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Characteristics of opportunistic species of the Corynebacterium and related coryneforms isolated from different clinical materials

An increasing contribution of opportunistic species of the *Corynebacterium* type entering into the composition of physiological flora has been observed in recent years. In favourable conditions it may cause infections. The most endangered group of persons are patients with hypoimmunity but, unfortunately, infections have been also found in so-called "immunocompetent" patients, in whom symptoms of hypoimmunity have not been found. A big differentiation of species within the *Corynebacterium* type resulted in the need to conduct thorough research in reference to their taxonomy, identification and evaluation of pathogenicity. Therefore, the traditional name *diphtheroids*, which suggests a direct relation to *C. diphtherae,* is more frequently replaced by taxonomists with a more descriptive name *coryneforms* (4, 5, 10). Upon the invention of identification methods based on phenotypic biochemical properties (APICoryne bioMerieux) of opportunistic species of the *Corynebacterium* type, many reports have appeared which are related to this type of infections. With time the species most frequently isolated from patients have been considered important opportunistic micro-organisms. They include *Corynebacterium jaikeium* (formerly *C. group JK*), *C. urealiticum* (*C. group D2*), *C. pseudodiphtheriticum, C. ulcerans, C. pseudo-tuberculosis, C. minutissimum,* (5, 8, 10, 13, 15). New reports, which are being constantly delivered about infections in people, draw our attention also to other species of the *Corynebacterium* type as the cause of opportunistic infections. A special problem is related to cases where isolated strains show a high antibiotic sensitivity (1, 3, 8, 10, 13, 15).

The aim of this work is to characterize isolated opportunistic strains of the Corynebacterium type from different clinical materials and to determine their antibiotic sensitivity as well as evaluate the identification methods by means of commercial tests.

MATERIAL AND METHODS

The examined group included 35 patients. In the examinations different diagnostic materials were used, sampled from patients in whom clinical symptoms of infection were found. They were: blood, sputum, urine, swabs from postfracture wounds and from postburn wounds, pus from abscesses, ear secretion, conjunctiva secretion, from tracheotomic cannula, from bronchoapirate, trachea swab, drain swab, dialysis fluids and throat swab. The blood collected for inoculation was directly added to BBl Septi-Chek (TSB) medium (Becton Dickinson).

The materials were treated with the routine microbiological diagnostics. Preparations, which were directly made from the collected samples (sputum, pus, secretions, dialysis fluids),

were stained with the Gram method. The growth of strains of the *Corynebacterium* type was observed on Columbia agar medium with 5% sheep blood after 24h and 48h of culture in oxygenic conditions and at the temperature 37^0C. Identification of species was performed by means of commercial biochemical tests APICoryne (bioMeriuex).

Determination of antibiotic sensitivity of the isolated strains was performed applying the disc diffusion method (according to Birby-Bauer) on the Muller-Hinton medium with an addition of 5% sheep blood using the following discs with antibiotics for the determination: Penicillin (10 units), Ampicillin (10 μg), Cephalotin (30), Cephazolin (30), Gentamicin (10), Tetracyclin (30), Amikacin (30), Erythromycin (15), Colistin (10), Chloramphenicol (30), Vancomycin (30), Nitrofurantoin (300), Trimethoprim/Sulfamethaxazol (23.75/1.25), Cefuroxime (30), Cefotaxime (30), Imipenem (10), Teicoplanin (30), Ciprofloxacin (5), Norfloxacin (10), Ofloxacin (5), Lincomycin (15), Piperacilin (30), Carbenicilin (100), Nalidixic acid (30), Clindamycin (2) (Becton – Dickenson). The zone of growth inhibition around the discs was read out according to NCCLS recommendations.

RESULTS

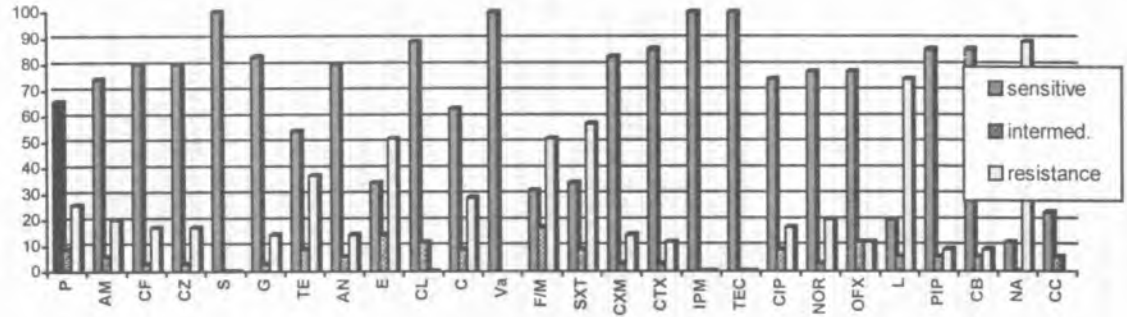
In direct sputum preparations stained by means of the Gram method Gram-positive club-like bacteria were observed, which showed a tendency to make cakes, or adhered to cell fragments that were present in the preparations. The growth of the examined strains on the Columbia agar medium with 5% sheep blood was observed after 24h in the form of small delicate colonies or bigger ones up to 1 mm, white or white-and-grey, non-shiny, which after 48h reached the size of 1–2 up to 3 mm and were whitish, round, of mat surface and did not produce hemolysis of beta-type (except *Arcanobacterium haemoliticum*). They were catalase-positive (except *A.arcanobacterium* and *Brevibacterium sp.*). Identification of the isolated strains (24h of culture) was made by means of APICoryne tests, according to the recommendations of the manufacturer. On the basis of the created numeric code, having read-out positive reactions, the identification of the species was made applying the computer program APILAB.

In the examined group of patients in which species of the *Corynebacteria* type or related coryneforms were isolated from the clinical material, the species of the *Corynebacteria* type and related coryneforms were found in the form of pure culture or their growth was abundant and the accompanying bacteria were in the form of only a few or several colonies. Such growth of culture confirmed the contribution of the examined species as the etiological factor of infection. Among the identified strains (35) – Table 1 – the *C. pseudodiphtheriticum* constituted the biggest number (11), next were the strains whose biochemical characteristics did not permit detailed identification in reference to the species as they entered into the range of characteristics of two species: *C.striatum/C.amycolatum* (8), *C.propinquum* (6). *Brevibacterium sp.* (2) and then one in each of *C.jeikeium*, *C.group G*, *C. amycolatum*, *C.group F1*, *C.accolens*, *Arcanobacterium haemoliticum*, *C.macqinleyi* and *C.afermentans.*

In all isolated species identification of antibiotic sensitivity was made (Fig. 1). Out of 25 identified antibiotics sensitivity of all strains (100%) was found to Vankomycin, Teicoplanin and Imipenem, whereas about 88-80% of strains were sensitive to Carbenicilin, Piperacilin, Cefotaxime, Cefuroxime, Gentamicin, Cephazolin, Cephalotin. The lowest sensitivity was shown by microorganisms to Nalidixic acid (11.4%), Lincomycin (20.0%), Clindamycin (22.8%), Nitrofurantoin (31.4%), Trimethoprim/Sulfamethaxazol (34.3%) and Erythromycin (34.3%).

Table 1. Characteristics of the isolated strains of the *Corynebacterium* type and related *coryneform* from different clinical materials

No	Different clinical materials	Isolated strains of the *Corynebacterium* type and related *coryneform*
1	blood	*C.striatum/C.amycolatum*
2	blood	*C.jeikeium*
3	urine	*C.pseudodiphtheriticum*
4	urine	*C.grupa G*
5	drain fluid	*C.striatum/C.amycolatum*
6	pus from a postburning wound	*C.striatum/C.amycolatum*
7	pus from a postfracture	*Brevibacterium sp.*
8	pus from an armpit	*C.amycolatum* (nieliczne *Staph.*kg-)
9	pus from an abscess (anus area)	*C.grupa F1* (*E.coli*)
10	from tracheotomic cannula	*C.striatum/C.amycolatum*
11	bronchoaspirate	*C.pseudodiphtheriticum*
12	ear secrection	*C.accolens* (nieliczne *Staph.*kg-)
13	ear secretion	*C.striatum/C.amycolatum* (nieliczne *Staph.*kg-)
14	ear secretion	*Brevibacterium sp.*(nieliczne *Staph.*kg-)
15	ar secretion	*C.propinquum* (nieliczne *Staph.*kg-)
16	sputum	*C.pseudohiphtheriticum* (*S.viridans, Neisseria sp.*)
17	sputum	*C.pseudodiphtheriticum* (*S.viridans, Neisseria sp.*)
18	sputum	*C.pseudodiphtheriticum* (*S.viridans, Neisseria sp.*)
19	sputum	*C,pseudodiphtheriticum* (nieliczne *S.aureus,S.viridans, Neisseria sp.*)
20	sputum	*C.pseudodiphtheriticum* (*S.viridans, Neisseria sp.*)
21	sputum	*C.pseudodiphtheriticum* (*S.viridans, Neisseria sp.*)
22	sputum	*C.propinquum* (*S.viridans, Neisseria sp.*)
23	sputum	*C.propinquum* (*S.viridans, Neisseria sp.*)
24	sputum	*C.striatum/C.amycolatum* (*S.viridans*)
25	throat swab	*Arcanobacterium haemoliticum* (*S.viridans,Neisseria sp*)
26	trachea secretion	*C.pseudodiphtheriticum* (nieliczny *S.viridans*)
27	conjunctiva swab	*C.propinquum* (poj.*Staph.*kg-)
28	conjunctiva swab	*C.macqinleyi* (poj.*Staph.*kg-)
29	conjunctiva swab	*C.striatum/C.amycolatum*
30	conjunctiva swab	*C.striatum/C.amycolatum*
31	conjunctiva swab	*C.propinquum* (poj.*Staph.*kg-)
32	pus secretion from sinusem	*C.pseudodiphtheriticum* (poj.*Staph.*kg-)
33	pus secretion from sinusem	*C.pseudodiphtheriticum*
34	dialysis fluid	*C.afermentans*
35	dialysis fluid	*C.propinquum* (poj.*Staph.*kg-)



P-Penicillin , AM-Ampicillin , CF-Cephalotin , CZ-Cephazolin , G-Gentamicin , TE-Tetracyclin , AN-Amikacin ,E- Erythromycin, CL-Colistin, C-Chloramphenicol , Va-Vancomycin , F/M-Nitrofurantoin , SXT-Trimethoprim/Sulfamethaxazol, CXM-Cefuroxime, CTX-Cefotaxime, IPM- Imipenem , TEC-Teicoplanin ,CIP- Ciprofloxacin , NOR-Norfloxacin , OFX-Ofloxacin ,L- Lincomycin , PIP-Piperacilin , CB-Carbenicilin, NA-Nalidixic acid , CC-Clindamycin

Fig. 1. Characteristics of antibiotic sensitivity of the isolated strains of the *Corynebacterium* type and related *coryneform* from different clinical materials

DISCUSSION

New studies which have appeared recently describing cases of isolation of different species of the *Corynebacterium* type and related *cyneforms* from clinical materials sampled from patients resulted in the increasing interest in this group of micro-organisms . Following taxonomic tests new species have been identified: *C.colyeae, C.singulare, C.durum, C.lipophiloflavum* (6, 7, 11, 12). Identification of this group of micro-organisms, unfortunately, may cause big difficulties and the recognition of infections caused by them depends considerably on possibilities of the laboratory identification on the level of hospital microbiological laboratory. It is obvious that there is a need to apply credible methods for quick identification of these bacteria. Such conditions seem to be fulfilled by the APICoryne set (bioMerieux), which makes it possible to determine species of the *Corynebacterium* and *coryneforms* which occur most frequently in clinical materials on the basis of the most characteristic biochemical properties.

In the examined group of patients (35 persons), in which infections with Gram-positive club-like bacteria have been found *C.pseudodiphtheriticum* occurred most frequently. In all cases identification of this species did not cause any problems. It is described as the cause of different infections of the opportunistic character, among others of lower airways, bacteraemia, of the urinary system (5, 8, 10, 13, 15).

Two species with close biochemical properties turned out to be a problem in identification with application of the APICoryne set: *C.striatum/C.amycolatum.* In such cases it is necessary to apply determination of additional properties, which in case of biochemically non-typical strains may not lead to identification. Since these species were isolated from such materials as among others blood, drain fluid, pus from wounds and secretion from tracheotomy cannula, where quick and thorough identification of the etiological factor permits to take up appropriate treatment, it is necessary to perform identification by means of genetic methods consisting in utilization of determination of the sequence of 16sRNA gene. This method is more and more frequently applied in many laboratories (2, 6, 7, 9, 11, 12).

In the examined group also the contribution of *C.propinquum* was observed in opportunistic infections (6 cases), the species closely related to *C.pseudodiphtheriticum*, which may occur in mucous membrane of the nasopharyngeal cavity (5, 10, 13, 15). Other species isolated from the patients (*Brevibacterium sp.* – 2 cases, and one: *C.afermentans C.jeikeium, C.grupa G, C.grupa F1, C.accolens, C.macqinleyi,*) that have also been described by other authors (5, 10, 13, 15) occurred with lower frequency. Most of them showed relatively high antibiotic sensitivity, except *C.jeikeium* (from blood), *Brevibacterium sp.* (ear secretion), *C.propinquum* (sputum), *C.pseudodiphtheriticum* (trachea secretion). An important case is the isolation of *Arcanobacterium haemoliticum*, bacteria Gram+, catalase- from the throat, which may result in pharyngitis, and its identification on the basis of morphological properties of colonies may be mixed up with beta-hemolytic streptococcus (1).

The highest sensitivity of the isolated strains was to Vankomycin, Teicoplanin and Imipenem, while the resistance was to Nalidixic acid, Lincomycin, Clindamycin, Nitrofurantoin, Trimethoprim/Sulfamethaxazol and Erythromycin. Positively, strains isolated from patients treated in hospital show higher antibiotic resistance (3, 4, 8, 14).

CONCLUSIONS

The research confirmed the contribution of species of the *Corynacterium* type and related *coryneform* in opportunistic infections. In the examined cases the species of *C. pseudodiphtheriticum* as well as *C. striatum* and *C. amycolatum* had the biggest contribution in the infections. Identification of

these species should be conducted not only on the basis of biochemical properties but also by means of a modern method of identification (examination of the sequence of 16sRNA gene).

In the examined strains the highest antibiotic sensitivity was found in Vankomycin, Teicoplanin and Imipenem.

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SUMMARY

Taking into account the increasing contribution of species, which enter into the composition of purely physiological flora of the organism, of the *Corynebacterium* type and related *coryneforms* in opportunistic infections in people, the analysis of strains was made from different clinical materials from patients. Their identification was made on the basis of biochemical properties and their antibiotic sensitivity was characterized. It was found that

strains with similar biochemical properties (*C.striatum, C.amycolatum*) should be identified by means of genetic methods, all the more that they were isolated from clinically important materials. Out of the examined strains the biggest number of infections were caused by *C.pseudodiphtheriticum*, next *C. striatum/C. amycolatum, Brevibacterium sp., C.propinquum, one: C.afermentans, C.jeikeium, C.group G, C.group F1, C.accolens, C.macqinleyi.* The highest sensitivity of isolated strains was to Vankomycin, Teicoplanin and Imipenem.

Charakterystyka oportunistycznych gatunków z rodzaju *Corynebacterium* i pokrewnych *coryneform* izolowanych z różnych materiałów klinicznych

Ze względu na obserwowany coraz większy udział w zakażeniach oportunistycznych gatunków wchodzących w skład flory fizjologicznej z rodzaju *Corynebacterium* i pokrewnych *coryneform* przeprowadzono analizę szczepów pochodzących od chorych z różnych materiałów klinicznych. Identyfikacje ich wykonano na podstawie właściwości biochemicznych oraz scharakteryzowano wrażliwość na antybiotyki. Stwierdzono, że szczepy o podobnych cechach biochemicznych (*C.striatum i C.amycolatum*) powinny być identyfikowane za pomocą metod genetycznych, tym bardziej że izolowane one były z ważnych klinicznie materiałów. Spośród badanych szczepów największa liczba przypadków zakażeń wywołana była przez *C.pseudodiphtheriticum*, następnie *C.striatum/C.amycolatum, Brevibacterium sp., C.propinquum, C.afermentans, C.jeikeium, C.grupa G, C.grupa F1, C.accolens, C.macqinleyi.* Najwyższą wrażliwość izolowanych szczepów zanotowano na: Wankomycynę, Teikoplaninę i Imipenem.