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Identification and antibiotic sensitivity of bacteria isolated from pharyngeal swabs in patients with infectious mononucleosis

Epstein-Barr virus (EBV) is a human herpesvirus which infects almost all the world's population subclinically during childhood and thereafter remains in the body for life.

The virus latently infects circulating B lymhpocytes, which, as relatively long-lived resting cells, are an ideal site for long-term residence. This infection is almost always controlled at subclinical levels, but under certain conditions EBV is associated with malignant tumors. EBV was first discivered in 1964 in B lymphocytes cultured from an African (endemic) Burkit's lymphoma (BL) (5), and the virus is now estimated to be present in around 96% of these tumors (8). Since its discovery, EBV has been found in a variety of other tumor types, in some of which a clear aetiological role for EBV has been established, whereas in others more work is required before a causal association can be made (3). In addition to BL, lymphoid tumors with an EBV association include B-lymphoproliferative disease in the immunocompromised host (2), subsets of Hodgkin's lymphoma (6) and certain types of T-cell lymphoma (13).

Persistently infected individuals shed EBV either constantly or intermittently into saliva and thereby spread the virus to uninfected individuals through close oral contact. Primary infection in aldolescence causes infectious mononucleosis (IM) in 30–50% of cases and induces both humoral and cellular immune responses that control but do not eliminate the EBV infection (12). IM usually presents with the acute onset of fever, painful lymph glands in the neck and sore throat. According to the Infectious Disease Society of America, EBV is one of the most frequent non--bacterial pathogens which causes exudative pharyngitis (14).

The aim of our study was to assess antibiotic sensitivity of the pathogenic and potentially pathogenic flora isolated from the pharyngeal swabs in patients with infectious mononucleosis.

MATERIAL AND METHODS

Assessments were done in 62 patients, aged 18–32, treated at the Outpatients' Clinic and the Department of Infectious Diseases, Medical University of Lublin in the years 2001–2004. The diagnosis of infectious mononucleosis (IM) was based on a clinical picture and hematological and biochemical results. Positive latex reaction to heterophilic antibodies (Elisa method, Biomed, Cracow, Poland) or the presence of anti-VCA IgM in titer > 20AU/ml (ELISA method, Organon, Technika) confirmed the diagnosis. The material consisted of pharyngeal swabs cultured according to the routine microbiological diagnostics on the following mediums: 5% blood agar, Chapmann's medium, McConkey's medium and *Haemophilus* agar (BioMerieux, Lyon, France). The phenotypic identification of the isolated strains was done with identification kits suitable for respective species: API Strep, API Staph, API E, API NH, API GN (BioMerieux, France). The antibiotic sensitivity was assessed with the disk diffusion method by Kirby-Bauer.

RESULTS

The pathogenic or potentially pathogenic flora was isolated in IM in 71% of the patients. Analysis of results showed the presence of *Haemophilus* bacilli in 41% of patients, out of which *H. parainfluenze* was present in 23% and *H. influenze* in 18%. *Staphylococcus aureus* was isolated in 29% of patients, *Escherichia coli* in 12%, *Streptococcus pyogenes* in 5% and *Enterobacter cloacae* the least often – in 2%. Table 1 shows antibiotic sensitivity of the isolated bacterial strains.

Table 1

Bacterial strain	*Haemophilus influenze/ parainfluenze*	*Staphylococcus--aureus MSSA*	*Escherichia coli*	*Streptococcus pyogenes*	*Enterobacter cloacae*
Penicillin				sensitive	
Ampicillin+sulbactam			sensitive	sensitive	
Amoxicillin+clavulanic acid		sensitive	sensitive		
Azytromycin	sensitive				
Cefuroxim		sensitive			sensitive
Cefamandol			sensitive		sensitive
Ceftazidim		sensitive	sensitive		sensitive
Cefotaxim	sensitive		sensitive		
Gentamycin		sensitive	sensitive		sensitive
Linkomycin		sensitive			
Clindamycin		sensitive			
Netromycin		sensitive			
Ciprofloxacin					sensitive
Pefloxacin		sensitive			
Piperacyllin			sensitive		
Co-trimoxazol			sensitive		
Erythromycin		sensitive		sensitive	
Vancomycin		sensitive			
Doxacyclin					sensitive

DISCUSSION

Pharyngoamygdalitis belongs to the most frequent clinical symptoms observed in IM. From the clinical point of view, the intensiveness of the disease may range from a chronic inflammation of mucous membranes to heavy types of angina, imitating even the pharyngeal diphtheria. Literature shows that purulent tonsillitis caused by EBV constitutes 19% of all viral infections and can imitate a bacterial infection (1,14). Our research including bacteriologic identification of pathogenic strains isolated from pharyngeal swabs in the acute phase of the infection showed that in 75% of patients the infection went along with the presence of pathogenic or potentially pathogenic flora, such as: staphylococci, streptococci, *Haemophilus* and *Enterobacteriacae* bacilli. It is interesting to notice that *Staphylococcus aureus* (MSSA) was isolated in every third patient. However, the common carrier state of this bacteria, amounting to 60% in populations, makes it

difficult to clearly estimate its possible role and impact on the clinical course of IM. Staphylococci are known to be responsible for development of an inflammatory process and the β-lactamases produced by them can cause inefficacy of some antibiotics (10).

Streptococcus pyogenes was isolated in 5% of patients and its presence correlated with heavy angina with wide-spread tonsillar deposits. Assessment of drug-sensitiveness showed high activity of penicillin, ampicillin / clavulanic acid and erythromycin against the isolated strain. It is important to stress that we obtained eradication with a precisely chosen antibiotic therapy. It was also interesting to notice that we isolated *Enterobacteriacae* bacilli from pharyngeal swabs cultures. The presence of *Escherichia coli* can be important pathogenically in 12% of patients. Literature reports, confirmed by clinical observations, indicate that a high rate of persistent colonization of the upper respiratory tract after an antibiotic therapy favours wide spreading of infection and pharyngitis (10). *E. coli* strains isolated by us were highly sensitive to most of commonly administered antibiotics,-active against both G (+) and G (-) bacteria.

Some antibiotics, including amoxicillin and cefotaxim, were proven to cause a decrease in resistance against digestive tract colonization by *Enterobacter cloacae* (4). Recent reports in literature indicate that the mentioned bacillus is more and more often an etiological factor of nosocomial infections (4). *Enterobacter cloacae* produces the chromosomal cephalosporynase, an enzyme particularly sensitive to inductive effects of β-lactamate antibiotics. This can explain failures while using the 1st generation cephalosporins in infections with this bacteria. We isolated *Enterobacter cloacae* in 2% of patients. Assessment of drug-sensitiveness of the isolated strain showed high activity of cephalosporin, doxacyclin and gentamycin. It must be stressed, however, that the use of cephalosporins leads to selection of mutant strains – the so-called "over-producers of the chromosomal cephalosporynase" – which eventually become resistant also to amino-glycosides and chinolones (4). Our research also shows that *Haemophilus* bacilli were the most often isolated, almost in every second patient. Similarly to the case of *Staphylococci*, the clinical interpretation of this fact is made difficult by a frequent carrier state of *Haemophilus* in adults, estimated on the level of 50% (10).

H. influenze can also be responsible for tonsillitis. In case of immunological suppression, activation and general infection with this bacillus was even observed in carriers. In IM there is a transitory suppression of immunoglobulin synthesis, which in normal state encloses bacteria present on tonsils (11). In IM therefore, the lack of these immunoglobulins facilitates bacterial adhesion and colonization. We cannot therefore exclude that the physiological flora, which constitutes a reservoir of potentially pathogenic bacteria, is the source of *Haemophilus* infection. Cephalosporins of generation III and IV are the treatment of choice in invasive infections with *H. influenze* (7). The strains isolated in swabs from our patients were sensitive only to cefotaxim and azytromycin.

In conclusion, it would be interesting to analyze the impact of pathogenic flora on the clinical course of IM on the basis of bacteriological results.

CONCLUSION

Bacteriological identification and assessment of antibiotic sensitivity of germs isolated from the pharyngeal swabs can be helpful in defining the therapeutic strategy in the course of infectious mononucleosis.

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SUMMARY

The aim of our study was to assess antibiotic sensitivity of the pathogenic and potentially pathogenic flora isolated from the pharyngeal swabs in patients with infectious mononucleosis. Assessments were done in 62 patients, aged 18–32. The diagnosis of infectious mononucleosis (IM) was based on a clinical picture and hematological, biochemical and serologic examination results. The material consisted of pharyngeal swabs cultured according to the routine microbiological diagnostics. The antibiotic sensitivity was assessed with the disk diffusion method by Kirby-Bauer. Bacteriological identification and assessment of antibiotic sensitivity of germs isolated from the pharyngeal swabs can be helpful in defining the therapeutic strategy in the course of infectious mononucleosis.

Identyfikacja i ocena wrażliwości na antybiotyki bakterii izolowanych z błony śluzowej gardła chorych na mononukleozę zakaźną

Celem pracy była ocena antybiotykowrażliwości flory bakteryjnej patogennej i potencjalnie patogennej, izolowanej z błony śluzowej gardła chorych na mononukleozę zakaźną (MZ).Badania przeprowadzono w grupie 62 chorych w wieku 18–32 lat. Rozpoznanie MZ ustalono na podstawie objawów klinicznych, parametrów hematologicznych i biochemicznych oraz badań serologicznych. Materiał do badań stanowiły wymazy z błony śluzowej gardła, które posiewano zgodnie ze stosowaną rutynowo diagnostyką mikrobiogiczną. Wrażliwość na antybiotyki oznaczono metodą dyfuzyjno-krążkową wg Kirby-Bauera.W przebiegu mononukleozy zakaźnej identyfikacja bakteriologiczna i ocena antybiotykowrażliwości szczepów izolowanych z błony śluzowej gardła może być pomocna w ustaleniu strategii postępowania terapeutycznego.