Antimicrobial Resistance Among Anaerobes - The European Experience

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Clinical usage of antimicrobial agents has been accompanied by the isolation of antimicrobial-resistant bacteria. During the last years there have been reports showing increasing numbers of anaerobic bacteria resistant to different antimicrobial agents in Europe. Resistance in anaerobic bacteria has a significant impact on the selection of antimicrobial agents for empirical therapy. The development of antibiotic resistance in anaerobic bacteria has been documented for beta-lactam drugs, clindamycin, macrolides, tetracyclines, fluoroquinolones and nitroimidazoles. The Bacteroides fragilis group is more resistant to antimicrobial agents than most other anaerobic bacteria. The Bacteroides genus and the genera Prevotella and Porphyromonas have become increasingly resistant to many anti-anaerobic agents. Fusobacterium strains resistant to beta-lactam drugs are relatively frequent. Resistant anaerobic cocci and Propionibacterium acnes have also been reported. Recently, fluoroquinolone-resistant Clostridium difficile strains producing three toxins (toxin A, toxin B and binary toxin) have been isolated from patients with severe C. difficile diseases.

The resistance mechanisms in anaerobic bacteria are: a) hydrolysis of the antimicrobial drug by several enzymes before reaching the site of action (most common, sometimes plasmid mediated); b) decreased permeability of the organisms; c) modification at the site of action of the antimicrobial agent; d) efflux mechanisms which eliminate the antimicrobial drug from the bacterial cell.

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Update on Clinically Significant Anaerobes (invited)

61.001

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Fluroquinolone-resistant Gonorrhea

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The rapid emergence of fluoroquinolone resistance among Neisseria gonorrhoeae isolates with a preexisting high prevalence of penicillin resistance in the past decade is of great concern. The prevalence of gonococcal resistance to ciprofloxacin has increased rapidly worldwide in the past few years, but this increase has varied considerably by country, ranging from 2.1% in Canada to 86.9% in China. In Taiwan, ciprofloxacin-resistant N. gonorrhoeae was first isolated in 1998. A remarkable increase in the prevalence of ciprofloxacin resistance was found between 2001 (66.7%) and 2003 (95.2%) at National Taiwan University Hospital. Previously established guidelines for the management of gonorrhoea in adults in the United States and the United Kingdom recommended the use of a fluoroquinolone (e.g., ofloxacin, ciprofloxacin, or levofloxacin) as a first-line option for gonorrhea therapy. Failure of gonococcal infections caused by N. gonorrhoeae strains with resistance to ciprofloxacin to respond to treatment with these agents has been well documented. Accordingly, a fluoroquinolone is no longer a first-line option for the treatment of gonorrhoea in many countries, particularly those with high incidence of fluoroquinolone resistance in gonococcal isolates. Spectinomycin and a cephalosporin (e.g., cefpodoxime or ceftriaxone) might be used as the first-line agent for the treatment of gonorrhea, although some gonococcal isolates exhibiting resistance to the above agents have been reported.

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Update on Clinically Significant Anaerobes (invited)

61.002

Clostridium difficile Type O27, Coping with a More Virulent Strain

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Clostridium difficile (CD) is an anaerobic bacterium capable of forming spores which confer resistance to heating, drying and chemical agents, including disinfectants. Spores of CD may survive in the environment for long periods and are resistant to alcohol. More than 150 PCR ribotypes and 24 toxinotypes have been recognized; epidemic ribotypes may have enhanced sporulation. Since 2003, increasing rates of CD infections (CDI) have been reported in North America and Europe involving a more severe course, higher mortality, increased risk of relapse and more complications. The outbreaks are difficult to control and require a multifaceted approach. The most important infection control measures act on interruption of transmission of spores to vulnerable patients from infected patients and from the environment. Vulnerable patients are mainly patients who receive antimicrobial treatment, and therefore fewer antibiotic prescriptions should lead to less vulnerable patients. At present, no sufficient evidence exists to propagate the use of probiotics to vulnerable patients for prevention of CDI. Transmission of spores occurs mainly via contact of contaminated health care workers to patients, directly by patient-to-patient transmission or by transmission from the contaminated environment to patients. There is no direct evidence that patients or healthcare workers who are symptom-free but colonised with C. difficile in the intestinal tract are significant sources of infection. Early diagnosis of CDI, prompt isolation of symptomatic patients and reducing antimicrobial treatment are essential first steps. The infection control measures include recommendations to isolate infected patient on a single room with designated toilet, to apply proper hand hygiene with soap and water,
to use appropriate protective clothing (gloves and aprons or gowns), to intensify environmental cleaning with a chlorine containing disinfectant and to take specific precautions for the use of devices (disposable or dedicated to individual patient). Patient isolation must continue at least until diarrhoea has ceased. Each hospital should have an appropriate surveillance system to recognize an increase of the incidence of CDI in an early stage. All infection control measures should be written in a local protocol so that additional measures can be carried out as soon as a problem with CDI arises. When outbreaks occur, additional recommendations include a reinforcement of general and hand washing measures, intensifying of testing patients with diarrhoea for C. difficile, reinforcement of environmental cleaning, information and education of health-care workers, cleaning department and visitors, cohorting of infected patients, and eventually closure of the unit followed by intensive environmental cleaning. Restricted antibiotic prescribing is also highly recommended to reduce polypharmacy and duration of administration. Second and third generations cephalosporins and more recently fluoroquinolones have been identified as potential risk factors. Although some hospitals report successes for enhanced environmental cleaning with potentially effective agents such as hydrogen peroxide vapour, the evidence is too scarce to consider this as an evidence-based approach.

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61.003

Molecular Biology of Protein Glycosylation in the Symbiotic Anaerobe Bacteroides Fragilis

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B. fragilis is one of the most abundant gram negative anaerobes living symbiotically in the human intestine, where it digests carbohydrates for the host and has been implicated in immune system development. It is also an opportunistic pathogen and a reservoir of antibiotic resistance genes. B. fragilis produces multiple capsular polysaccharides and fucosylated glycoproteins. Mutant strains deficient in production of either of these cannot compete with wild-type in colonization of germ-free mice, indicating that both types of molecule are vital for symbiosis. Here we characterize the molecular biology of the protein glycosylation system. A cell lysate was enriched for glycosylated proteins by lectin affinity chromatography and proteins identified by mass spectrometry. These candidates were expressed from a plasmid in B. fragilis with a C-terminal His tag, purified and glycosylation confirmed by periodate reactivity and release of oligosaccharides. The glycoproteins include a secreted lipoprotein and several soluble periplasmic proteins, the first time that the latter has been observed in a bacterial species. Deletion of a genetic region containing a gene resembling an O-antigen flippase and multiple glycosyltransferases reduced the MW of the glycoproteins, indicating that these genes are involved in protein glycosylation and suggesting that it occurs in the periplasm. The smallest and most abundant glycoprotein was investigated in detail. Similar glycans were released by beta-elimination and hydrazinolysis, consistent with O-linkage to Ser or Thr. Point mutations in peptides that were rarely or never observed by mass spectrometry, and therefore likely to be glycosylated, identified one Ser and three Thr residues as probable glycosylation sites. Deletion of the signal peptide prevented glycosylation of the protein, consistent with it occurring in the periplasm. We continue to investigate the biochemistry and genetics of the glycosylation system, the biological functions of the glycoproteins and the role of glycosylation.

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61.004

Anaerobes As Biofilms

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Although anaerobes are predominant in humans, largely outnumbering aerobic bacteria, experimental data concerning their biofilm forming ability are still relatively few and essentially focused on the anaerobic flora of the mouth (including dental plaque, buccal mucosa and tongue biofilm) and the vaginal mucosa.

As the oral environment is concerned, its complexity has induced the development of a number of artificial mouth models able to simulate the different conditions of microbial growth within the microcosms of the oral cavity. With regards to the vaginal mucosa, recent studies have elucidated that the biofilm phenotype confers to Gardnerella vaginalis a survival advantage in the presence of hydrogen peroxide and lactic acid producing resident lactobacilli; on the other hand, the ability of probiotic strains of Lactobacillus to interfere with Gardnerella vaginalis and disrupt its biofilm, has been recently reported as a promising tool to reduce the need for antibiotics in the treatment of bacterial vaginosis.

As the intestinal tract is concerned, investigations by microscopic and FISH techniques have shown that mucosal bacteria, including bacteroides and bifidobacteria, occur in microcolonies and are distributed throughout the mucus layer.

Our group is currently investigating the role of anaerobes in the occlusion of biliary stents. SEM observations revealed that biliary sludge occluding the lumen of the 18 so far examined polyethylene stents was constituted by a multi-species (aerobes and anaerobes) microbial biofilm immersed in an amorphous material containing also dietary fibers and crystals of bile salts. The ability of the isolated anaerobic strains, belonging to the species Bacteroides, Clostridium, Fusobacterium, Peptostreptococcus, Prevotella and Veillonella, to form biofilm has been assessed in vitro. On the