

**CULTURAL AND BIOCHEMICAL CHARACTERISTICS
OF *ACINETOBACTER* SPP. STRAINS ISOLATED
FROM HOSPITAL UNITS**

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Abstract. The *A. baumannii* and *A. lwoffii* strains, isolated from clinical and environment samples in hospital units, were investigated from the biochemical characteristics and their susceptibility to antimicrobial agent points of view. The nonfermenter, Gram-negative coccobacilli were classified in *A. baumannii* and *A. lwoffii* species on the basis of the catalase positive, oxidase negative, nonmotility, the fermentative/oxidative test and utilization of nutritive substrates. The percentages of susceptibility to antimicrobial agents varied according to the class of antimicrobial drugs. Only 16.6% of strains were sensitive to ampicillin, 25% to ceftriaxone, ceftazidime, gentamicin and kanamycin. Most of the strains were sensitive to ciprofloxacin and to imipenem (83.3% and 91.6% respectively).

Key-words: *Acinetobacter*, strains, biochemical characteristics, susceptibility, antibiotics

Rezumat. Tulpini de *A. baumannii* și *A. lwoffii*, izolate din probe clinice și probe din mediul ambiant din unități spitalicești, au fost investigate privind comportamentul biochimic și sensibilitatea la agenți antimicrobieni. Tulpinile de coccobacili Gram-negativi, nonfermentativi, au fost identificate ca *A. baumannii* și *A. lwoffii* pe baza testelor catalază pozitivă, oxidază negativă, imobilitate, testul oxidare/fermentare pe mediul Hugh-Leifson și activitatea pe diferite substraturi nutritive. Testarea sensibilității la substanțe antimicrobiene a evidențiat procentaje variabile de sensibilitate/rezistență, în funcție de clasele de antibiotice. Numai 16,6% din tulpini au fost sensibile la ampicilină, 25% sensibile la ceftriaxonă, ceftazidim, gentamicină și kanamicină. 83,3% și 91,6% dintre tulpini au fost sensibile la ciprofloxacin și, respectiv la imipenem.

Cuvinte cheie: *Acinetobacter*, tulpini, caractere biochimice, sensibilitate, antibiotice

INTRODUCTION

Bacteria classified as members of the genus *Acinetobacter* have suffered a long history of taxonomic changes. The genus *Acinetobacter* is now defined as including gram-negative coccobacilli, with a DNA G+C content of 39 to 47 mol%, that are strictly aerobic, nonmotile, catalase positive and oxidase negative (1,2). Studies have revealed that the genus consists

of at least 12 DNA hybridization groups which are known as genospecies. Genospecies 1 is the type species *A. calcoaceticus* and is isolated from soil. Genospecies 2 is *A. baumannii* and includes those isolates previously referred to as *A. calcoaceticus* var. *anitratum* being the most prevalent ones in human clinical species (2,3). Genospecies 4, 5, 6, 7 and 8 are named *A. haemolyticus*, *A. junii*, *A. johnsonii*,

A. lwoffii respectively. Genospecies 3, 6, 9, 10, 11 and 12 are unnamed (2). In the book of R. Weyant et al., 1996, *Acinetobacter* group was divided into 16 genomospecies (3).

Acinetobacter species play a significant role in the colonization and infection of inpatients. They have been involved in a variety of nosocomial infections, including bacteremia, urinary tract infection and secondary meningitis. The main role as agent of nosocomial pneumonia, particularly ventilator-associated pneumonia was confined to hospital intensive care units. Some rare cases of community-acquired infections caused by *Acinetobacter* sp. have been reported. A propensity to tolerate drying and resistance to multiple classes of antibiotics are the key factors in enabling the organism to survive and spread in the nosocomial environment (1-7). In general, the treatment of infections with *Acinetobacter* sp. is often extremely difficult because of the widespread resistance to the major

groups of antibiotics of these species (1, 8-14).

The aim of our paper was to present the cultural and biochemical characteristics and the sensitivity to antimicrobial agents of some *Acinetobacter* strains which were isolated in our laboratory in the period of 2001-2004.

MATERIAL AND METHODS

A number of 24 Gram-negative strains, nonfermentative coccobacilli, were tested in order to be taxonomic framed. These strains were isolated from clinical and environmental specimens (table 1).

A. baumannii was isolated from skin (4 strains) and cordon (1 strain) of newborn in two large maternities from north-eastern of Romania, nasal swabs of adult patients (2 strains) in dialysis sections, the hand swabs of hospital workers (2 strains) and hospital environment (swabs on surfaces of different machines, wash-hand basins, floors, tables, UV lamps) (8 strains).

Table 1. *Acinetobacter* strains isolated from hospital environment samples

Species	Clinical samples	Environmental samples	Total
<i>Acinetobacter baumannii</i>	9	8	17
<i>Acinetobacter lwoffii</i>	3	4	7
Total	12	12	24

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A. lwoffii strains were also isolated from skin (2 strains) of new-born, nasal swabs of adult patients (1 strain) and from hospital environment (4 strains).

All strains were isolated on usual culture medium. Biochemical capabilities of these organisms were tested on different mediums for proteic, sacharolitic and lipidic metabolism. The oxidation-fermentation (O-F) basal medium with 1% carbohydrate - was used to detect oxidative activity.

The susceptibility against 10 antimicrobial agents was investigated by disc diffusion method according to NCCLS, 1999 guidelines used as the reference method for antimicrobial susceptibility testing (8). The antimicrobial agents were: ampicillin (AMP), cephalotin (CF), ceftriaxone (CTX), ceftazidime (CAZ), gentamycin (GM), kanamycin (KA), chloramphenicol (CH), trimethoprim (TM), ciprofloxacin (CIP), imipenem (IMP).

RESULTS AND DISCUSSION

The nonfermenter isolates were classified in *A. baumannii* and *A. lwoffii* species based on catalase positive, oxidase negative, nonmotile and utilization of many substrates. The both species appear as coccobacilli on Gram stain. *A. baumannii* strains grew well on usual culture mediums and produced colonies by 2-3mm diameter at 18-24 hours. The colonies were comparable to those of enterobacteria. They produced a pale yellow to white-greyish pigment on the solid medium. The colonies were not pigmented when they grew on blood agar.

A. lwoffii produced smaller colonies on usual, non pigmented mediums

In table 2 there are presented the biochemical characteristics of *Acinetobacter* sp. strains which were isolated in our laboratory. The fermentation / oxidation test on Hugh Leifson medium was very important for differentiation between *A. baumannii* and *A. lwoffii*.

A. baumannii strains presented a large metabolic activity. They had the capacity to produce acid from glucose, xylose, galactose, manose, rhamnose and lactose. The production of acid from maltose and urea test are variable reactions. All strains were positive to Simmons citrate. The negative reactions: the acid production from manitol and sucrose, esculin hydrolysis, H₂S on TSI, nitrate reduction, methyl red and Voges-Proskauer.

A. lwoffii strains had a limited metabolic activity. They were catalase positive and had negative reactions for more substrates. The classification in the species of *Acinetobacter* group is often difficult to many microbiological laboratories due to the necessity of additional tests. At the same time, it is now generally accepted that nucleic acid hybridization and sequencing studies provide the best available and most rational methods for designating species and determination relationships between different organisms (1-4). On the basis of the DNA relatedness criteria 19 DNA-DNA homology groups – genomic species have been recognized within the genus. Seven of the genomic species have been given species names.

Table 2. The biochemical characteristics of *Acinetobacter* sp. strains

Test, substrate	<i>A.baumannii</i> (n=17)	<i>A.lwoffii</i> (n=7)
Morphology	coccobacilli	coccobacilli
Motility	nonmotile	nonmotile
Fermentative or oxidative	O	NO
Catalase	+	+
Oxidase	-	-
Growth on		
MacConkey agar	+	+
SS agar	V	V
Acid from:		
Glucose	+	-
Xylose	+	-
Mannitol	-	-
Sucrose	-	-
Galactose	+	-
Manose	+	-
Rhamnose	+	-
Lactose	+	-
Maltose	V	-
Esculin hydrolysis	-	-
TSI acid:		
Slant	-	-
Butt	-	-
H ₂ S: on TSI	-	-
Simmons citrate	+	V
Urea, Christensen	V	-
Nitrate reduction	-	-
Methyl red	-	-
Voges-Proskauer	-	-

Key reactions: O= oxidative; NO = nonoxidizer; + = positive reaction;
- = negative reactions; V= variable reactions.

Groups 1, 2, 3 and 13 TU have an extremely close relationship and are referred to some groups as the *A. calcoaceticus* – *A. baumannii* complex (1). Recent genetic studies have identified at least 16 separate *Acinetobacter* genomospecies. Table 3 shows some data concerning classification of *Acinetobacter* species and their characteristics. The information on

cellular fatty acid composition facilitates the classification of this group of bacteria (3). *A. baumannii* is the *Acinetobacter* genomic species of greatest clinical importance. Suspicion of infection can be revealed by repeated isolation of another genomic species, especially if clinical symptoms are also present (1-4).

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Table 3. Some common characteristics of *Acinetobacter* genomospecies (Weyant S.R. et al., 1996)

Genomospecies number	Proposed name	Reference strain ^a	Glucose oxidation ^b	Hemolysis	Gelatin	Growth temp(°C)		
						37	41	44
Genomospecies 1	<i>A.calcoaceticus</i>	ATCC23055	+	-	-	+	-	-
Genomospecies 2	<i>A.baumannii</i>	ATCC19606	95	-	-	+	+	+
Genomospecies 3		ATCC17922	+	-	-	+	+	-
Genomospecies 13			+	-	-	+	+	50
Genomospecies 10		ATCC17924	+	-	-	+	-	-
Genomospecies 14 and 13			+	+	75	25	-	-
Genomospecies 15			50	-	-	+	50	-
Genomospecies 14			+	+	+	+	-	-
Genomospecies 4	<i>A.haemolyticum</i>	ATCC 17906	52	+	96	+	-	-
Genomospecies 6		ATCC17979	66	+	+	+	-	-
Genomospecies 12	<i>A.radioresistens</i> ^c	ATCC43998	33	-	-	+	-	-
Genomospecies 5	<i>A.junii</i>	ATCC17908	-	-	-	+	90	-
Genomospecies 7	<i>A.johnsonii</i>	ATCC17909	-	-	-	-	-	-
Genomospecies 8	<i>A.lwoffii</i>	ATCC15309	-	-	-	+	-	-
Genomospecies 9		ATCC9957	-	-	-	+	-	-
Genomospecies 11		ATCC11171	-	-	-	+	-	-
Genomospecies 15			-	+	+	+	-	-
Genomospecies 16			-	+	+	+	-	-
Genomospecies 17			-	+	+	+	-	-

a = Type strain; b = numbers represent percentage of positives as reported by authors; c = this name was proposed by Nishimura et al., 1989; + and - represent 100% positive and 100% negative.

Susceptibility of the studied *Acinetobacter* strains to antimicrobial agents was ranged between 16.6% for AMP and 91.6% for IMP (fig. 1).

Percentages of 20.8% of the strains were sensitive to CF, 25% to CTX, CAZ, GE and KA, and 41.6% to CH and TM, 66.6% to TE. Most of the strains were sensitive to CIP and IMP (83.3 % and 91.6% respectively). Other studies have shown a larger resistance of *Acinetobacter* sp. strains. More recently, the resistance to multiple antibiotics and to IMP has been increasingly. High resistance

percentages of *A. baumannii* strains to IMP were reported by Joshi et al., and Taneja et al. (29% and 36.4% respectively). Bayuga et al., reported 45% multiresistant *A. baumannii* strains and Joshi et al. showed that 75% of the isolates were multidrug resistant and more than 70% were lactamases-producers (9-16). It is very important to test the susceptibility of *A. baumannii* strains to antibiotics due to special properties of these species for rapid developing multiple anti-microbial resistances (1, 9-16).

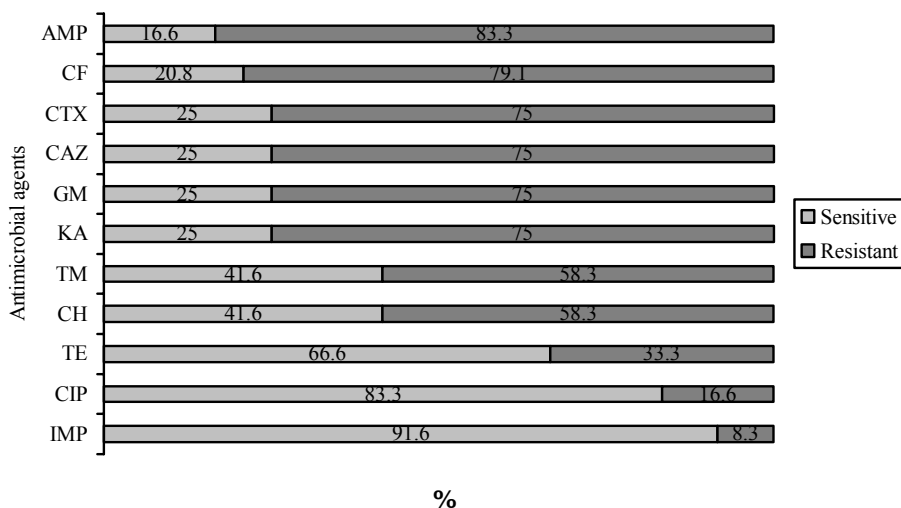


Fig.1 Percentages of sensitive/resistant *Acinetobacter* sp. strains

The *Acinetobacter* species presence in hospital environment is of special importance. These organisms are often transferred from the hands and nostrils of health workers of hospital personnel to patients and result in significant morbidity, especially in intensive care and rehabilitation units (2,4-7). It was demonstrated that the hands of medical staff and the surface area can be an important source during nosocomial outbreaks (1,5-7).

Additionally, a variety of support care equipment, such as respirators and instruments used for invasive procedures, can be contaminated with *Acinetobacter* and continue to be a source for outbreaks of infection (4-7). In some institutions, where epidemic infections with *Acinetobacter* sp. were circumscribed, the common contaminated objects in the environment could usually be identified as the possible way

transmission as well as personnel's hands. In these cases, the implementation of standard principles for preventing hospital – acquired infections will result in the prompt eradication of the outbreak. In other hospitals, infections have become endemic, and the clinical and microbiological epidemiology of these infections remain obscure (7,10).

CONCLUSIONS

24 strains of Gram-negative, non-fermentative coccobacilli isolated from clinical and environmental specimens were taxonomic framed in *Acinetobacter* genus.

The strains were classified as *A. baumannii* (17 strains) and *A. lwoffii* (7 strains) based on catalase positive, oxidase negative, nonmotile, fermentative/oxidative test and the utilization of many substrates.

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The percentages of susceptibility to antimicrobial agents varied depending on the class of antimicrobial drugs. Only 16.6% of strains were sensitive to AMP, 20.8% to CF, 25% to CTX, CAZ, GE and KA, and 41.6% to CH and TM. A higher percentage of the strains were sensitive to TE (66.6%). Most of the strains were sensitive to CIP and to IMP (83.3% and 91.6%). It is very important to test the susceptibility of *A. baumannii* strains to antibiotics because of the special property of these species in developing rapid multiple resistances to antimicrobial agents.

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