into the colonic epithelium and the lamina propria. The resulting colitis and ulceration of the mucosa result in bloody, mucoid stools, and/or febrile diarrhea.

Clinical Presentation

Shigellosis has two basic clinical presentations: (1) watery diarrhea associated with vomiting and mild to moderate dehydration, and (2) dysentery characterized by a small volume of bloody, mucoid stools, and abdominal pain (cramps and tenesmus) (Table 22-1). Volunteer challenge studies show that shigellosis can be evoked by an extremely small inoculum (10-100 organisms), and the time of onset of symptoms is somewhat influenced by the size of the challenge. The salient point is that shigellosis

is an acute infection with onset of symptoms usually occurring within 24-48 hours of ingestion of the etiologic agent. The average duration of symptoms in untreated adults is 7 days, and the organism may be cultivated from stools for 30 days or long-er.

The clinical features of shigellosis are summarized in Figure 22-1. Watery diarrhea occurs as a prodrome, or as the sole clinical manifestation, in a majority of patients infected with S sonnei. Diarrhea is often a prodome of the dysentery characterizing infection with other species of Shigella. Recently discovered enterotoxins secreted by S flexneri may contribute to the diarrheal phase as the etiologic agents traverse the small intestine. However, diarrhea is most common in patients who have colitis involving the transverse colon or cecum. These patients evidence net water secretion and impaired absorption in the inflamed colon. In patients experiencing dysentery, involvement is most severe in the distal colon, and the resulting inflammatory colitis is evidenced in frequent scanty stools reflecting the ileocecal fluid flow. Dysentery is also characterized by the daily loss of 200-300 ml of serum protein into the feces. This loss of serum proteins results in depletion of nitrogen stores that exacerbates malnutrition and growth stunting. Depletion of immune factors also increases the risk of concurrent, unrelated infectious disease and contributes to substantial mortality.



FIGURE 22-1 Pathogenesis of shigellosis in humans.

Possible complications of shigellosis include bacteremia, convulsions and other neurological complications, reactive arthritis, and hemolytic-uremic syndrome. Bacteremia occasionally accompanies S dysenteriae serotype 1 infections in malnourished infants, but this complication is uncommon in otherwise healthy individuals. Convulsions have been reported in up to 25% of Shigella infections involving children

under the age of 4 years. Both high fever and a family history of seizures are risk factors for a convulsive episode. Ekiri syndrome, an extremely rare, fatal encephalopathy has also been described in Japanese children with S sonnei or S flexneri infections. Reactive arthritis, a self-limiting sequela of S flexneri infection, occurs in an incidence as high as 2% in individuals expressing the HLA-B27 histocompatibility antigen. Hemolytic-uremic syndrome, characterized by a triad of microangiopathic hemolytic anemia, thrombocytopenia, and acute renal failure, is a rare complication in children infected with S dysenteriae serotype 1.

Structure, Classification, and Antigenic Types

Organisms of the genus Shigella belong to the tribe Escherichia in the family Enterobacteriaceae. In DNA hybridization studies, Escherichia coli and Shigella species cannot be differentiated on the polynucleotide level; however, the virulence phenotype of the latter species is a distinctive distinguishing feature. Enteroinvasive E coli (EIEC), are very similar to shigellae biochemically and they also evoke diarrhea and/or dysentery. Some EIEC are also serologically related to shigellae. For example, EIEC serotype O124 agglutinates in S dysenteriae serotype 3 antiserum.

The genus Shigella is differentiated into four species: S dysenteriae (serogroup A, consisting of 12 serotypes); S flexneri (serogroup B, consisting of 6 serotypes); S. boydii (serogroup C, consisting of 18 serotypes); and S sonnei (serogroup D, consisting of a single serotype). Serogoups A, B, and C are very similar physiologically while S. sonnei can be differentiated from the other serogroups by positive b-D-galactosidase and ornithine decarboxylase biochemical reactions. The identification of shigellae by species in the clinical laboratory is usually accomplished by slide agglutination using commercially available, absorbed rabbit antisera.

Pathogenesis

Pathology

The rectosigmoidal lesions of shigellosis resemble those of ulcerative colitis. With frequencies indicated in Figure 22-2, there is proximal extension of erythema, edema, loss of vascular pattern, focal hemorrhage, and adherent layers of purulent exudate. Biopsy specimens from affected areas are typically edematous, with capillary congestion, focal hemorrhage, crypt hyperplasia, goblet cell depletion, mononuclear and polymorphonuclear (PMN) cell infiltration, shedding of epithelial cells and erythrocytes, and microulcerations.

The pathogenic mechanism that underlies these pathological manifestations is diagrammed in Figure 22-3. This cartoon incorporates experimental observations from tissue cultures and from animal models of shigellosis such as rabbit ligated ileal loops injected with virulent organisms. In the latter model, Shigella infection is initiated at the membranous (M) cells that are associated with macroscopic lymphoid follicles (Peyer's patches). Biopsy studies in rhesus monkeys suggest that shigellae also infect microscopic lymphoid follicles of the primate colon. During the early stages of infection, bacteria are transcytosed through the M cells into the subepithelial space. In the subepithelial space, the organisms are phagocytosed by resident macrophages.



FIGURE 22-2 Gross pathology of shigellosis.

However, virulent shigellae are not killed and digested in the macrophage phagolysome. The bacteria lyse the phagosome and initiate apoptosis (programmed cell death). During this process, the infected macrophage releases the inflammatory cytokine IL-1, which elicits infiltration of PMN.



FIGURE 22-3 Histopathology of acute colitis following peroral infection with shigellae. The organisms are initially ingested by membranous (M) cells that are associated with lymphoid microfollicles in the colon. After transcytosis through the M cell, the bacteria are deposited into the subepithelial space where they are phagocytosed by macrophages. The macrophage phagosome is subsequently degraded, and the intracellular shigellae cause release of IL-1 that evokes an influx of polymorphonuclear leukocytes (PMN). Eventually the infected macrophages undergo apoptosis (programmed cell death), and the bacteria are released onto the basolateral surface of adjacent colonic enterocytes. In addition, PMN transmigration through the epithelium disrupts tight junctions, allowing shigellae to migrate into the subepithelial space. The bacteria infect enterocytes by induced endocytosis, and the endocytic vacuoles are subsequently degraded. The intercellular shigellae attach to actin in the enterocyte junctional complex, multiply, and spread to contiguous enterocytes by induced actin polymerization. Ultimately, the infected enterocytes die, and the resulting necrosis of the epithelium, in conjunction with the continuing inflammatory response, constitutes the lesions of shigellosis.

Transmigration of infiltrating PMNs through the tight junctions of local epithelial cells and into the intestinal lumen allows the reverse migration of shigellae from the lumen into the subepithelial spaces. These organisms then infect the columnar epithelial cells by inducing endocytic uptake at the basolateral surface. Immediately after infection of enterocytes, intracellular shigellae lyse endocytic vacuoles and attach to the actin cytoskeleton in the area of the junctional complex. As these organisms multiply within the enterocyte cytoplasm, occasional daughter cells induce polar nucleation of filamentous actin resulting in a "tail" that propels the shigellae into protrusions impinging on contiguous enterocytes. Plasma membranes enveloping the organisms are again lysed, and the organisms are deposited within the contiguous host cell resulting in intercellular bacterial spread.

In summary, shigellosis can be characterized as an acute inflammatory bowel disease initiated by the uptake of only a few organisms into lymphoid follicles. Intracellular replication and intercellular spread leads to an amplified inflammatory cascade at the initial site of entry, and as this inflammation persists and expands, the infiltration of PMN facilitates the entry of additional bacteria into the epithelium. The inflammatory infiltrate can also cause detachment of sheets of epithelial cells in areas devoid of lymphoid structures or bacterial cells.

Genetics of Virulence

Shigella are exquisitely adapted for reproduction within the colonic epithelium of the human host. Many of the bacterial virulence determinants that mediate the complex interactions between these bacteria and mammalian host cells have been identified by genetic and immunological means. These virulence determinants are encoded by large extra-chromosomal elements (plasmids) that are functionally identical in all Shigella species and in EIEC. A complex of two plasmid-encoded determinants, designated Invasion Plasmid Antigens (Ipa) B and C, is recognized by antibody in the sera of convalescent patients. Ipa proteins are maximally expressed in conditions approximating the intestinal lumen (e.g., bile salts, high osmolarity, and human body

temperature), and release of the IpaBC complex is triggered by contact with the mammalian host cell. This complex induces the endocytic uptake of shigellae by M cells, epithelial cells, and macrophages. IpaB also mediates lysis of endocytic vacuoles in epithelial cells or macrophages. In the latter case, Ipa proteins also cause release of the IL-1 cytokine and macrophage apoptosis. Another plasmid-encoded virulence determinant is secreted at the poles of Shigella daughter cells as these organisms multiply within the cytoplasm of infected host cells. This InterCellular Spread (IcsA) protein elicits polymerization of filamentous actin. Formation of this actin tail provides a motive force for shigellae impinging on the plasma membrane of the infected cell. The resulting protrusions deform the plasma membrane of contiguous cells. The IcsB plasmid-encoded protein then lyses the plasma membranes, resulting in intercellular bacterial spread. Biochemical characterization of the interaction between these Shigella virulence determinants and host cell components is a remaining research challenge. Characterizing and enhancing the neutralizing potential of antibody recognizing these protein virulence determinants is also an important research goal.

Toxins

Spent medium from S flexneri or EIEC cultures elicits fluid accumulation in rabbit ligated ileal loops and ion secretion in isolated ileal tissue. Using these assays, enterotoxins designated ShET1 and ShET2 have been identified, and the genetic loci encoding these toxins have been localized to the chromosome and plasmid, respectively. ShET1 is neutralized by convalescent sera from volunteers challenged with S flexneri 2a, suggesting that this toxic moiety is expressed by shigellae growing in the human intestine. The ShET1 locus is present on the chromosome of S flexneri 2a, but it is only occasionally found in other serotypes. In contrast, ShET2 is more widespread and detectable in 80% of shigellae representing all four species. These enterotoxins may elicit the diarrheal prodrome that often precedes bacillary dysentery; however, their role in the disease process remains to be defined by controlled challenge studies using toxin-negative mutants.

S dysenteriae serotype 1 expresses Shiga toxin, an extremely potent, ricin-like, cytotoxin that inhibits protein synthesis in susceptible mammalian cells. This toxin also has enterotoxic activity in rabbit ileal loops, but its role in human diarrhea is unclear, since shigellae apparently express a number of enterotoxins. Experimental infection of rhesus monkeys with S dysenteriae 1, and with a Shiga toxin-negative mutant, suggests that this cytotoxin causes capillary destruction and focal hemorrhage that exacerbates dysentery (see Table 22-1). More importantly, Shiga toxin is associated with the hemolytic-uremic syndrome, a complication of infections with S dysenteriae 1. Closely related toxins are expressed by enterohemorrhagic E coli (EHEC) including the potentially lethal, food-borne O157-H7 serotype.

Host Defense

Shigellae are remarkably infectious enteric pathogenes that can cause disease after the ingestion of as few as 10 organisms. Nonetheless, shigellosis is normally an acute, self-limiting disease that exemplifies the regenerative capacity of the intestinal epithelium. Shigella virulence probably reflects both the efficient uptake by the follicle associated epithelium (M cells) and the amplifying effect of the inflammatory cascade generated by apoptic macrophages. Tenesmus and evacuation of mucus by intestinal goblet cells may effectively eliminate both extracellular shigellae and infected enterocytes from the intestinal lumen, but this defensive response, in conjunction with PMN infiltration, also constitutes the definitive sign of bacillary dysentery. In endemic areas, shigellosis is essentially a childhood disease, and the incidence decreases drastically in the indigenous population over 5 years of age. Controlled volunteer challenge studies in North American adults also indicate that prior infection with S flexneri protects against reinfection with the homologous serotype (70% efficacy). Serotype-specific immune protection against shigellosis suggests that antibody recognizing the O-polysaccharide of LPS protects against clinical symptoms. Ingested bovine colostrum containing antibody recognizing the O-polysaccharide of S flexneri 2a passively protects volunteers challenged with the homologous Shigella serotype. These observations have encouraged the development of a number of parenteral and mucosally administered O-polysaccharide vaccines that are currently in safety and/or efficacy trials. These vaccines offer the possibility of effective control of shigellosis independent of the needed improvements in the public health infrastructure of developing countries, but licensure and delivery of practical Shigella vaccines remains a distant prospect.

Epidemiology

Humans are the primary reservoir of Shigella species, with captive subhuman primates as accidental hosts. In developing countries with prevailing conditions of inadequate sanitation and overcrowded housing, the infection is transmitted most often by the excreta of infected individuals via direct fecal-oral contamination. Flies may contribute to spread from feces to food. The most common species, S dysenteriae and S flexneri, are also the most virulent. In developed countries, sporadic commonsource outbreaks, predominantly involving S sonnei, are transmitted by uncooked food or contaminated water. The latter outbreaks usually involve semipublic water systems such as those found in camps, trailer parks, and Indian reservations. Direct fecal-oral spread can also occur in institutional environments such as child day-care centers. mental hospitals, and nursing homes. Homosexual men are also at increased risk for direct transmission of Shigella flexneri infections, and chronic, recrudescent illness complicating HIV infection has been reported.

<u>Diagnosis</u>

Clinical

Patients presenting with watery diarrhea and fever should be suspected of having shigellosis. The diarrheal stage of the infection cannot be distinguished clinically from other bacterial, viral, and protozoan infections. Nausea and vomiting can accompany Shigella diarrhea, but these symptoms are also observed during infections with nontyphoidal salmonellae and enterotoxigenic E coli. Bloody, mucoid stools are highly indicative of shigellosis, but the differential diagnosis should include EIEC, Salmonella enteritidis, Yersinia enterocolitica, Campylobacter species, and Entamoeba histolytica. Although blood is common in the stools of patients with amebiasis, it is usually dark brown rather than bright red, as in Shigella infections.

scopic examination of stool smears from patients with amebiasis should reveal erythrophagocytic trophozoites in the absence of PMN, whereas bacillary dysentery is characterized by sheets of PMN. Sigmoidoscopic examination of a shigellosis patient reveals a diffusely erythematous mucosal surface with small ulcers, whereas amebiasis is characterized by discrete ulcers in the absence of generalized inflammation.

Laboratory

Although clinical signs may evoke the suspicion of shigellosis, diagnosis is dependent upon the isolation and identification of Shigella from the feces. Positive cultures are most often obtained from blood-tinged plugs of mucus in freshly passed stool specimens obtained during the acute phase of disease. Rectal swabs may also be used to culture shigellae if the specimen is processed rapidly or is deposited in a buffered glycerol saline holding solution. Isolation of shigellae in the clinical laboratory typically involves an initial streaking for isolation on differential/selective media with aerobic incubation to inhibit the growth of the anaerobic normal flora. Commonly used primary isolation media include MacConkey, Hektoen Enteric Agar, and Salmonella-Shigella (SS) Agar. These media contain bile salts to inhibit the growth of other Gram-negative bacteria and pH indicators to differentiate lactose fermenters (Coliforms) from non-lactose fermenters such as shigellae. A liquid enrichment medium (Hajna Gram-negative broth) may also be inoculated with the stool specimen and subcultured onto the selective/differential agarose media after a short growth period. Following overnight incubation of primary isolation media at 37° C, colorless, non-lactose-fermenting colonies are streaked and stabbed into tubed slants of Kligler's Iron Agar or Triple Sugar Iron Agar. In these differential media, Shigella species produce an alkaline slant and an acid butt with no bubbles of gas in the agar. This reaction gives a presumptive identification, and slide agglutination tests with antisera for serogroup and serotype confirm the identification.

Some E coli biotypes of the normal intestinal flora closely resemble Shigella species (i.e. they are nonmotile, delayed lactose fermenters). These coliforms can usually be differentiated from shigellae by the ability to decarboxylate lysine. However, some coliforms cause enteroinvasive disease because they carry the Shigella-like virulence plasmid, and these pathogens are conventionally identified by laborious serological screening for EIEC serotypes. Sensitive and rapid methodology for identification of both EIEC and Shigella species utilizes DNA probes that hybridize with common virulence plasmid genes or DNA primers that amplify plasmid genes by polymerase chain reaction (PCR). Enzyme-linked immunosorbent assay (ELISA) using antiserum or monoclonal antibody recognizing Ipa proteins can also be used to screen stools for enteroinvasive pathogens. These experimental diagnostic techniques are useful for epidemiological studies of enteroinvasive infections, but they are probably too specialized for routine use in the clinical laboratory.

Treatment

Although severe dehydration is uncommon in shigellosis, the first consideration in treating any diarrheal disease is correction of abnormalities that result from isotonic dehydration, metabolic acidosis, and significant potassium loss. The oral rehydration

treatment developed by the World Health Organization has proven effective and safe in the treatment of acute diarrhea, provided that the patient is not vomiting or in shock from severe dehydration. In the latter case, intravenous fluid replacement is required until initial fluid and electrolyte losses are corrected. With proper hydration, shigellosis is generally a self-limiting disease, and the decision to prescribe antibiotics is predicated on the severity of disease, the age of the patient, and the likelihood of further transmission of the infection. Effective antibiotic treatment reduces the average duration of illness from approximately 5-7 days to approximately 3 days and also reduces the period of Shigella excretion after symptoms subside. Absorbable drugs such as ampicillin (2 g/day for 5 days) are likely to be effective when the isolate is sensitive. Trimethoprim (8 mg/kg/day) and sulfamethoxazole (40 mg/kg/day) will eradicate sensitive organisms quickly from the intestine, but resistance to this agent is increasing. Ciprofloxacin (1 g/day for 3 days) is effective against multiple drug resistant strains, but this antibiotic is not approved by the United States Food and Drug Administration for use in children less than 17 years of age because there is a theoretical risk of cartilage damage. Opiates, such as paregoric, induce intestinal stasis and may promote bacterial invasion, prolonging the febrile state.

<u>Control</u>

As is the case with other intestinal infections, the most effective methods for controlling shigellosis are provision of safe and abundant water and effective feces disposal. These public health measures are, at best, long range strategies for control of enteric infections in developing countries. The estimated five million deaths annually attributed to diarrheal disease in these countries, in addition to the malabsorption and growth stunting among survivors, require more immediate and practical approaches. The most effective intervention strategy to minimize morbidity and mortality would involve comprehensive media and personal outreach programs consisting of the following components: (1) education of all residents to actively avoid fecal contamination of food and water and to encourage hand washing after defecation; (2) encourage mothers to breast-feed infants; (3) promote the use of oral rehydration therapy to offset the effects of acute diarrhea; (4) encourage mothers to provide convalescent nutritional care in the form of extra food for children recovering from diarrhea or dysentery.

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Campylobacter and Helicobacter

General Concepts

Campylobacter Jejuni and other Enteric Campylobacters

Clinical Manifestations

Campylobacter species cause acute gastroenteritis with diarrhea, abdominal pain, fever, nausea, and vomiting. Recently, Campylobacter infections have been identified as the most common antecedent to an acute neurological disease, the Guillain-Barré syndrome.

<u>Structure</u>

Campylobacter species are Gram-negative, microaerophilic, non-fermenting, motile rods with a single polar flagellum; they are oxidase-positive and grow optimally at 37° or 42° C.

Classification and Antigenic Types

Campylobacter species have many serogroups, based on lipopolysaccharide (O) and protein (H) antigens. However, only a few serogroups account for most human isolates in a given geographic region. *C jejuni* possesses several common surface-exposed antigens, including porin protein and flagellin.

Pathogenesis

The bacteria colonize the small and large intestines, causing inflammatory diarrhea with fever. Stools contain leukocytes and blood. The role of toxins in pathogenesis is unclear. *C jejuni* antigens that cross-react with one or more neural structures may be responsible for triggering the Guillian-Barre syndrome.

Host Defenses

Nonspecific defenses such as gastric acidity and intestinal transit time are important. Specific immunity, involving intestinal immunoglobulin (IgA) and systemic antibodies, develops. Persons deficient in humoral immunity develop severe and prolonged illnesses.

Epidemiology

C jejuni and C coli infections are endemic worldwide and hyperendemic in developing countries. Infants and young adults are most often infected. Disease incidence peaks in the summer. Domestic and wild animals are the reservoirs for the organisms. Outbreaks are associated with contaminated animal products or water.

Diagnosis

Observation of darting motility in fresh fecal specimens or of vibrio forms on Gram stain permit presumptive diagnosis; definitive diagnosis is established by stool culture, and occasionally by blood culture.

<u>Control</u>

Control depends on measures to prevent transmission from animal reservoirs to humans.

Helicobacter Pylori and other Gastric Helicobacter-like Organisms

Clinical Manifestations

Helicobacter pylori is associated with chronic superficial gastritis (stomach inflammation) and plays a role in the pathogenesis of peptic ulcer disease. Increasing evidence indicates that H pylori infection is important in causing gastric carcinoma and lymphoma. Acute infection may cause vomiting and upper gastrointestinal pain; hypochlorhydria and intense gastritis develop. Chronic infection usually is asymptomatic.

Structure

This Gram-negative curved or spiral rod is distinguished by multiple, sheathed flagellae and abundant urease.

Classification and Antigenic Types

The antigenic structures are not completely defined and no universal typing scheme has been developed; strains may be differentiated by genotypic methods including restriction endonuclease analysis, and polymerase chain reaction (PCR).

Pathogenesis

Helicobacter pylori is sheltered from gastric acidity in the mucus layer and a small proportion of cells adheres to the gastric epithelium. The microorganism does not appear to invade tissue. Production of urease, a vacuolating cytotoxin, and the cagA-encoded protein is associated with injury to the gastric epithelium.
Host Defenses

Local and systemic humoral immune responses are essentially universal, but are not able to clear infection.

Epidemiology

H pylori infection has a worldwide distribution; about 1/3 of the world's population is infected. The prevalence of infection increases with age. The major, if not exclusive, reservoir is humans but the exact modes of transmission are not known. H pylori has now been isolated from feces and dental plaque.

Diagnosis

Examination of gastric biopsy or stained smears allows presumptive diagnosis; definitive diagnosis is made by culture. Recently, non-invasive techniques such as the urea breath test and serologic tests have been developed to diagnose H pylori infection, with accuracy exceeding 95 percent.

<u>Control</u>

Several indications have emerged for the use of antimicrobial therapies that eradicate H pylori infection. No vaccine is yet available.

Other Pathogenic Camplyobacter and Helicobacter Species

Campylobacter fetus causes bacteremia in compromised hosts and self-limited diarrhea in previously healthy individuals. Helicobacter cinaedi and H fennelliae cause enteric and extraintestinal diseases and are more common in homosexual men and in travelers.

INTRODUCTION

Campylobacter and Helicobacter are Gram-negative microaerophilic bacteria that are widely distributed in the animal kingdom. They have been known as animal pathogens for nearly 100 years. However, because they are fastidious and slow-growing in culture, they have been recognized as human gastrointestinal pathogens only during the last 20 years. They can cause diarrheal illnesses, systemic infection, chronic superficial gastritis, peptic ulcer disease, and can lead to gastric carcinoma.

Table 23-1 lists the Campylobacter species known to be pathogenic for humans. Campylobacter jejuni, and, less often, C coli and C lari are the most common bacterial causes of acute diarrheal illnesses in developed countries. Helicobacter pylori (formerly known as Campylobacter pylori), which was first cultured from gastric biopsy tissues in 1982, causes chronic superficial gastritis and is associated with the pathogenesis of peptic ulcer disease and gastric cancer. Campylobacter fetus subspecies fetus occasionally causes systemic illnesses in compromised hosts.

Campylobacter Jejuni and other Enteric Campylobacters

Clinical Manifestations

The symptoms and signs of Campylobacter enteritis are not distinctive enough to differentiate it from illness caused by many other enteric pathogens. Symptoms range from mild gastrointestinal distress lasting 24 hours to a fulminating or relapsing colitis that mimics ulcerative colitis or Crohn's disease (Figure 23-1). The predominant symptoms experienced by individuals in developed countries are diarrhea, abdominal pain, fever, nausea, and vomiting. A history of grossly bloody stools is common, and many patients have at least one day with eight or more bowel movements; fecal leukocytes are usually present. A cholera-like illness with massive watery diarrhea may also occur. Campylobacter enteritis usually is self-limiting with gradual improvement in symptoms over several days. Most patients recover within a week, but 10%-20% experience relapse or a prolonged severe illness. Toxic megacolon, pseudomembranous colitis, and massive lower gastrointestinal hemorrhage also have been described. Mesenteric adenitis and appendicitis have been reported in children and young adults. Bacteremia is uncommon (<1%) in immunocompetent patients with *C jejuni* infection.



Figure 23-1 Pathogenesis of Campylobacter and Helicobacter infection in humans.

Among populations in developing countries, infection by *C jejuni* and closely related organisms is associated with much milder illness, without bloody diarrhea, fever or fecal leukocytes. Asymptomatic infection is much more common than in the developed countries, especially in older children and adults. However, when travelers from developed countries acquire *C jejuni* infections in developing countries, the symptoms are those associated with an inflammatory process. This indicates that organisms in the developing countries are fully pathogenic. Guillain-Barré syndrome is an uncommon consequence of *C jejuni* infection that is present 2-3 weeks after the diarrheal illness. However, because of the high incidence of Campylobacter infection, it has been estimated to be the trigger of 20 to 40 percent of all cases of Guillain-Barré syndrome.

Structure

Campylobacter jejuni, like all Campylobacter species, is a microaerophilic, non-fermentative Gram-negative organism. The name Campylobacter, meaning "curved rod," describes the appearance of the organisms (Figure 23-2). In young cultures, or-

ganisms are comma shaped, spiral, S shaped, or gull-winged shaped; as cultures age or are subjected to atmospheric or temperature stresses, round or coccoid forms appear.

C jejuni, which is structurally similar to other Gram-negative bacilli, is motile, with a single flagellum at one or both poles of the cell. The cell envelope has an inner bipolar lipid cell membrane, a thin peptidoglycan layer, an outer bipolar lipid layer with the lipid moiety of a lipopolysaccharide layer embedded in it, and the carbohydrate portion extending to the surface of the cell. Interspersed in the outer membrane layer are membrane proteins, some of which are exposed to the surface and are antigenic for infected hosts. Many Campylobacter species contain surface proteins that are external to the outer membrane. Campylobacter lipopolysaccharide has endotoxin activity similar to that of other Gram-negative bacteria.

All Campylobacter species except H pylori are similar in structure and appearance. The Campylobacter and Helicobacter species and subspecies may be differentiated by biochemical markers.

Classification and Antigenic Types

Based on heat-labile antigens, at least 108 serogroups of both *C jejuni* and C coli have been described. In addition, 47 and 18 different heat-stable somatic (O) antigens have been described among isolates of *C jejuni* and C coli organisms, respectively. Although geographic differences in the prevalence of serogroups exist, 10 O-groups account for about 70% of human infections. Similarly, only a few serogroups account for most human isolates in any geographic region. Serotyping has been of value in numerous epidemiologic investigations.

Despite the antigenic diversity of these organisms, C *jejuni* possesses several common surface-exposed antigens which have been used for development of serological tests. Major antigens include the porin protein (Mr 45,000), flagellin (Mr 63,000), and a group of proteins around Mr 30,000 that appear important in adhesion. The flagellar proteins undergo antigenic phase variation.

Pathogenesis

As with other enteric pathogens, the attack rate of *C jejuni* varies with the ingested dose. In outbreaks of Campylobacter enteritis, the incubation period has ranged from 1-7 days, with most illness developing 2-4 days after infection. Infection leads to multiplication of organisms in the intestines. Patients shed 106 to 109 Campylobacter per gram of feces, concentrations similar to those shed in Salmonella and Shigella enteric infections. The sites of tissue injury include the small and large intestines, and the lesions show an acute exudative and hemorrhagic inflammation. Patients frequently have colonic involvement consisting of inflammation of the lamina propria with neutrophils, eosinophils, and mononuclear cells. Destruction of epithelial glands with crypt abscess formation occurs in severe cases (Figure 23-3). The pathologic lesions seen in Campylobacter colitis are difficult to distinguish from those in ulcerative colitis. Therefore, before ulcerative colitis can be diagnosed, infection by Campylobacter and related organisms should be ruled out.

The mechanisms by which *C jejuni* causes illness are uncertain. Cellular infiltration in colonic biopsy specimens of patients with Campylobacter infections and the occa-

sional presence of bacteremia suggest that these organisms may be invasive. That most Campylobacter enteritis in developed countries is associated with fever and the presence of fecal leukocytes and blood in the stool also is consistent with the invasive characteristics of the organisms. *C jejuni* is invasive in vitro in chicken embryo cells and causes bacteremia in experimentally infected mice, rabbits, calves, chickens and monkeys.

Some *C jejuni* isolates elaborate very low levels of cytotoxins similar to Shiga toxin. Some isolates have been reported to elaborate an enterotoxin similar to cholera toxin. Enterotoxin production has been more frequently observed in isolates from developing countries, where infection by *C jejuni* has been associated with watery diarrhea. However, the clinical significance of the toxigenicity of these organisms is still unclear. Strains lacking detectable enterotoxin production and with low level in vitro cytotoxin production were fully virulent in volunteers. Campylobacter jejuni may adhere in vitro in several tissue culture lines. This may be important in intestinal colonization or may enhance tissue invasion. A superficial antigen (PEB1) that appears to be the major adhesin is conserved among *C jejuni* strains. However, the actual in vivo significance of adherence remains undefined.

Host Defenses

C *jejuni* and related organisms are capable of infecting healthy persons as well as immunocompromised patients. The minimal infection-causing dose of C *jejuni* is not known, although volunteers who have ingested as few as 800 organisms have become ill. Campylobacter jejuni can be killed by hydrochloric acid, suggesting that normal gastric acidity may be an important barrier against infection.

Neutrophils often are observed in the feces of patients infected with *C jejuni*, and colonic biopsy specimens from patients with Campylobacter colitis have shown marked infiltration with neutrophils, suggesting that these cells may be important in host defense. In mice, macrophages are important for clearance of bacteremia, and, in vitro, *C jejuni* antigens stimulate a T-cell response.

Acutely infected persons frequently develop elevated specific serum Immunoglobulin A (IgA), IgG, and IgM titers, which may persist for several weeks. Experimentally infected animals and humans manifest specific intestinal IgA production. Whether the antibody response eliminates the infection or protects against reinfection is not known. However, upon challenge with *C jejuni*, human volunteers with elevated specific serum IgA levels were likely to develop asymptomatic infection with only a brief duration of pathogen excretion. In contrast, hypogammaglobulinemic persons and those with acquired immune deficiency syndrome (AIDS) are at increased risk for severe, recurrent or bacteremic infections. *C jejuni* isolates are usually susceptible to complement-mediated killing by normal serum. Regardless of the exact host defense mechanisms involved, most *C jejuni* infections resolve spontaneously.

Epidemiology

In developed countries, *C jejuni* is an important cause of diarrhea, particularly in children and young adults (Figure 23-4). Between 3 and 14 percent of patients with diarrhea who seek medical attention are infected with *C jejuni*. Prolonged asymptomatic carriage is rare. The attack rate is highest in children less than 1 year old, and

gradually decreases throughout childhood. A second peak occurs in young adults (18 to 29 years old). Although *C jejuni* enteritis occurs throughout the year, the highest isolation rates occur in summer, as with other enteric pathogens. In contrast, up to 40 percent of healthy children in developing countries may carry the organism at any time. This is an age-related phenomenon, with the highest excretion rates in very young children. Case-to-infection ratios decline with age, which probably is indicative of acquisition of immunity due to recurrent exposure.

Diagnosis

Campylobacter enteritis is hard to distinguish from enteritis caused by other pathogens. The presence of neutrophils or blood in the feces of patients with acute diarrheal illnesses is an important clue to Campylobacter infection. Darting motility in a fresh fecal specimen observed by dark-field or phase-contrast microscopy or characteristic vibrio forms visible after Gram staining permit a presumptive diagnosis. The diagnosis is confirmed by isolating the organism from a fecal culture or, rarely, from a blood culture (Figure 23-5). Because of its growth requirement for microaerobic atmosphere, special laboratory methods are needed to isolate *C jejuni*. Plating methods must be selective to inhibit the growth of competing microorganisms in the fecal flora. The traditional approach to isolating C jejuni has been to use media that contain antibiotics to which C jejuni is resistant but most members of the usual flora are susceptible. However, owing to their motility and small diameter, Campylobacter organisms have been isolated by filtration methods that do not use antibioticcontaining media. Use of filters (pore size 0.6µm) in conjunction with non-selective media improves stool culture yields of both C jejuni and the atypical enteric Campylobacters. Polymerase chain reaction (PCR)-based techniques have been developed for rapid detection, culture confirmation and for typing of *C jejuni* strains.



Figure 23-5 Detection of *C jejuni* and related enteric bacteria.

Because Campylobacter is microaerophilic, cultures must be incubated in an environment with reduced oxygen, optimally between 5 and 10 percent. The optimal temperature for growth is 42° C for *C jejuni*, and 37° C for many of the other enteric Campylobacters. When selective methods are used, suspicious colonies can be readily identified by their spreading character, mucoid appearance, and grayish color. The series of biochemical reactions outlined in Table 23-2 can differentiate the Campylobacter species. Serologic methods for diagnosis are only research tools at the present. A non-radioactive gene probe is available for rapid identification of *C jejuni* and C coli from isolated colonies.

Control

Control of Campylobacter enteritis depends largely on interrupting the transmission of the organism to humans from farm and domestic animals, food of animal origin, or contaminated water. Individuals can reduce the risk of Campylobacter infection by properly cooking and storing meat and dairy products, avoiding contaminated drinking water and unpasteurized milk, and washing their hands after contact with animals or animal products.

Fluid and electrolyte replacement are the cornerstones for treatment. Specific treatment with antimicrobial agents indicated for persons with severe or prolonged symptoms. However, for mild infections, the efficacy of treatment with antimicrobial agents has not yet been demonstrated. When treatment is required, erythromycin or ciprofloxacin appear to be the agents of choice. The presence of several surfaceexposed, broadly specific proteins may permit vaccine development.

Other Pathogenic Campylobacter Species

Campylobacter fetus subsp fetus, well known as an animal pathogen, may cause bacteremia and other extraintestinal infections in compromised hosts, as well as an uncommon self-limited diarrheal illness in previously healthy persons. Recognized complications of C fetus infection include meningitis, endocarditis, pneumonia, thrombophlebitis, septicemia, arthritis, and peritonitis.

Virtually all C fetus isolates from humans possess lipopolysaccharide molecules with long polysaccharide side chains. Two major serogroups, A and B, have been identified. A microcapsule of high molecular-weight, antigenically related surface-array proteins has been associated with serum and phagocytosis resistance. These proteins apparently mediate serum resistance by inhibiting the binding of complement component C3b, thereby conferring to the organism a significant survival advantage. C fetus can undergo antigenic variation, switching the particular S-layer protein expressed.

Helicobacter Pylori and other Gastric Helicobacter-Like Organisms

Clinical Manifestations

Helicobacter pylori has repeatedly been shown to be associated with chronic superficial gastritis (CSG), which involves the antrum and the fundus of the stomach (Figure 23-1). Essentially all infected persons develop chronic superficial gastritis and it has clearly been shown worldwide that H pylori is the major cause of this lesion. Most of the patients with H pylori-induced chronic superficial gastritis are asymptomatic. The organisms are present on the luminal surface of mucus-secreting cells and

within gastric pits, but do not invade tissue. Colonization of the affected areas of the gastric mucosa may be patchy (heavily colonized areas may be adjacent to those with no colonization). Organisms are generally not present over areas of intestinal metaplasia in the gastric mucosa. This CSG is nearly always present in patients with either gastric or duodenal ulcers. Essentially all patients with duodenal ulcers harbor H pylori in the duodenum. In duodenal ulcer disease, H pylori is associated with gastric metaplasia, but not with normal duodenal mucosa. The association of H pylori infection and gastric metaplasia is highly associated with active duodenitis.

H pylori causes the most common form of chronic gastritis (CSG), and chronic gastritis is a well known risk factor for the development of gastric carcinoma. The epidemiologic characteristics of H pylori infection are similar to those observed in the epidemiology of adenocarcinoma of the stomach. In addition, the development of intestinal metaplasia and atrophic gastritis, two risk factors for gastric cancer, are associated with H pylori infection. All these data and prospective epidemiologic studies indicate that infection of humans with H pylori is causally associated with the risk of developing gastric cancer. H pylori infection also is associated with risk of developing gastric lymphoma.

Structure

H pylori differs genetically from members of the genus Campylobacter, and has been reclassified from Campylobacter (where it was initially placed) to the separate genus Helicobacter. H pylori organisms are microaerophilic, nonsporulating, Gramnegative curved rods, $3.5 \mu m$ long and 0.5 to $1 \mu m$ wide, with a spiral periodicity in fresh cultures and spherical (coccoid) forms present in older cultures. H pylori further differs from Campylobacter species in having multiple polar sheathed flagellae (Figure 23-6), a unique composition of cell wall fatty acids, and a smooth surface. Most Campylobacter species contain either unipolar or bipolar single unsheathed flagellae and have a wrinkled surface. Unlike most campylobacters, H pylori produces urease and does not grow when incubated below 30° C. Growth is best on chocolate or blood agar plates after incubation for 2 to 5 days; for liquid media, either a blood or a hemin source appears essential.

Classification and Antigenic Types

The antigenic nature of H pylori has not been completely defined. The whole-cell and outer-membrane profiles of all H pylori isolates have major similarities and are substantially different from those of *C jejuni* and C fetus. However, H pylori has strain-specific protein and lipopolysaccharide antigens, so it may be possible to type the organism. Simple systems for biotyping and serotyping H pylori are not yet available, but strains can be differentiated by genotypic-based techniques such as restriction endonuclease analysis, polymerase chain reaction, and restriction fragment length polymorphism (RFLP).

Pathogenesis

Helicobacter pylori are readily killed by brief exposure to hydrochloric acid solutions with pH below 4.0. This is paradoxical for an organism whose primary residence is the gastric lumen. However, several factors may explain this phenomenon. Firstly, H pylori lives in the mucus layer overlaying the gastric mucosa, a niche protected against gastric acid. This mucus is relatively thick and viscous and maintains a pH gradient from approximately pH 2 adjacent to the gastric lumen to pH 7.4 immediately above the epithelial cells. Secondly, H pylori is among the most efficient producers of urease. An important effect of this metabolic activity is the release of ammonia, which buffers acidity. Third, H pylori is highly motile even in very viscous mucus. This motility may allow organisms to migrate to the most favorable pH gradient. Finally, acute H pylori infection is associated with hypochlorhydria. H pylori only overlays gastric-type but not intestinal-type epithelial cells; a proportion of the bacterial cells are adherent.

Inflammatory infiltrates with polymorphonuclear leukocytes, eosinophils, and an increased number of lymphocytes are observed in the epithelium and lamina propria (Figure 23-7). The exact mechanism by which H pylori causes tissue injury is unknown. At present there is little evidence for direct tissue invasion by H pylori. For pathogenic organisms that do not invade tissue, the lesions are likely to reflect a response to extracellular products such as exotoxins. An 87kDa cytotoxin that induces vacuolation of eukaryotic cells is expressed in vitro by about 50% of strains. However, vacA, the gene encoding this toxin, is present in all strains but has substantial variability. Strains from patients with ulcers are more often toxin-producing than are strains from patients with gastritis only. H pylori also appears to affect the gastric mucus layer in which it resides. Isolates cultured in vitro produce an extracellular protease. This proteolytic activity affects the ability of mucus to retard diffusion of hydrogen ions. Mucus depletion over inflamed tissues is characteristic of H pyloriassociated gastritis. Ammonia, produced by urease, is known to be toxic to eukaryotic cells and may potentiate mucosal injury. H pylori strains from patients with duodenal ulceration more frequently express a highly antigenic protein of 120-128kDa than H pylori strains from patients with gastritis. The gene encoding this protein, termed cagA, only is present in about 60% of H pylori strains.

Host Defenses

Although gastric acid plays an important role in protection against many enteric organisms, it is not a sufficient barrier to prevent colonization of the gastric mucosa by H pylori. Infected persons develop high titers of serum and local IgA and IgG antibodies to H pylori. Longitudinal serologic studies show that H pylori can persist for years or longer despite these high antibody levels. It is not known whether these specific antibodies play any protective role, such as inhibiting adherence or promoting opsonophagocytosis. The role of cell-mediated immunity to these persistent pathogens has only been explored in recent years. Activation of mononuclear cells by H pylori induces production of tumor necrosis factor a, Interleukin-1 and other cytokines. Differences among infected hosts in cell-mediated immunity are possible determinants of outcome variability.

Epidemiology

H pylori is found worldwide and affects persons from diverse socioeconomic strata. The prevalence of these infections, as documented by both histologic and serologic studies, rises with age, as does gastritis. Person-to-person transmission is the major, if not exclusive, source of infection. H pylori has been isolated from dental plaque, and DNA products may be detected in saliva by PCR. H pylori has been isolated from feces. These data indicate potential routes of transmission of H pylori. H pylori is frequently isolated from asymptomatic persons who have no dyspeptic or ulcerrelated symptoms. H pylori infection is more common among populations from developing areas than in more industrialized countries. Moreover, high prevalence of infection has been observed among persons in settings where sanitary conditions are suboptimal suggesting that fecal-oral transmission occurs. Infection, defined by seropositivity, persists for years and possibly for life. The annual incidence of infection in adult populations in developed countries is approximately 1 percent. On occasion, transmission occurs from person to person via contaminated endoscopes.

Other gastric Helicobacter-like organisms have now been observed in a variety of animals, including rodents, primates, swine, and ferrets, but with the exception of primates and possibly cats, these isolates are clearly different from human isolates. Human exposure to non-human primates is not sufficiently frequent to explain the wide prevalence of H pylori infection in humans. Food-borne transmission would not be unusual for an enteric pathogen, but no other environmental reservoirs of H pylori have been identified.

Diagnosis

H pylori can be presumptively identified in freshly prepared gastric biopsy smears by phase-contrast microscopy, based on the characteristic motility of the microorganisms, and by staining histologic sections from gastric biopsies with Gram (carbol fuchsin counterstain), Warthin-Starry silver, Giemsa, or acridine orange stains. Organisms also can be seen directly in fixed tissue stained with hematoxylin and eosin. H pylori may be isolated from gastric tissue or from biopsies of esoageal or duodenal tissue containing gastric metaplasia (Figure 23-8) using nonselective media, such as chocolate agar, or antibiotic-containing selective media, such as those of Skirrow or Goodwin. Spiral organisms that are oxidase-, catalase-, and urease-positive can be identified as H pylori. Culture allows determination of antimicrobial susceptibilities. In gastric biopsies, H pylori also can be diagnosed presumptively, on the basis of the presence of preformed urease. DNA probe and PCR methodologies have been developed as well.

All of the above tests require endoscopy and biopsy. A non-invasive technique known as the urea breath test has been developed to diagnose H pylori infection. Infection can also be diagnosed accurately by detecting serum antibodies to H pylori antigens. These methods may be more sensitive than diagnostic methods involving biopsies. These non-invasive methods will greatly facilitate diagnosis in individual patients, aid studies of the epidemiology of this infection, and be useful for evaluation of the efficacy of antimicrobial therapy. A number of kits now are commercially available.

Control

Antimicrphobial therapy for treatment of this infection has emerged as the most important means to resolve H pylori infection. Antimicrobial therapy is now one of the primary therapies for duodenal ulceration. Studies to identify the best combinations of antibiotics are being done. However, for most cases of H pylori-associated nonulcer dyspepsia, data related to efficacy of antimicrobial therapy are not clear.



Figure 23-8 Detection methods for H pylori

Other Pathogenic Helicobacter Species

Helicobacter cinaedi and Helicobacter fennelliae are two newly recognized Helicobacter species, formerly identified as Campylobacter species which have been associated with enteric and extraintestinal diseases; they are more common in homosexual men, and in travelers to developing countries.

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Cholera, Vibrio cholerae O1 and O139, and Other Pathogenic Vibrios

Cholera and Vibrio cholerae

Clinical Manifestations

Cholera is a potentially epidemic and life-threatening secretory diarrhea characterized by numerous, voluminous watery stools, often accompanied by vomiting, and resulting in hypovolemic shock and acidosis. It is caused by certain members of the species Vibrio cholerae which can also cause mild or inapparent infections. Other members of the species may occasionally cause isolated outbreaks of milder diarrhea whereas othersthe vast majorityare free-living and not associated with disease.

Structure, Classification, and Antigenic Types

Vibrios are Gram-negative, highly motile curved rods with a single polar flagellum. They tolerate alkaline media that kill most intestinal commensals, but they are sensitive to acid. Numerous free-living vibrios are known, some potentially pathogenic. Until 1992, cholera was caused by only two serotypes, Inaba (AC) and Ogawa (AB), and two biotypes, classical and El Tor, of toxigenic O group 1 V cholerae. These organisms may be identified by agglutination in O group 1-specific antiserum directed against the lipopolysaccharide component of the cell wall and by demonstration of their enterotoxigenicity. In 1992, cholera caused by serogroup O139 (synonym "Bengal"; the 139th and latest serogroup of V cholerae to be identified by 1) absence of agglutination in O group 1 specific antiserum; 2) by agglutination in O group 139 specific antiserum; and 3) by the presence of a capsule.

Pathogenesis

Cholera is transmitted by the fecal-oral route. Vibrios are sensitive to acid, and most die in the stomach. Surviving virulent organisms may adhere to and colonize the small bowel, where they secrete the potent cholera enterotoxin (CT, also called "choleragen"). This toxin binds to the plasma membrane of intestinal epithelial cells and releases an enzymatically active subunit that causes a rise in cyclic adenosine 51-monophosphate (cAMP) production. The resulting high intracellular cAMP level causes massive secretion of electrolytes and water into the intestinal lumen.

Host Defenses

Gastric acid, mucus secretion, and intestinal motility are the prime nonspecific defenses against V cholerae. Breastfeeding in endemic areas is important in protecting infants from disease. Disease results in effective specific immunity, involving primarily secretory immunoglobulin (IgA), as well as IgG antibodies, against vibrios, somatic antigen, outer membrane protein, and/or the enterotoxin and other products.

Epidemiology

Cholera is endemic or epidemic in areas with poor sanitation; it occurs sporadically or as limited outbreaks in developed countries. In coastal regions it may persist in shellfish and plankton. Long-term convalescent carriers are rare. Enteritis caused by the halophile V parahaemolyticus is associated with raw or improperly cooked seafood.

Diagnosis

The diagnosis is suggested by strikingly severe, watery diarrhea. For rapid diagnosis, a wet mount of liquid stool is examined microscopically. The characteristic motility of vibrios is stopped by specific antisomatic antibody. Other methods are culture of stool or rectal swab samples on TCBS agar and other selective and nonselective media; the slide agglutination test of colonies with specific antiserum; fermentation tests (oxidase positive); and enrichment in peptone broth followed by fluorescent antibody tests, culture, or retrospective serologic diagnosis. More recently the polymerase chain reaction (PCR) and additional genetically-based rapid techniques have been recommended for use in specialized laboratories.

Control

Control by sanitation is effective but not feasible in endemic areas. A good vaccine has not yet been developed. A parenteral vaccine of whole killed bacteria has been used widely, but is relatively ineffective and is not generally recommended. An experimental oral vaccine of killed whole cells and toxin B-subunit protein is less than ideal. Living attenuated genetically engineered mutants are promising, but such strains can cause limited diarrhea as a side effect. Antibiotic prophylaxis is feasible for small groups over short periods.

Other Vibrio Infections

Other serogroups of V cholerae may cause diarrheal disease and other infections but are not associated with epidemic cholera. Vibrio parahaemolyticus is an important cause of enteritis associated with the ingestion of raw or improperly prepared seafood. Other Vibrio species, including V vulnificus, can cause infections of humans and other animals including fish. Campylobacter species (formerly included with vibrios) can cause enteritis. C pylori, now known as Helicobacter pylori, is associated with gastric and duodenal ulcers (see Ch. 23).

INTRODUCTION

Vibrios are highly motile, gram-negative, curved or comma-shaped rods with a single polar flagellum. Of the vibrios that are clinically significant to humans, Vibrio cholerae O group 1, the agent of cholera, is the most important. Vibrio cholerae was first isolated in pure culture by Robert Koch in 1883, although it had been seen by other investigators, including Pacini, who is credited with describing it first in Florence, Italy, in 1854.

Cholera is a life-threatening secretory diarrhea induced by an enterotoxin secreted by V cholerae. Cholera and the cholera enterotoxin are increasingly recognized as the prototypes for a wide variety of non-invasive diarrheal diseases, collectively known as the enterotoxic enteropathies; of these, diarrhea due to enterotoxigenic strains of Escherichia coli (see Ch. 26) is the most important. Cholera remains a major epidemic disease. There have been seven great pandemics. The latest, which started in 1961, invaded the Western Hemisphere (for the first time this century) with a massive outbreak in Peru in 1991. There have since been more than a million cases in Central and South America as well as a few imported cases in the U.S. and Canada. V cholerae serogroup O139, which arose in October of 1992 in India and Bangladesh, may become the cause of the 8th great pandemic of cholera.

Other vibrios may also be clinically significant in humans, and some are known to cause diseases in domestic animals. Nonpathogenic vibrios are widely distributed in the environment, particularly in estuarine waters and seafoods. For this reason, isolation of a vibrio from a patient with diarrheal disease does not necessarily indicate an etiologic relationship.

<u>Vibrio Cholerae</u>

Clinical Manifestations

Following an incubation period of 6 to 48 hours, cholera begins with the abrupt onset of watery diarrhea (Fig. 24-1). The initial stool may exceed 1 L, and several liters of fluid may be secreted within hours, leading to hypovolemic shock. Vomiting usually accompanies the diarrheal episodes. Muscle cramps may occur as water and electrolytes are lost from body tissues. Loss of skin turgor, scaphoid abdomen, and weak pulse are characteristic of cholera. Various degrees of fluid and electrolyte loss are observed, including mild and subclinical cases. The disease runs its course in 2 to 7 days; the outcome depends upon the extent of water and electrolyte loss and the adequacy of water and electrolyte repletion therapy. Death can occur from hypovolemic shock, metabolic acidosis, and uremia resulting from acute tubular necrosis.



FIGURE 24-1 Pathophysiology of cholera.

Structure, Classification, and Antigenic Types

The cholera vibrios are Gram-negative, slightly curved rods whose motility depends on a single polar flagellum. Their nutritional requirements are simple. Fresh isolates are prototrophic (i.e., they grow in media containing an inorganic nitrogen source, a utilizable carbohydrate, and appropriate minerals). In adequate media, they grow rapidly with a generation time of less than 30 minutes. Although they reach higher population densities when grown with vigorous aeration, they can also grow anaerobically. Vibrios are sensitive to low pH and die rapidly in solutions below pH 6; however, they are quite tolerant of alkaline conditions. This tolerance has been exploited in the choice of media used for their isolation and diagnosis.

Until 1992, the vibrios that caused epidemic cholera were subdivided into two biotypes: classical and El Tor. Classical V cholerae was first isolated by Koch in 1883. Subsequently, in the early 1900s, some vibrios resembling V cholerae were isolated from Mecca-bound pilgrims at the quarantine station at El Tor, in the Sinai peninsula, that had been established to try to control cholera associated with pilgrimages to Mecca. These vibrios resembled classical V cholerae in many ways but caused lysis of goat or sheep erythrocytes in a test known as the Greig test. Because the pilgrims from whom they were isolated did not have cholera, these hemolytic El Tor vibrios were regarded as relatively insignificant except for the possibility of confusion with true cholera vibrios. In the 1930s, similar hemolytic vibrios were associated with relatively restricted outbreaks of diarrheal disease, called paracholera, in the Celebes. In 1961, cholera caused by El Tor vibrios erupted in Hong Kong and spread virtually worldwide. Although in the course of this pandemic most V cholerae biotype El Tor strains lost their hemolytic activity, a number of ancillary tests differentiate them from vibrios of the classical biotype.

The operational serology of the cholera vibrios which belong in O antigen group 1 is relatively simple. Both biotypes (El Tor and classical) contain two major serotypes, Inaba and Ogawa (Fig. 24-2). These serotypes are differentiated in agglutination and vibriocidal antibody tests on the basis of their dominant heat-stable lipopolysaccharide somatic antigens. The cholera group has a common antigen, A, and the sero-types are differentiated by the type-specific antigens, B (Ogawa) and C (Inaba). An additional serotype, Hikojima, which has both specific antigens, is rare. V cholerae O139 appears to have been derived from the pandemic El Tor biotype but has lost the characteristic O1 somatic antigen; it has gained the ability to produce a polysac-charide capsule; it produces the same cholera enterotoxin; and it seems to have re-tained the epidemic potential of O1 strains.



FIGURE 24-2 Vibrio cholerae (O group 1 antigen).

Other antigenic components of the vibrios, such as outer membrane protein antigens, have not been extensively studied. The cholera vibrios also have common flagellar antigens. Cross-reactions with Brucella and Citrobacter species have been reported. Because of DNA relatedness and other similarities, other vibrios formerly called "nonagglutinable" are now classified as V cholerae. The term nonagglutinable is a misnomer because it implies that these vibrios are not agglutinable; in fact, they are not agglutinable in antisera against the O antigen group 1 cholera vibrios, but they are agglutinable in their own specific antisera. More than 139 serotypes are now recognized. Some strains of non-O group 1 V cholerae cause diarrheal disease by means of an enterotoxin related to the cholera enterotoxin and, perhaps, by other mechanisms, but these strains have not been associated with devastating outbreaks like those caused by the true cholera vibrios. Recently, vibrio strains that agglutinate in some O group 1 cholera diagnostic antisera but not in others have been isolated from environmental sources. Volunteer feeding experiments have shown that these atypical O group 1 vibrios are not enteropathogenic in humans. Recent studies using specific toxin gene probes indicate that these environmental isolates not only are nontoxigenic, but also do not possess any of the genetic information encoding cholera toxin, although some isolates from diarrheal stools do.

The cholera vibrios cause many distinctive reactions. They are oxidase positive. The O group 1 cholera vibrios almost always fall into the Heiberg I fermentation pattern; that is, they ferment sucrose and mannose but not arabinose, and they produce acid but not gas. Vibrio cholerae also possesses lysine and ornithine decarboxylase, but not arginine dihydrolase. Freshly isolated agar-grown vibrios of the El Tor biotype, in contrast to classical V cholerae, produce a cell-associated mannose-sensitive hemagglutinin active on chicken erythrocytes. This activity is readily detected in a rapid slide test. In addition to hemagglutination, numerous tests have been proposed to differentiate the classical and El Tor biotypes, including production of a hemolysin, sensitivity to selected bacteriophages, sensitivity to polymyxin, and the Voges-Proskauer test for acetoin. El Tor vibrios originally were defined as hemolytic. They differed in this characteristic from classical cholera vibrios; however, during the most recent pandemic, most El Tor vibrios (except for the recent isolates from Texas and Louisiana) had lost the capacity to express the hemolysin. Most El Tor vibrios are Voges-Proskauer positive and resistant to polymyxin and to bacteriophage IV, whereas classical vibrios are sensitive to them. As both biotypes cause the same disease, these characteristics have only epidemiologic significance. Strains of the El Tor biotype, however, produce less cholera enterotoxin, but appear to colonize intestinal epithelium better than vibrios of the classical variety. Also, they seem some what more resistant to environmental factors. Thus, El Tor strains have a higher tendency to become endemic and exhibit a higher infection-to-case ratio than the classical biotype.

Pathogenesis

Recent studies with laboratory animal models and human volunteers have provided a detailed understanding of the pathogenesis of cholera. Initial attempts to infect healthy American volunteers with cholera vibrios revealed that the oral administra-

tion of up to 1011 living cholera vibrios rarely had an effect; in fact, the organisms usually could not be recovered from stools of the volunteers. After the administration of bicarbonate to neutralize gastric acidity, however, cholera diarrhea developed in most volunteers given 104 cholera vibrios. Therefore, gastric acidity itself is a powerful natural resistance mechanism. It also has been demonstrated that vibrios administered with food are much more likely to cause infection.

Cholera is exclusively a disease of the small bowel. To establish residence and multiply in the human small bowel (normally relatively free of bacteria because of the effective clearance mechanisms of peristalsis and mucus secretion), the cholera vibrios have one or more adherence factors that enable them to adhere to the microvilli (Fig. 24-3). Several hemagglutinins and the toxin-coregulated pili have been suggested to be involved in adherence but the actual mechanism has not been defined. In fact, there may be multiple mechanisms. The motility of the vibrios may affect virulence by enabling them to penetrate the mucus layer. They also produce mucinolytic enzymes, neuraminidase, and proteases. The growing cholera vibrios elaborate the cholera enterotoxin (CT or choleragen), a polymeric protein (Mr 84,000) consisting of two major domains or regions. The A region (Mr 28,000), responsible for biologic activity of the enterotoxin, is linked by noncovalent interactions with the B region (Mr 56,000), which is composed of five identical noncovalently associated peptide chains of Mr 11,500. The B region, also known as choleragenoid, binds the toxin to its receptors on host cell membranes. It is also the immunologically dominant portion of the holotoxin. The structural genes that encode the synthesis of CT reside on a transposon-like element in the V cholerae chromosome, in contrast to those for the heat-labile enterotoxins (LTs) of E coli (Ch. 25), which are encoded by plasmids. The amino acid sequences of these structurally, functionally, and immunologically related enterotoxins are very similar. Their differences account for the differences in physicochemical behavior and the antigenic distinctions that have been noted. There are at least two antigenically related but distinct forms of cholera enterotoxin, called CT-1 and CT-2. Classical O1 V cholerae and the Gulf Coast El Tor strains produce CT-1 whereas most other El Tor strains and O139 produce CT-2. Vibrio cholerae exports its enterotoxin, whereas the E coli LTs occur primarily in the periplasmic space. This may account for the reported differences in severity of the diarrheas caused by these organisms.

Studies in adult American volunteers have shown that 5μ g of CT, administered orally with bicarbonate, causes 1 to 6 L of diarrhea; 25μ g causes more than 20 L.

The molecular events in these diarrheal diseases involve an interaction between the enterotoxins and intestinal epithelial cell membranes (Fig. 24-4). The toxins bind through region B to a glycolipid, the GM1 ganglioside, which is practically ubiquitous in eukaryotic cell membranes. Following this binding, the A region, or a major portion of it known as the A1 peptide (Mr 21,000), penetrates the host cell and enzymatically transfers ADP-ribose from nicotinamide adenine dinucleotide (NAD) to a target protein, the guanosine 5'-triphosphate (GTP)-binding regulatory protein associated with membrane-bound adenylate cyclase. Thus, CT (and LT) resembles diphtheria toxin in causing transfer of ADP-ribose to a substrate. With diphtheria

toxin, however, the substrate is elongation factor 2 and the result is cessation of host cell protein synthesis. With CT, the ADP-ribosylation reaction essentially locks adenylate cyclase in its "on mode" and leads to excessive production of cyclic adenosine 51-monophosphate (cAMP). Pertussis toxin, another ADP-ribosyl transferase, also increases cAMP levels, but by its effect on another G-protein, Gi (Fig. 24-5). The subsequent cAMP-mediated cascade of events has not yet been delineated, but the final effect is hypersecretion of chloride and bicarbonate followed by water, resulting in the characteristic isotonic voluminous cholera stool. In hospitalized patients, this can result in losses of 20 L or more of fluid per day. The stool of an actively purging, severely ill cholera patient can resemble rice waterthe supernatant of boiled rice. Because the stool can contain 108 viable vibrios per ml, such a patient could shed 2 X 1012 cholera vibrios per day into the environment. Perhaps by production of CT, the cholera vibrios thus ensure their survival by increasing the likelihood of finding another human host. Recent evidence suggests that prostaglandins may also play a role in the secretory effects of cholera enterotoxin. Recent studies in volunteers using genetically-engineered Tox- strains of V cholerae have revealed that the vibrios have putative mechanisms in addition to CT for causing (milder) diarrheal disease. These include Zot (for Zonula occludens toxin) and Ace (for accessory cholera enterotoxin), and perhaps others, but their role has not been established conclusively. Certainly CT is the major virulence factor and the act of colonization of the small bowel may itself elicit an altered host response (e.g., mild diarrhea), perhaps by a transmembrane signaling mechanism.



FIGURE 24-4 Mechanism of action of cholera enterotoxin. Cholera toxin approaches target cell surface. B subunits bind to oligosaccharide of GM1 ganglioside.

Conformational alteration of holotoxin occurs, allowing the presentation of the A subunit to cell surface. The A subunit enters the cell. The disulfide bond of the A subunit is reduced by intracellular glutathione, freeing A1 and A2. NAD is hydrolyzed by A1, yielding ADP-ribose and nicotinamide. One of the G proteins of adenylate cyclase is ADP-ribosylated, inhibiting the action of GTPase and locking adenylate cyclase in the "on" mode (Modified from Fishman PH: Mechanism of action of cholera toxin: events on the cell surface. p. 85. In Field M, Fordtran JS, Schultz SG (eds): Secretory Diarrhea. Waverly Press, Baltimore, 1980, with permission.)



FIGURE 24-5 Comparison of activities of cholera enterotoxin (CT) with pertussis toxin (PT). The a-subunits of Gs and Gi, with GTP-binding sites, are ADPribosylated, respectively, by A1 peptide of CT or by the A subunit of PT, preventing, respectively, the hydrolysis of Gs-GTP to GDP or the responsiveness of Gi to inhibitory hormones, both effectively producing increases in adenylate cyclase activity. (Modified from Gill DM, Woolkalis M: Toxins which activate adenylate cyclase. CIBA Found Symp 112:57, 1985, with permission.)

Various animal models have been used to investigate pathogenic mechanisms, virulence, and immunity. Ten-day-old suckling rabbits develop a fulminating diarrheal disease after intraintestinal inoculation with virulent V cholerae or CT. Adult rabbits are relatively resistant to colonization by cholera vibrios; however, they do respond, with characteristic out pouring of fluid, to the intraluminal inoculation of live vibrios or enterotoxin in surgically isolated ileal loops. Suckling mice are susceptible to intragastric inoculation of vibrios and to orally administered toxin. Adult conventional mice are also susceptible to orally administered toxin, but resist colonization except in isolated intestinal loops. Interestingly, however, germ-free mice can be colonized for months with cholera vibrios. They rarely show adverse effects, although they are susceptible to cholera enterotoxin. Dogs have been used experimentally, although they are relatively refractory and require enormous inocula to elicit choleraic manifestations. Chinchillas also are susceptible to diarrhea following intraintestinal inoculation with moderate numbers of cholera vibrios. Infections initiated by extraintestinal routes of inoculation (e.g., intraperitoneal) largely reflect the toxicity of the lipopolysaccharide endotoxin. The intraperitoneal infection in mice has been used to assay the protective effect of conventional killed vibrio vaccines (no longer widely used).

Various animals, including humans, rabbits, and guinea pigs, also respond to intradermal inoculation of relatively minute amounts of CT with a characteristic delayed (maximum response at 24 hours), sustained (visible up to 1 week or more), erythematous, edematous induration associated with a localized alteration of vascular permeability. In laboratory animals, this response can be measured after injecting a proteinbinding dye, such as trypan blue, that extravasates to produce a zone of bluing at the site of intracutaneous inoculation of toxin. This observation has been exploited in the assay of CT and its antibody and in the detection of other enterotoxins.

In addition, because of the broad spectrum of activity of CT on cells and tissues that it never contacts in nature, various in vitro systems can be used to assay the enterotoxin and its antibody. In each, the toxin causes a characteristically delayed, but sustained, activation of adenylate cyclase and increased production of cAMP, and it may cause additional, readily recognizable, morphologic alterations of certain cultured cell lines. The cells most widely used for this purpose are Chinese hamster ovary (CHO) cells, which elongate in response to picogram doses of the toxin, and mouse Y-1 adrenal tumor cells, which round up. Cholera toxin has become an extremely valuable experimental probe to identify other cAMP-mediated responses. It also activates adenylate cyclase in pigeon erythrocytes, a procedure that was used by D. Michael Gill to define its mode of action.

These assays and models also have been applied in the study of an expanding number of CT-related and unrelated enterotoxins. These include the LTs of E coli, which are structurally and immunologically similar to it and are effective in any model that is responsive to CT. The family of small molecular weight heat-stable enterotoxins (ST) of E coli, which activate guanylate cyclase, and which are rapidly active in the infant mouse and certain other intestinal models, are clearly unrelated to CT. CTrelated enterotoxins have been reported from certain nonagglutinable (non-O group I) Vibrio strains and a Salmonella enterotoxin was shown to be related immunologically to CT. CT-like factors from Shigella and V parahaemolyticus have thus far been demonstrated only in sensitive cell culture systems. Other enterotoxins and enterocytotoxins, which elicit cytotoxic effects on intestinal epithelial cells, also have been described from Escherichia, Klebsiella, Enterobacter, Citrobacter, Aeromonas, Pseudomonas, Shigella, V parahaemolyticus, Campylobacter, Yersinia enterocolitica, Bacillus cereus, Clostridium perfringens, C difficile, and staphylococci. Escherichia coli, some vibrio strains, and some other enteric bacteria produce cytotoxins that, like Shiga toxin of Shigella dysenteriae, act on Vero (African green monkey kidney) cells in vitro. These toxins have been called Shiga-like toxins, Shiga toxinlike toxins, Vero toxins, and Vero cytotoxins. The classic staphylococcal enterotoxins perhaps should more properly be called neurotoxins, as they seem to affect the central nervous system rather than the gut directly to cause fluid secretion or histopathologic effects.

Host Defenses

Infection with cholera vibrios results in a spectrum of responses. These range from no observed manifestations except perhaps a serologic response (the most common) to acute purging, which must be treated by hospitalization and fluid replacement therapy; this is the classic response. The reasons for these differences are not entirely clear, although it is known that individuals differ in gastric acidity and that hypochlorhydric individuals are most prone to cholera. Whether individuals differ in the availability of intestinal receptors for cholera vibrios or for their toxin has not been established. Prior immunologic experience of subjects at risk is certainly a major factor. For example, in heavily endemic regions such as Bangladesh, the attack rate is relatively low among adults in comparison with children. In neoepidemic areas, cholera is more frequent among the working adult population. Resistance is related to the presence of circulating antibody and, perhaps more importantly, local immunoglobulin A (IgA) antibody against the cholera bacteria or the cholera enterotoxin or both. Intestinal IgA antibody can prevent attachment of the vibrios to the mucosal surface and neutralize or prevent binding of the cholera enterotoxin. For reasons that are not clear, individuals of blood group O are slightly more susceptible to cholera. Breastfeeding is highly recommended as a means of increasing immunity of infants to this and other diarrheal disease agents.

Recovery from cholera probably depends on two factors: elimination of the vibrios by antibiotics or the patient's own immune response, and regeneration of the poisoned intestinal epithelial cells. Treatment with a single 200-mg dose of doxycycline has been recommended. As studies in volunteers demonstrated conclusively, the disease is an immunizing process. Patients who have recovered from cholera are solidly immune for at least 3 years.

Cholera vaccines consisting of killed cholera bacteria administered parenterally have been used since the turn of the century. However, recent controlled field studies indicate that little, if any, effective immunity is induced in immunologically virgin populations by such vaccines, although they do stimulate preexisting immunity in the adult population in heavily endemic regions. Controlled studies have likewise shown that a cholera toxoid administered parenterally was ineffective in preventing cholera. Probably the natural disease should be simulated to induce truly effective immunity although a parenterally administered conjugate vaccine consisting of the polysaccharide of the vibrio LPS covalently linked to cholera toxin has given promising results in preliminary studies. Studies in volunteers have shown that orally administered, chemically mutagenized or genetically engineered mutants which do not produce CT or produce only its B subunit protein can induce immunity against subsequent challenge. However, most of these candidate vaccines also produce unacceptable side effectsprimarily mild to moderate diarrhea. An exception is strain CVD103-HgR (a mercury resistant A-B+ derivative of classical biotype Inaba serotype strain 569B). This strain has minimal reactogenicity but does not colonize well and therefore has to be given in higher doses. Field studies with this strain are in progress. Combined

preparations of bacterial somatic antigen and toxin antigen have been reported to act synergistically in stimulating immunity in laboratory animals; that is, the combined protective effect is closer to the product than to the sum of the individual protective effects. However, a large field study evaluating such nonviable oral vaccines in Bangladesh revealed that neither the whole-cell bacterin nor the killed vibrios supplemented with the B-subunit protein of the cholera enterotoxin induced sufficient long term protection, especially in children, to justify their recommendation for public health use. No clear-cut advantage of the inclusion of the B-subunit was demonstrated.

In any case, even if these vaccines were effective, the requirement for large and repeated doses would make them too expensive for use in the developing areas that are usually afflicted with epidemic cholera. Moreover, they were clearly less effective in childrenthe primary target population in heavily endemic areas. Neither the killed whole cell vaccine nor strain CVD103-HgR could be expected to protect against the new O139 serovar.

Epidemiology

Humans apparently are the only natural host for the cholera vibrios. Cholera is acquired by the ingestion of water or food contaminated with the feces of an infected individual. Previously, the disease swept the world in six great pandemics and later receded into its ancestral home in the Indo-Pakistani subcontinent. In 1961, the El Tor biotype (a subset distinguished by physiologic characteristics) of V cholerae, not previously implicated in widespread epidemics, emerged from the Celebes (now Sulawesi), causing the seventh great cholera pandemic. In the course of their migration, the El Tor biotype cholera vibrios virtually replaced V cholerae of the classic biotype that formerly was responsible for the annual cholera epidemics in India and East Pakistan (now Bangladesh). The pandemic that began in 1961 is now heavily seeded in Southeast Asia and in Africa. It has also invaded Europe, North America, and Japan, where the outbreaks have been relatively restricted and self-limited because of more highly developed sanitation. Several new cases were reported in Texas in 1981 and sporadic cases have since been reported in Louisiana and other Gulf Coast areas. This now endemic focus appears to be due to a clone which is unique from the pandemic strain. In 1991, the pandemic strain hit Peru with massive force and has since spread through most of the Western Hemisphere, causing more than a million cases. Fortunately, mortality has been less than 1 percent because of the effectiveness of oral rehydration therapy. The vibrios surprised us again, in 1992, with the emergence of O139 in India and Bangladesh. For a while it appeared that O139 would replace O1 (both classical and El Tor) but it has exhibited quiescent periods when O1 reemerges.

Cholera appears to exhibit three major epidemiologic patterns: heavily endemic, neoepidemic (newly invaded, cholera-receptive areas), and, in developed countries with good sanitation, occasional limited outbreaks. These patterns probably depend largely on environmental factors (including sanitary and cultural aspects), the prior immune status or antigenic experience of the population at risk, and the inherent properties of the vibrios themselves, such as their resistance to gastric acidity, ability to colonize, and toxigenicity. In the heavily endemic region of the Indian subcontinent, cholera exhibits some periodicity; this may vary from year to year and seasonally, depending partly on the amount of rain and degree of flooding. Because humans are the only reservoirs, survival of the cholera vibrios during interepidemic periods probably depends on a relatively constant availability of low-level undiagnosed cases and transiently infected, asymptomatic individuals. Long-term carriers have been reported but are extremely rare. The classic case occurred in the Philippines, where "cholera Dolores" harbored cholera vibrios in her gallbladder for 12 years after her initial attack in 1962. Her carrier state resolved spontaneously in 1973; no secondary cases had been associated with her well-marked strain. Recent studies, however, have suggested that cholera vibrios can persist for some time in shellfish, algae or plankton in coastal regions of infected areas and it has been claimed that they can exist in "a viable but nonculturable state."

During epidemic periods, the incidence of infection in communities with poor sanitation is high enough to frustrate the most vigorous epidemiologic control efforts. Although transmission occurs primarily through water contaminated with human feces, infection also may be spread within households and by contaminated foods. Thus, in heavily endemic regions, adequate supplies of pure water may reduce but not eliminate the threat of cholera.

In neoepidemic cholera-receptive areas, vigorous epidemiologic measures, including rapid identification and treatment of symptomatic cases and asymptomatically infected individuals, education in sanitary practices, and interruption of vehicles of transmission (e.g., by water chlorination), may be most effective in containing the disease. In such situations, spread of cholera usually depends on traffic of infected human beings, although spread between adjacent communities can occur through bodies of water contaminated by human feces. John Snow was credited with stopping an epidemic in London, England, by the simple expedient of removing the handle of the "Broad Street pump" (a contaminated water supply) in 1854, before acceptance of the "germ theory" and before the first isolation of the "Kommabacillus" by Robert Koch.

In such developed areas as Japan, Northern Europe, and North America, cholera has been introduced repeatedly in recent years, but has not caused devastating outbreaks; however, Japan has reported secondary cases and, in 1978, the United State experienced an outbreak of about 12 cases in Louisiana. In that outbreak, sewage was infected, and infected shellfish apparently were involved. Interestingly, the hemolytic vibrio strain implicated was identical to one that caused an unexplained isolated case in Texas in 1973.

Diagnosis

Rapid bacteriologic diagnosis offers relatively little clinical advantage to the patient with secretory diarrhea, because essentially the same treatment (fluid and electrolyte replacement) is employed regardless of etiology. Nevertheless, rapid identification of the agent can profoundly affect the subsequent course of a potential epidemic outbreak. Because of their rapid growth and characteristic colonial morphology, V cholerae can be easily isolated and identified in the bacteriology laboratory, provided,

first, that the presence of cholera is suspected and, second, that suitable specific diagnostic antisera are available. The vibrios are completely inhibited or grow somewhat poorly on usual enteric diagnostic media (MacConkey agar or eosin-methylene blue agar). An effective selective medium is thiosulfate-citrate-bile salts-sucrose (TCBS) agar, on which the sucrose-fermenting cholera vibrios produce a distinctive yellow colony. However, the usefulness of this medium is limited because serologic testing of colonies grown on it occasionally proves difficult, and different lots vary in their productivity. This medium is also useful in isolating V parahaemolyticus. They can also be isolated from stool samples or rectal swabs from cholera cases on simple meat extract (nutrient) agar or bile salts agar at slightly alkaline pH values. Following observation of characteristic colonial morphology with a stereoscopic microscope using transmitted oblique illumination, microorganisms can be confirmed as cholera vibrios by a rapid slide agglutination test with specific antiserum. Classic and El Tor biotypes can be differentiated at the same time by performing a direct slide hemagglutination test with chicken erythrocytes: all freshly isolated agar-grown El Tor vibrios exhibit hemagglutination; all freshly isolated classic vibrios do not. In practice, this can be accomplished with material from patients as early as 6 hours after streaking the specimen in which the cholera vibrios usually predominate. However, to detect carriers (asymptomatically infected individuals) and to isolate cholera vibrios from food and water, enrichment procedures and selective media are recommended. Enrichment can be accomplished by inoculating alkaline (pH 8.5) peptone broth with the specimen and then streaking for isolation after an approximate 6-hour incubation period; this process both enables the rapidly growing vibrios to multiply and suppresses much of the commensal microflora.

The classic case of cholera, which includes profound secretory diarrhea and should evoke clinical suspicion, can be diagnosed within a few minutes in the prepared laboratory by finding rapidly motile bacteria on direct, bright-field, or dark-field microscopic examination of the liquid stool. The technician can then make a second preparation to which a droplet of specific anti-V cholerae O group 1 antiserum is added. This quickly stops vibrio motility. Another rapid technique is the use of fluorescein isothiocyanate-labeled specific antiserum (fluorescent antibody technique) directly on the stool or rectal swab smear or on the culture after enrichment in alkaline peptone broth. For cultural diagnosis, both nonselective and selective (TCBS) media may be used. Although demonstration of typical agglutination essentially confirms the diagnosis, additional conventional tests such as oxidase reaction, indole reaction, sugar fermentation reactions, gelatinase, lysine, arginine, and ornithine decarboxylase reactions may be helpful. Tests for chicken cell hemagglutination, hemolysis, polymyxin sensitivity, and susceptibility to phage IV are useful in differentiating the El Tor biotype from classic V cholerae. Tests for toxigenesis may be indicated.

Diagnosis can be made retrospectively by confirming significant rises in specific serum antibody titers in convalescents. For this purpose, conventional agglutination tests, tests for rises in complement-dependent vibriocidal antibody, or tests for rises in antitoxic antibody can be employed. Convenient microversions of these tests have been developed. Passive hemagglutination tests and enzyme-linked immunosorption assays (ELISAs) have also been proposed.

Cultures that resemble V cholerae but fail to agglutinate in diagnostic antisera (nonagglutinable or non-O group 1 vibrios) present more of a problem and require additional tests such as oxidase, decarboxylases, inhibition by the vibriostatic pteridine compound 0/129, and the "string test." The string test demonstrates the property, shared by most vibrios and relatively few other genera, of forming a mucus-like string when colony material is emulsified in 0.5 percent aqueous sodium deoxycholate solution. Additional tests for enteropathogenicity and toxigenesis may be useful. Genetically based tests such as PCR are increasingly being used in specialized laboratories.

Control

Treatment of cholera consists essentially of replacing fluid and electrolytes. Formerly, this was accomplished intravenously, using costly sterile pyrogen-free intravenous solutions. The patient's fluid losses were conveniently measured by the use of buckets, graduated in half-liter volumes, kept underneath an appropriate hole in an army-type cot on which the patient was resting. Antibiotics such as tetracycline, to which the vibrios are generally sensitive, are useful adjuncts in treatment. They shorten the period of infection with the cholera vibrios, thus reducing the continuous source of cholera enterotoxin; this results in a substantial saving of replacement fluids and a markedly briefer hospitalization. Note, however, that fluid and electrolyte replacement is all-important; patients who are adequately rehydrated and maintained will virtually always survive, and antibiotic treatment alone is not sufficient.

Recently it has been recognized that almost all cholera patients and others with similar severe secretory diarrheal disease can be maintained by fluids given orally if the solutions contain a usable energy source such as glucose. Because of this discovery, packets containing appropriate salts are distributed by such organizations as WHO and UNICEF to cholera-afflicted areas, where they are dissolved in water as needed. One such formulation, called ORS for oral rehydration salts, contains NaCl, 3.5 g; KCl,1.5 g; NaHCO3, 2.5 g (or trisodium citrate, 2.9 g); and glucose, 20.0 g. This mixture is dissolved in 1 L of water and taken orally in increments. Flavoring may be added. Improved versions of ORS, including rice-based formulations that reduce stool output and can be made at home, have been recommended. Unfortunately, this technique, which will save countless millions of lives in developing countries, has not yet been widely accepted by practicing physicians in developed countries.

The possibility of pharmacologic intervention (e.g., a pill that will stop choleraic diarrhea after it has started), has been considered. Two drugs, chlorpromazine and nicotinic acid, have been effective in experimental animals, although the precise mechanism of action has yet to be defined.

Like smallpox and typhoid, choleraunder natural circumstancesappears to affect only humans; therefore, V cholerae as an etiologic entity could conceivably disappear with the last human infection. Nevertheless, the spectrum of cholera-like diarrheal diseases probably will persist for some time.

Cholera is essentially a disease associated with poor sanitation. The simple application of sanitary principlesprotecting drinking water and food from contamination with human feceswould go a long way toward controlling the disease. However, at present, this is not feasible in the underdeveloped areas that are afflicted with epidemic cholera or are considered to be cholera receptive. Meanwhile, development of a vaccine that would effectively prevent colonization and manifestations of cholera would be extremely helpful. As indicated above, such vaccines are presently being tested. Antibiotic or chemotherapeutic prophylaxis is feasible and may be indicated under certain circumstances. It also should be mentioned that the incidence of cholera is significantly higher in formula-fed than in breast-fed babies.

Present information indicates that V parahaemolyticus enteritis could be almost completely prevented by applying appropriate procedures to prevent multiplication of the organisms in contaminated seafood, such as keeping it refrigerated continually.

Other Vibrio Infections

Other vibrios may be clinically significant also. These include non-O group 1 V cholerae. Vibrio parahaemolyticus, a halophilic (salt-loving) vibrio associated with enteritis is acquired by ingestion of raw or improperly cooked seafoods. Another halophilic vibrio, which ferments lactose and for this reason was called the L + vibrio, has recently been identified as V vulnificus. It has been associated with wound infections as well as fatal septicemias. Other groups of vibrios, previously referred to as group F and EF-6, have recently been classified into species: V fluvialis, V hollisae, V furnissia, and V damsela. Vibrio mimicus is a recently described sucrosenegative species. Vibrio fetus, a group of anaerobic to microaerophilic spirally curved rods associated with venereally transmitted infertility and abortion in domestic animals, is now called Campylobacter jejuni and is considered to belong in the family Spirillaceae rather than in the family Vibrionaceae. Campylobacter jejuni has been associated with dysentery-like gastroenteritis, duodenal and gastric ulcers, as well as with other types of infection, including bacteremic and central nervous system infections in humans (see Ch. 23). Another vibrio-like organism, Helicobacter pylori (formerly known as C pylori) causes gastritis and predisposes to duodenal ulcers and gastric cancer. Although some similarities in habitat and other properties occur, members of the family Vibrionaceae are separated taxonomically from members of the family Enterobacteriaceae. The oxidase test (vibrios are usually oxidase positive) is particularly useful. Other vibrios exist, and some of these may be responsible for diseases in fish and other lower animals. As vibrios are widely distributed in the environment, particularly in estuarine waters and in seafoods, reports of their isolation from patients with diarrheal disease do not necessarily always imply an etiologic relationship.

Cholera-like vibrios have been reported in Maryland's Chesapeake Bay but have not been associated with any human cases despite more than 15 years of extensive surveillance. These vibrios are probably nonpathogenic nonagglutinable (non-O group 1) vibrios, or the atypical O group 1 vibrios mentioned above, which do not contain the genes for toxin production, do not colonize, and are avirulent. Relatively little is known about the epidemiology of nonagglutinable vibrios. When sought, these vibrios have been found widely in brackish surface waters (sewers, marshes, bogs, and coastal areas), and are generally more numerous in warmer months. They appear to be free-living aquatic organisms; whether particular subsets are potential pathogens is not yet clear. Strains isolated from humans with diarrheal disease more frequently give positive responses in assays for enterotoxins or enteropathogenicity, but the pathogenic mechanism of other isolates associated with shellfish remains undefined. An epidemiologic pattern is more evident with V parahaemolyticus, which is clearly part of the normal flora of coastal and estuarine waters throughout the world. Although originally recognized in Japan, V parahaemolyticus enteritis has been reported virtually worldwide within the last decade. Its reported frequency varies widely, partly because of inherent differences in distribution and partly because many laboratories do not use the appropriate culture medium (TCBS) to isolate these organisms. Two types of clinical syndromes, both usually selflimited, have been observed. The most common is a watery diarrhea, perhaps with associated abdominal cramps, nausea, vomiting, and fever, with a modal incubation period of 15 hours. A dysenteric syndrome with a short incubation period of 2 1/2 hours also has been described. In Japan, about 24 percent of reported cases of food poisoning are attributed to V parahaemolyticus. The disease occurs primarily during summer, possibly reflecting the increased presence of the organism in the marine environment during those months, as well as the enhanced opportunity for it to multiply in unrefrigerated foods. It appears to be transmitted exclusively by food, primarily raw or improperly prepared seafood. As growth of this organism is inhibited at temperatures below 15° C, rapid cooling and refrigeration of seafoods that are eaten raw would vastly reduce the incidence of disease. The organisms are killed by heating to 65° C for 10 minutes; therefore, properly handled cooked seafood should present no problem. The role played in virulence and pathogenesis by the thermostable direct hemolysin, which is responsible for the positive Kanagawa phenomenon (a hemolytic reaction around colonies growing on a particular blood agar medium), is not yet fully defined. This hemolysin is clearly associated with pathogenicity, but whether it is merely an associated marker or intimately involved in the disease process awaits further research. Be this as it may, only strains that possess the Kanagawa hemolysin are considered pathogenic. In laboratory studies, the isolated hemolysin has been reported to be cytotoxic, cardiotoxic, and lethal.

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Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus

General Concepts

Clinical Manifestations

The genera Escherichia, Klebsiella, Enterobacter, Serratia, and Citrobacter (collectively called the coliform bacilli) and Proteus include overt and opportunistic pathogens responsible for a wide range of infections. Many species are members of the normal intestinal flora. Escherichia coli (E coli) is the most commonly isolated organism in the clinical laboratory.

Enteric Infections: E coli is a major enteric pathogen, particularly in developing countries. The principal groups of this organism responsible for enteric disease include the classical enteropathogenic serotypes (EPEC), enterotoxigenic (ETEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), and enteroggregative (EAEC) strains which are described in detail in Chapter 25.

Nosocomial Infections: Coliform and Proteus bacilli currently cause 29 percent of nosocomial (hospital-acquired) infections in the United States. In order of decreasing frequency, the major sites of nosocomial infection are the urinary tract, surgical sites, bloodstream, and pneumonias. This group of nosocomial pathogens are responsible for 46% of urinary tract and 24% of surgical site infections, 17% of the bacteremias, and 30% of the pneumonias. E coli is the premier nosocomial pathogen.

Community-Acquired Infections: E coli is the major cause of urinary tract infections, including prostatitis and pyelonephritis; Proteus, Klebsiella, and Enterobacter species are also common urinary tract pathogens. Proteus mirabilis is the most frequent cause of infection-related kidney stones. Klebsiella pneumoniae causes a severe pneumonia; K rhinoscleromatis causes rhinoscleroma; and K ozaenae is associated with ozena, an atrophic disease of the nasal mucosa.

Structure, Classification, and Antigenic Types

The coliforms and Proteus are Gram negative bacilli. All genera except Klebsiella are flagellated. Some strains produce capsules. Virulence often depends on the presence of attachment pili (which can be characterized by specific hemagglutinating reactions). Sex pili also may be present. The major classes of antigens used in defining strains are H (flagellar), O (somatic), and K (capsular).

Pathogenesis

E coli enteropathogens have diverse mechanisms for disease production which include different toxins and colonization factors (see Ch. 25). Specific serotypes of coliforms and Proteus with particular virulence factors often preferentially infect specific extraintestinal sites. E coli bacilli in extraintestinal infections have soluble and cell-bound hemolysins, siderophores, capsules, and adherence pili.

Host Defenses

Coliforms and Proteus species rarely cause extraintestinal disease unless host defenses are compromised. Disruption of the normal intestinal flora by antibiotic therapy may allow resistant nosocomial strains to colonize or overgrow. The skin and mucosae may be breached by disease, trauma, operation, venous catheterization, tracheal intubation, etc. Immunosuppressive therapy also increases the risk of infection.

Epidemiology

The epidemiology of coliform and Proteus infections involves many reservoirs and modes of transmission. The infecting organism may be endogenous or exogenous. Transmission may be direct or indirect; vehicles include hospital food and equipment, intravenous solutions, and the hands of hospital personnel. Nosocomial strains progressively colonize the intestine and pharynx with increasing length of hospital stay, resulting in an increased risk of infection.

Diagnosis

The clinical picture depends on the site of infection; diagnosis relies on culturing the organism and on biochemical and/or serologic identification. A variety of phenotypic (i.e., biotyping, serotyping, antibiograms, bacteriocin and phage typing) and geno-typic (i.e., plasmid analysis, RFLP, ribotyping, and PCR) methods are used for epidemiological investigations.

Control

The most effective way to reduce transmission of nosocomial organisms is for all hospital personnel to wash hands meticulously after attending to each patient. Vaccines and hyperimmune sera are not currently available. Various antibiotics are the backbone of treatment; drug resistance (often multiple) due to conjugative plasmids is a major problem.

INTRODUCTION

The Gram-negative bacilli of the genera Escherichia, Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus (Table 26- 1) are members of the normal intestinal flora of humans and animals and may be isolated from a variety of environmental sources. With the exception of Proteus, they are sometimes collectively referred to as the coliform bacilli because of shared properties, particularly the ability of most species to ferment the sugar lactose.

Many of these microorganisms used to be dismissed as harmless commensals. Today, they are known to be responsible for major health problems worldwide. A limited number of species, including E coli, K pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, S marcescens, and P mirabilis, are responsible for most infections produced by this group of organisms. The increasing incidence of the coliforms, Proteus, and other Gram-negative organisms in diseases reflects in part a better understanding of their pathogenic potential but more importantly the changing ecology of bacterial disease. The widespread and often indiscriminate use of antibiotics has created drug-resistant Gram-negative bacilli that readily acquire multiple resistance through transmission of drug resistance plasmids (R factors). Also, development of new surgical procedures, health support technology, and therapeutic regimens has provided new portals of entry and compromised many host defenses.

Clinical Manifestations

As opportunistic pathogens, the coliforms and Proteus take advantage of weakened host defenses to colonize and elicit a variety of disease states (Fig. 26-1). Together,

the many disease syndromes produced by these organisms are among the most common infections in humans requiring medical intervention.



FIGURE 26-1 Sites of colonization and extraintestinal disease production by the coliforms and Proteus.

Enteric Infections

The role of E coli as a major enteric pathogen, particularly in developing countries, is discussed in detail in Ch. 25. However, the different types of E coli associated with enteric infections and which are classified into five groups according to their virulence properties are briefly described here: Enteropathogenic (EPEC) serotypes in the past were associated with serious outbreaks of diarrhea in newborn nurseries in the US. They remain an important cause of acute infantile diarrhea in developing countries. Disease is rare in adults. Enteroinvasive (EIEC) types produce disease resembling shigellosis in adults and children. Enterotoxigenic (ETEC) types are a major cause of traveler's diarrhea, and of infantile diarrhea in developing countries. Enterohemorrhagic E. coli (EHEC) occur largely as a single serotype (O157:H7) causing sporadic cases and outbreaks of hemorrhagic colitis characterized by bloody diarrhea. EHEC also may cause hemolytic uremic syndrome (HUS), an association of hemolytic anemia, thrombocytopenia, and acute renal failure. Enteroaggregative (EAEC) types exhibit a characteristic aggregative pattern of adherence and produce persistent gastroenteritis and diarrhea in infants and children in developing countries. **Nosocomial Infections**

The etiology of nosocomial infections has markedly changed during past decades. Streptococci were the major nosocomial pathogens in the preantibiotic era. However, following the introduction and use of sulfonamides and penicillin, Staphylococcus aureus became the predominant pathogen in the 1950's. Aerobic gram negative rods gained prominence as nosocomial pathogens with widespread use of aminoglycosides and first generation cephalosporins through the early 1970's. Subsequent widespread use of broad spectrum cephalosporins was associated with changes in the frequency and etiology of nosocomial infections into the 1980's with the trend towards certain gram-positive pathogens. For example, in nosocomial bloodstream infections from 1980 to 1989 marked increases in the incidence of coagulase-negative staphylococci, S. aureus, enterococci, and Candida albicans infections occurred.

The coliforms and Proteus were responsible for 29 percent of nosocomial (hospitalacquired) infections in the United States from 1990 through 1992 based on data from hospitals participating in the National Nosocomial Infections Survey (NNIS) (Table 26-2). Estimates of nosocomial infections in US hospitals suggest that about 5 percent of the estimated 40 million annual admissions, or 2 million patients, had at least one nosocomial infection. Thus, the coliforms and Proteus probably are responsible for hospital-acquired infections in approximately 600,000 patients each year. Aside from the enormous cost measured in human life, nosocomial infections prolong the duration of hospitalization by an average of 4 days and increase the cost of medical care by \$4.5 billion a year in 1992 dollars.

The highest numbers of nosocomial infections in the NNIS occur in surgical and medicine services. Among surgical patients, highest rates of nosocomial infections occur with surgery on the stomach (21%) and bowel (19%), craniotomies (18%), coronary artery bypass graft procedures (11%) and other cardiac surgery (10%). High rates also are observed with burn (15%) and high-risk nursery patients (14%). In order of decreasing frequency, the major sites of nosocomial infection are the urinary tract, surgical sites, bloodstream, and lower respiratory tracts. The coliforms and Proteus were responsible for 46% of urinary tract and 24% of surgical site infections, 17% of the bacteremias, and 30% of the pneumonias from 1990 through 1992. Escherichia coli, the predominant nosocomial pathogen, is the major cause of infection in the urinary tract and is common in other body sites. Staphylococcus aureus and Pseudomonas aeruginosa are currently the most common pathogens in nosocomial pneumonias, followed by Enterobacter and Klebsiella. Coagulase-negative staphylococci have replaced E coli as the predominant pathogen in primary bloodstream infections. The major causes of surgical site infections are S aureus, coagulase- negative staphylococci, and enterococci.

Other coliform bacilli and Proteus have been incriminated in various hospitalacquired infections. Klebsiella, Enterobacter, and Serratia species are frequent causes of bacteremia at some medical centers and also are frequently involved in infections associated with respiratory tract manipulations, such as tracheostomy and procedures using contaminated inhalation therapy equipment. Klebsiella and Serratia species commonly cause infections following intravenous and urinary catheterization and infections complicating burns. Proteus species frequently cause nosocomial infections of the urinary tract, surgical wounds, and lower respiratory tract. Less frequently, Proteus species cause bacteremia, most often in elderly patients. A series of nationwide outbreaks of bacteremia (1970 to 1971 and 1973), caused by contaminated commercial fluids for intravenous injections, involved Enterobacter cloacae, Enterobacter agglomerans, and C freundii.

The role of Citrobacter species in human disease is not as great as that of the other coliforms and Proteus. Citrobacter freundii and C diversus (C koseri) have been isolated predominantly as superinfecting agents from urinary and respiratory tract infections. Citrobacter septicemia may occur in patients with multiple predisposing factors; Citrobacter species also cause meningitis, septicemia, and pulmonary infections in neonates and young children. Neonatal meningitis produced by C diversus, while uncommon, is associated with a very high frequency of brain abscesses, death, and mental retardation in survivors. Although E coli and group B streptococci cause most cases of neonatal meningitis, the most common cause of brain abscesses in neonatal meningitis is P mirabilis.

Immunocompromised patients often develop non-hospital-acquired infections with coliforms. For example, group B streptococci and E coli are responsible for most cases of neonatal meningitis, with the latter accounting for about 40 percent of cases. Infections seen in cancer patients with solid tumors or malignant blood diseases frequently are caused by E coli, Klebsiella, Serratia, and Enterobacter species. Such infections often have lethal course. Individuals who are immunosuppressed by therapy (e.g., cancer patients or transplant recipients) or by congenital defects of the immune system may develop Klebsiella, Enterobacter, and Serratia infections. Many additional factors such as diabetes, trauma, and chronic lung disease may predispose to infection by coliforms and other microbes.

Community-Acquired Infections

The coliform organisms and Proteus species are major causes of diseases acquired outside the hospital; many of these diseases eventually require hospitalization. Escherichia coli causes approximately 85 percent of cases of urethrocystitis (infection of the urethra and bladder), about 80 percent of cases of chronic bacterial prostatitis, and up to 90 percent of cases of acute pyelonephritis (inflammation of the renal pelvis and parenchyma). Approximately one half of females have had a urinary tract infection by their late twenties due to E coli from their fecal flora. Proteus, Klebsiella, and Enterobacter species are among the other organisms most frequently involved in urinary tract infections. Proteus, particularly P mirabilis, is believed to be the most common cause of infection-related kidney stones, one of the most serious complications of unresolved or recurrent bacteriuria.

Klebsiella was first recognized clinically as an agent of pneumonia. Klebsiella pneumoniae accounts for a small percentage of pneumonia cases; however, extensive damage produced by the organism results in high case fatality rates (up to 90 percent in untreated patients). Klebsiella rhinoscleromatis is the agent of rhinoscleroma, a chronic destructive granulomatous disease of the respiratory tract that is endemic in Eastern Europe and Central America. Klebsiella ozaenae, a rare cause of serious infection, is classically associated only with ozena, an atrophy of nasal mucosal membranes with a mucopurulent discharge that tends to dry into crusts; however, recent studies indicate that the organism may cause various other diseases including infections of the urinary tract, soft tissue, middle ear, and blood.

Distinctive Properties Structure and Antigens

The generalized structure and antigenic composition of coliform bacilli, as well as of Proteus and other members of the family Enterobacteriaceae, are depicted schematically in Figure 26-2. A more detailed figure of the structure is presented in Chapter 2. The major antigens of coliforms are referred to as H, K, and O antigens. The coliforms and Proteus are divided into serotypes on the basis of combinations of these antigens; different serotypes may have different virulence properties or may preferentially colonize and produce disease in particular body habitats. The H antigen determinants are flagellar proteins. Escherichia coli, Enterobacter, Serratia, Citrobacter, and Proteus organisms are peritrichous (i.e., they have flagella that grow from many places on the cell surface). Klebsiella species are nonmotile and nonflagellated and thus have no H antigens.



FIGURE 26-2 Structure and antigenic composition of coliforms and Proteus species.

Some strains of coliform and Proteus species have pili (fimbriae). Pili are associated with adhesive properties and, in some cases, are correlated with virulence. Different pilial colonization factors generally are detectable as hemagglutinins that can be distinguished by the type of erythrocyte agglutinated and by the susceptibility of the hemagglutination to inhibition by the sugar mannose. Sex pili, which have receptors for "male" specific bacterial viruses and are genetically determined by extrachromosomal plasmids, are important in coliform ecology and in the epidemiology of diseases produced by coliforms and Proteus species in that sex pili are involved in genetic transfer by conjugation (e.g., chromosome-mediated and plasmid-mediated drug resistances or virulence factors).

Major Surface Antigens

K antigens (capsule antigens) are components of the polysaccharide capsules. Certain K antigens (e.g., K88 and K99 of E coli) are pilus-like proteins. The K antigens often block agglutination by specific O antisera. In the past, K antigens routinely were differentiated into A, L, and B groups on the basis of differences in their lability to heat; however, these criteria are subject to difficulties that make the distinction tenuous. Some Citrobacter serotypes produce Vi (virulence) antigen, a K antigen also found in Salmonella typhi. Species of Proteus, Enterobacter, and Serratia apparently have no regular K antigens. However, the K antigens are important in the pathogenesis of some coliforms. A diffuse slime layer of variable thickness (the M antigen) also may be produced but, unlike the K antigens, it is nonspecific and is serologically cross-reactive among different organisms.

The outer membrane of the bacterial cell wall of these species contains receptors for bacterial viruses and bacteriocins (plasmid-encoded, antibiotic like bactericidal proteins called colicins in E coli that are active against the same or closely related species). The outer membrane also contains lipopolysaccharide (LPS), of which the lipid A portion is endotoxic and the O (somatic) antigen is serotype specific. The serologic specificity of the O antigens is based on differences in sugar components, their linkages, and the presence or absence of substituted acetyl groups. Loss of the O antigen by mutation results in a smooth-to-rough transformation, which often involves changes in colony type and saline agglutination, as well as loss of virulence . Certain strains of P vulgaris (OX-19, OX-2, and OX-K) produce O antigens that are shared by some rickettsiae. These Proteus strains are used in an agglutination test (the Weil-Felix test) for serum antibodies produced against rickettsiae of the typhus and spotted fever groups (see Ch. 38).

Toxins

Enterotoxigenic strains of Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus also have been isolated from infants and children with acute gastroenteritis. The enterotoxins of at least some of these organisms are of the heat-labile and heat-stable types and have other properties in common with the E coli toxins (see Ch. 25). However, the importance of the coliforms and Proteus, other than E coli, in enteric infections is questionable

Pathogenesis

The process of disease production by coliforms is, in many cases, poorly understood. Production of disease by coliforms or Proteus species in extraintestinal sites often involves specific serotypes of the organisms and special virulence factors. For example, respiratory tract infections by K pneumoniae predominantly involve capsular types 1 and 2, whereas urinary tract infections often involve types 8, 9, 10, and 24. Similarly, only a few polysaccharide K antigens (types 1, 2, 3, 5, 12, and 13) of E coli are found with high frequency in urinary tract and other extraintestinal infections. These observations suggest that different serotypes may have specific pathogenicities. An alternative explanation is that such strains may simply be the most prevalent types in the normal gut flora.

There is good evidence for specific pathogenicity in E coli strains that cause extraintestinal infections (Table 26-3). Approximately 80 percent of E coli isolates involved in neonatal meningitis carry the K1 antigen, a fact attributable, at least in part, to the higher resistance to phagocytosis of K1-positive strains. Certain O antigens (O7 and O18) are found in combination with K1, usually in strains that are isolated from cases of neonatal bacteremia and meningitis and that show increased resistance to the bactericidal effects of serum complement. Interestingly, the E coli K1 antigen, composed of neuraminic acid, shows immune cross-reactivity with the group B meningococcal polysaccharide capsule.

	Virulence Factor	Proposed Role(s) in Pathogenesis
	Col V plasmid	Codes for a siderophore (aerobactin)
		for Fe chelation
		Increases bacterial resistance to serum
	Hemolysin	Damages host cells
	(0.0808) (S. 6 (800))	Releases Fe from red blood cells
	Enterochelin	Chelates Fe for bacterial uptake
	K1 antigen	Impedes phagocytosis
	00000000000000	Blocks binding of C3b opsonin
	P-pili	Allow bacteria to bind to P blood group antigens on urinary tract cells (especially in kidneys)
	Type 1 pili	Allow bacteria to bind to (1) bladder epitehlium,(2) Tamm-Horsfall glycoprotein, and (3) D-mannose residues on a variety of cells

TABLE 26-3 Virulence Factors of E coli Isolates from Extraintestinal Infections

Escherichia coli strains isolated from extraintestinal infections often possess a number of properties not usually found in random fecal isolates. These include production of soluble and cell-bound hemolysins, the colicin V plasmid, production of the siderophores aerobactin and enterochelin, and special pilial antigens for adherence to target cells. The hemolysin kills host cells and makes iron more available by releasing hemoglobin-bound iron from lysed red cells. To strip iron from the host ironbinding proteins (transferrin and lactoferrin), E coli produces siderophores of both the hydroxamate (aerobactin) and phenolate (enterochelin) types. Common or type 1 pili may mediate adherence to bladder cells; P-pili are virulence factors for strains causing pyelonephritis; S-pili, which recognize O-linked sialo-oligosaccharides of glycophorin A, are associated with meningitis and urinary tract infections. Certain afimbrial adhesions and outer membrane proteins also have been associated with urinary tract infections.

The enzyme urease, produced by Proteus, and to a lesser extent by Klebsiella species, is thought to play a major role in the production of infection-induced urinary stones. Urease hydrolyzes urea to ammonia and carbon dioxide. Alkalinization of the urine by ammonia can cause magnesium phosphate and calcium phosphate to become supersaturated and crystallize out of solution to form, respectively, struvite and apatite stones. Bacteria within the stones may be refractory to antimicrobial therapy. Large stones may interfere with renal function. The ammonia produced by urease activity may also damage the pithelium of the urinary tract. Except in cases of bacteremia and other systemic infection, there is little evidence that endotoxin plays a role in most coliform and Proteus diseases. Humans with coliform bacteremia show many of the typical effects of endotoxin, including fever, depletion of complement, release of inflammatory mediators, lactic acidosis, hypotension, vital organ hypoperfusion, irreversible shock, and death.

Host Defenses

It cannot be overemphasized that coliforms (except for E coli in enteric diseases) and Proteus species are unlikely to cause disease unless the local or generalized host defenses fail in some way. The normal gastrointestinal flora, which includes E coli and, frequently, other coliforms and Proteus species in small numbers, is important in preventing disease through bacterial competition. Prolonged antibiotic therapy compromises this defense mechanism by reducing susceptible components of the normal flora, permitting nosocomial coliform strains or other bacteria to colonize or overgrow.

The organisms may breach anatomic barriers through third-degree burns, ulcers associated with solid tumors of the skin and mucous membranes, intravenous catheters, and surgical or instrumental procedures on the biliary, gastrointestinal, and genitourinary tracts. The lungs may be violated by instrumentation, as in tracheal intubation, or even by aerosols from contaminated nebulizers or humidifiers, which carry organisms to the terminal alveoli.

Corticosteroid administration, radiotherapy, and the increased steroid levels associated with pregnancy tend to decrease host control over infections (e.g., by depressing the immune response). Cytotoxic drugs also are immunosuppressive. Cancer-or drug-induced neutropenia is an important predisposing factor in bacteremia. Devitalized tissue or foreign bodies may be a source of organisms and may also shelter the organisms from phagocytes and antimicrobial factors.

The interaction of multiple predisposing factors often determines the clinical course and outcome of coliform or Proteus infection. For example, the mortality of bacteremia increases progressively when the underlying disease (e.g., cancer or diabetes) is rated as nonfatal, ultimately fatal (death within 5 years), or rapidly fatal (death within 1 year). Similarly, coliform and Proteus infections commonly are more severe in the very old and very young.

Epidemiology

The epidemiology of coliform and Proteus infections is complex and involves multiple reservoirs and modes of transmission. Klebsiella, Enterobacter, Serratia, Citrobacter, and Proteus species live in water, soil, and occasionally food and, in many cases, form part of the intestinal flora of humans and animals. Escherichia coli is believed not to be free living, and its presence in environmental samples is taken as indicating recent fecal contamination. In fact, water quality is determined by the presence of the rapid lactose fermenting E coli, Klebsiella, and Enterobacter (coliform counts) and E coli (fecal coliform counts) using special selective media.

Coliform and Proteus organisms causing infection may be exogenous or endogenous. While most nosocomial infections appear to arise from endogenous flora, studies of hospitalized adults and infants have shown that the intestinal tract is progressively
colonized by nosocomial coliforms with increasing length of hospitalization. Patients being treated with antibiotics, severely ill patients, and (probably) infants are more likely to be colonized, and other sites of colonization such as the nose and throat may be important in such patients. Colonized patients have a higher risk of nosocomial infection than patients who are not colonized.

The bacteria may be acquired indirectly via various vehicles or by direct contact . A variety of vehicles have been implicated in the spread of nosocomial pathogens. For example, Klebsiella, Enterobacter, and Serratia species have all been recovered in large numbers from hospital food, particularly salads, with the hospital kitchen being a primary source . An outbreak of urinary tract infections due to multiply drug-resistant S marcescens was associated with contaminated urine- measuring containers and urinometers. Serious outbreaks or individual cases of bacteremia due to coliforms have been associated with extrinsic contamination of intravenous fluids or caps during manufacture and with extrinsic contamination of intravenous fluids and administration sets in the hospital environment. Other medical devices and medications have served as vehicles for the spread of nosocomial pathogens. Occasionally, transmission may be via members of the hospital staff who are colonized with nosocomial pathogens in the rectum or vagina or on the hands; however, passive carriage on the hands of medical personnel constitutes the major mode of transmission.

Certain properties of the coliforms may be important in the epidemiology of hospital-acquired infections. Coliform bacteria other than E coli frequently are found in tap water or even distilled or deionized water. They may persist or actively multiply in water associated with respiratory therapy or hemodialysis equipment. Klebsiella, Enterobacter, and Serratia species, like Pseudomonas species, may exhibit increased resistance to antiseptics and disinfectants. The same group of coliforms has a selective ability over other common nosocomial pathogens (including E coli, Proteus species, Pseudomonas aeruginosa, and staphylococci) to proliferate rapidly at room temperature in commercial parenteral fluids containing glucose.

Diagnosis

Because the coliforms and Proteus can cause many types of infection, the clinical symptoms rarely permit a diagnosis. Culturing and laboratory identification are usually required. Selected characteristics that are useful in the differentiation of coliform bacilla and Proteus species found in human clinical specimens are shown in Table 26-4. The organisms have simple nutritional requirements and grow well on mildly selective media commonly used for members of the Enterobacteriaceae, but not on some moderately and highly selective enteric plating media (Salmonella-Shigella, bismuth sulfite, and brilliant green agar). Extraintestinal specimens such as urine, purulent material from wounds or abscesses, sputum, and sediment from cerebrospinal fluid should be plated for isolation on blood agar and a differential medium such as MacConkey or eosin-methylene blue agar. The finding of more than 105 organisms/ml in clean voided midstream urine is often taken as "significant bacteriuria." However, in acutely symptomatic females and with other types of specimens (i.e., those obtained by catheterization or suprapubic aspiration) from either sex, a more appropriate threshold, particularly in the presence of pus cells and the absence of epi-

thelial cells, might be more than 102 colonies of a known uropathogen/ml. Because urine is a good growth medium for many microbes, specimens should be refrigerated (4oC) if transport to the laboratory is delayed longer than 30 minutes, unless a urine transport container with preservative is used.

Isolation of certain coliforms or Proteus species from fecal specimens may be facilitated by adding a moderately selective medium such as xylose-lysine-desoxycholate (XLD) or Hektoen enteric agar. Use of tetrathionate or selenite broth for enrichment of enterotoxigenic strains from feces is not recommended because both media inhibit various genera of coliforms. The strong (E coli, K pneumoniae, Enterobacter aerogenes) and occasionally the slow or weak (Serratia, Citrobacter) lactose-fermenting coliforms produce characteristic pigmented colonies on the enteric plating media. A striking characteristic of Proteus species is their propensity to swarm over the surface of most plating media, making the isolation of other organisms in mixed cultures difficult. The swarming growth appears as a rapidly spreading thin film, sometimes with changing patterns of whirls and bands. Sorbitol MacConkey agar is useful for screening EHEC (commonly E. coli O157:H7) on which sorbitol-negative colonies are nonpigmented and considered suspicious for the organism. Unless the physician specifically requests that the laboratory look for the possibility of E coli as an enteropathogen, tests for pathogenic strains, including toxin assays, serotyping, and serogrouping, will not be done.

In cases of suspected bacteremia, replicate bottles (one cultured aerobically, the other anaerobically) containing 25 to 100 ml of appropriate medium with anticoagulant (e.g., sodium polyanetholesulfonate) are inoculated with 10-ml portions of blood. It is usually necessary to take multiple specimens, both before and after antibiotic therapy is started. It is important to take specimens after antibiotic treatment is started so that therapeutic failure can be recognized while the bacteremia may still be amendable to more aggressive medical or surgical treatment.

All of the coliforms and Proteus species are Gram negative, facultative anaerobic, non-spore- forming rods that are typically motile, except for Klebsiella, which is nonmotile. The oxidase test is negative, and nitrates are reduced to nitrites. Proteus species and all coliforms ferment glucose, but fermentation of other carbohydrates varies. Lactose usually is fermented rapidly by Escherichia, Klebsiella and some Enterobacter species and more slowly by Citrobacter and some Serratia species. Proteus, unlike the coliforms, deaminates phenylalanine to phenylpyruvic acid, and it does not ferment lactose. Typically, Proteus is rapidly urease positive. Some species of Klebsiella, Enterobacter, and Serratia produces a positive urease reaction, but they do so more slowly. A battery of tests for biochemical properties is required to identify the coliforms and Proteus to the species level. Commercial identification systems are now widely used by most US clinical laboratories and consist of "kits" or miniaturized biochemical tests which are read manually (e.g., API-20E and BBL Crystal) or automatically (e.g., Vitek or MicroSCAN).

The coliforms are characterized by great antigenic diversity caused by various combinations of specific H, K, and O antigens. For example, approximately 50 H, 90 K, and 160 O antigens have been identified among various strains of E coli. In contrast, Klebsiella, with no H antigens, has 10 O antigens and approximately 80 K antigens. Serologic identification of the coliforms and Proteus species, commonly by reference laboratories, is an extremely important epidemiologic tool. Similarly, other phenotying methods including biotyping (biochemical profiles), antibiograms (patterns of resistance to antimicrobal agents), and bacteriocin and phage typing have been widely used in epidemiologic studies, particularly of multiresistant isolates of coliforms and Proteus. Recently, genotyping methods such as plasmid profiles (determined by agarose gel electrophoresis), RFLP (restriction fragment link polymorphism) of total DNA, pulsed-field gel electrophoresis, targeted analysis of DNA polymorphism, ribotype, and arbitrarily primed PCR (polymerase chain reaction) have been used in epidemiological studies. In hospital-acquired infections, for example, the same or a small serologic number of plasmid types or suggests single sources of infection. The finding of multiple serotypes or plasmid profiles suggests multiple sources of infection or endogenous infections.

Control

Prevention of coliform and Proteus infections, particularly those that are hospital acquired, is difficult and perhaps impossible. Sewage treatment, water purification, proper hygiene, and other control methods for enteric pathogens will reduce the incidence of E coli enteropathogens. However, these control measures are rarely available in less developed regions of the world. Breast-feeding is an effective means of limiting outbreaks of enteropahogens in infants. Aggressive infection control committees in hospitals can do much to reduce nosocomial infections through identification and control of predisposing factors, education and training of hospital personnel, and limited microbial surveillance. Except for investigations of potential outbreaks, routine culturing of personnel, patients, and the environment is not warranted. Selective decontamination of the digestive tract with a suitable nonabsorbable antimicrobial regimen may be useful during outbreaks caused by nosocomial coliforms and Proteus. Meticulous hand washing after each patient contact a highly effective means of reducing the transmission of nosocomial pathogens (Fig. 26-3)is infrequently or poorly performed by some hospital personnel. In a study conducted in an intensive care unit following an educational campaign on the importance of hand washing, the compliance was 17 percent for physicians, 100 percent for nurses, 82 percent for respiratory technicians, and 88 percent for diagnostic services personnel.

Active or passive immunization against coliforms and Proteus species is not practiced. However, vaccines or hyperimmune sera for the six common Gram negative pathogens (E coli, Klebsiella, Enterobacter, Serratia, Pseudomonas aeruginosa, and Proteus) probably would have a major impact on morbidity and mortality from nosocomial infections. In a trial, the mortality was reduced markedly in a group of patients with Gram-negative bacteremia who had been given antiserum against a mutant E coli with an exposed lipopolysaccharide core.

Ampicillin, sulfonamides, cephalosporins, tetracycline, trimethoprimsulfamethoxazole, nalidixic acid, ciprotloxacin, and nitrofurantoin have been useful in treating urinary tract infections by coliforms and Proteus species.



FIGURE 26-3 Major routes of transmission and prevention of spread of nosocomial pathogens.

Gentamicin, amikacin, tobramycin, ticarcillin/clavulate, imipenem, aztreonam, and a variety of third-generation cephalosporins may be effective for systemic infections; however, laboratory tests for drug susceptibility are essential. For example, resistence of E coli to ampicillin, and first generation cephalosporins is increasing rapidly to the extent that they can no longer be considered primary drugs of choice in empirical treatment of urinary tract infections. Likewise, emergence of coliforms with chromosomal or plasmid-encoded extended spectrum B-lactamase activity is causing global problems with resistance to third generation cephalosporins. Some coliforms have multiple resistance due to the presence of R plasmids transmissible by conjugation. Conjugative resistance plasmids allow the transfer of resistance genes among species and genera that normally do not exchange chromosomal DNA (Ch. 5). In some cases, resolution of the infection may require drainage of abscesses or other surgical intervention.

Measures commonly used to control epidemics of antibiotic resistant Gram-negative bacilli have included: (1) closing the unit to new admissions until control of the outbreak is underway; (2) reinforcing hand-washing practices; (3) gown and glove isolation, often combined with isolation of patients in separate quarters; and (4) restricting the use of the antibiotic to which the offending clone is resistant.

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