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Specific identification and antibiotic-sensitivity of *Acinetobacter* strains

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Abstract

80 Acinetobacter strains were studied. Their differentiation according to the specific belonging showed that 55 strains belonged to *A. calcoaceticus* var. *anitratum* species and 25 strains to *A. calcoaceticus* var. *lwoffi*. It was established that Acinetobacter strains are multi dry resistant. In particular they showed 100% resistance to ampicillin, carbenicillin, erythromycin, methicillin, oxacillin, tetracycline, oleandomycin, streptomycin, rondomycin, linkomycin. According to the obtained data claphoran and phortum are the most effective.

Keywords: Bacteriophage; Biological properties; Pyocyanic bacteria; Sewage waters; Diagnostics.

1. Introduction

Internal infections in hospitals are one of the major problems in modern medicine. As a result of the decline in the body's resistance to the underlying disease, which is preceded by antibiotic- and immunotherapy, the nature and features of surgical intervention, anesthesia, artificial blood circulation, and other peculiarities, patients are prone to infectious complications caused by conditionally-pathogenic bacteria [1, 2]. In recent years, the group of aerobic, non-fermentable, gram-negative bacteria characterized by resistance to antibiotics and other prophylactic drugs has acquired partly great clinical significance [3]. The frequency of their detection in the material of patients with purulent-septic processes in the material in the data of various authors ranged from 5 to 30% [4].

Acinetobacter-like bacteria are among the most common pathogens of the family of gram-negative intestinal bacteria [5].

When developing diagnostic schemes for differentiation of non-fermented Gram-negative bacteria of different species and groups, preference is given to tests that are more accessible to practical laboratories and have a taxonomic value. One of them is the property to produce acid from glucose cytochrome oxidase under aerobic and anaerobic conditions. Determination of lysis activities of cytochromoxidases and sugars in combination with the study of cultural and morphological properties allows us to pre-differentiate *Acinetobacter* from other non-fermentable bacteria, while identification of their species poses some difficulties. Differentiation of *Acinetobacter* strains is of great importance because these triggers are more likely to cause diseases such as septic shock, various purulent-inflammatory processes associated with the low efficacy of antibiotic therapy, and its untimely use [6]. It should be noted that bacteria of the genus *Acinetobacter* are characterized by high antibiotic resistance [7].

Intensive and not always justified use of antibiotics for therapeutic and prophylactic purposes, which promotes the formation of polyresistant microorganisms, their spread in the clinic, unfavorable changes in the structure of the hospital microflora - is one of the reasons for the unchanged frequency of infections within hospitals. Decreased susceptibility to antibiotics of the gastrointestinal tract, respiratory tract and skin microflora in the microbial biocenosis

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disrupts the relationship between individual species of microorganisms and the possibility of more intensive reproduction is given to gram-negative conditionally pathogenic bacteria which usually have natural resistance to a number of antibiotics [8].

The study of the identification and resistance to antibiotics of *Acinetobacter* strains is undoubtedly of practical interest. The aim of the work was to study the biochemical properties of collectible and clinical strains of *Acinetobacter* by traditional methods and examining susceptibility of these strains to antibiotics.

2. Material and methods

The objects of study were collectible cultures of *Acinetobacter calcoacetius* (strain 5593), *Acinetobacter lwoffi* (strain 5581), obtained from the collection of the G. Eliava Institute of Bacteriophage, Microbiology and Virology, as well as clinical strains isolated from patients in Telavi.

We studied their movement, the character of growth under different conditions of cultivation in simple and complex food areas. Using traditional methods, the presence of cytochrome oxidases, catalase were determined, production of indole and hydrogen sulfide was examined, the ability (property) to reduce nitrates to nitrites was studied as well as gelatin cleavage, urease production, utilization of citrate and sodium acetate (on Simmons citrate agar). The lysis activity of sugars was studied by adding 1% of carbohydrates (glucose, maltose, sucrose, lactose, arabinose, xylose, galactose, ramnose, fructose, mannitol, mannose) to the Hugh-leifson agar. Antibiotic susceptibility was studied by the paper disc method (oriented) and double serial dilutions (minimum inhibitory concentration - MDC) [9, 10, 11 12].

3. Results and discussion

To identify the cultural and biochemical properties of *Acienetobacter*, studies were conducted to identify the species of *Acienetobacter*. Both biochemical variants of *A. calcoaceticus* were formed on solid nutrient soils (blood and flesh-peptide agar, as well as in the Endo agar).

Table 1 Cultural and biochemical properties of non-fermentable species of bacteria belonging to the genus Acinetobacter

Biochemical properties	***Acinetobacter***	
	anitratus	*lwoffi*
Hemolysis	0	0
Oxidase	0	0
Pigment formation	0	0
Movement	0	0
Growth on MacConkey's Agar at 42°C	100	100
Oxidation		
Glucose	100	0
10% of lactose	100	0
Maltose	4	0
Xylose	68	0
Galactose	86	0
Production		
H_2S	0	0
Indole	0	0
Nitrate reductase	68	3
Gelatin	6	5
Ureaze	40	21
Arginine decarboxylase	0	0
Arginine dihydrolase	0	3

Catalase	100	100
Citrate utilization	100	60
Total number of cultures	55	25

We obtained round colonies with straight edges, sometimes without oily consistency, without hemolysis. For identification of *Acienetobacter calcoaceticus* var. *Anitratus, A. calcoaceticus* var. *lwoffi* 5 main signs were selected: presence of pigment formation, oxidases, movement, ability to oxidize glucose. 100% of the cultures of *Acinetobacter* were identical to each other. They are coccobacilli, predominantly a pair of seedless, capsule-free. All cultures were oxidase negative and non-pigmented. The ability to oxidize glucose depended on the biochemical variant: 100% of the culture of *A. calcoaceticus* var. *anitratus* oxidized glucose (as opposed to *A. calcoaceticus* var. *lwoffi*).

Division within the species also revealed 100% ability of *A. anitratus* strains to oxidize 10% concentration of lactose, xylose and galactose, whereas *A. lwoffi* did not possess this ability. 100% of the culture of *A. anitratus* and 60% of *A. lwoffi* were disposed of citrate (Table 1).

By defining additional signs for more detailed characterization and differentiation of the species, it was revealed that all cultures of *Acinetobacter* were catalytic, had the ability to grow at 42°C, did not produce indole, hydrogen sulfide. Cultures were grown in the MacConkey's Agar. Less similar results were obtained while examining the ability to reduce nitrates, the hydrolysis of urea and gelatin. Some cultures of *A. lwoffi* had arginine + dihydrolase in contrast to the standard cultures.

Table 2 Resistance of *A. calcoaceticus* strains to antibiotics

№	**Antibiotics**	**Number of resistant strains in%**	
		A. calcoaceticus var. *anitratus*	*A. calcoaceticus* var. *lwoffi*
1	Methicillin	100	100
2	Oxacillin	100	100
3	Ampicillin	100	100
4	Carbenicillin	100	100
5	Erythromycin	100	100
6	Oliandomycin	100	100
7	Lincomycin	100	100
8	Tetracycline	100	100
9	Rondomycin	100	100
10	Streptomycin	100	100
11	Monomycin	34	88
12	Kanamycin	45	72
13	Neomycin	34	88
14	Gentamicin	72	68
15	Polymyxin	38	40
16	Chloramphenicol	100	100
17	Ristomycin	100	100
18	Claforan	12	12
19	Fortum	0	0
20	Kefzol	100	100
	Strain number	55	25

In addition to differentiating *Acinetobacter* strains, we identified susceptibility to 20 antibiotics of *A. calcoaceticus* cultures isolated from patients in Telavi. Multiple resistance has been identified even with more active drugs such as gentamicin, polymyxin, carbenicillin. In the study of antibiotic susceptibility by the disc method, low activity of claforan and fortum was noted (Table 2).

Determination of antimicrobial activity in relation to *A. calcoaceticus* by MCD method carbenicillin, kanamycin, claforan, fortum comparison of the obtained data showed that the lowest activity against these microorganisms had carbenicillin and the largest – fortum.

Claforan was characterized by a favorable rate in the antibiotic study group. In 60.8% of claforan MDC strains ranged from 20-80 μg/ml, in 32.3% of the above strains growth was inhibited at the 200-400 μg/ml level (Table 3).

Table 3 Distribution by degree of sensitivity (MCD) of 25 clinical strains of *Acinetobacter calcoaceticus*

№	Antibiotics	MDC mcg / ml						
		1-10	20	40	70-80	100	200	400
1	Kanamycin	0	0	0	0	0	0	25
2	Claforan	0	3	3	6	0	1	7
3	Fortum	20	5	0	0	0	0	0
4	Carbenicillin	0	0	0	1	3	1	20

4. Conclusion

Thus, based on the obtained data, it was able to determine species belonging to *A. calacoaceticus* var. *anitratus*, *A. calcoaceticus* var. *lwoffi*. *Acinetpbacter* strains are characterized by high resistance to many antibiotics, which are more commonly used to treat infectious diseases caused by these non-fermenting microorganisms.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest amongst the authors.

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