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Original Contribution

EPIDEMIOLOGY OF ACINETOBACTER BAUMANNII INFECTIONS IN MULTIPROFILE HOSPITAL

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ABSTRACT
Nineteen non duplicate A.baumannii strains, isolated from different clinical samples and samples, from the environment in ICU at Military Medical Academy /MMA/, Sofia, were investigated. The strains were estimated as phenotipicaly equal according to their biochemical characteristic and the resistance' pattern to antimicrobial drugs. The data, obtained by RAPD PCR with DAF 4 primer show 7 discrete clusters / A- G / of strains formed according to their genotype. Group B includes 9 A.baumannii strains. Two of the strains / NN 2,3 / are isolated from nebulizers of oxygen providing system in ICU, six strains from tracheal secretions also from patients in ICU and one strain / N 11 / from drainage in patient originating from chest surgery. The generated PCR fingerprinting demonstrates that the strains investigated are probably clonal related that means that the nebulisers are possible source of infection and that probably an epidemic strains can spread between the patients in different units in the hospital.

Key words: Acinetobacter baumannii, epidemiology, PCR fingerprinting, clonal relation

INTRODUCTION

Numerous studies have documented the presence of Acinetobacter spp in the hospital environment, but rates of positive cultures may vary widely, depending on the epidemiological setting. (1). Acinetobacter baumannii /A.baumannii / is now one of the most frequently encountered nosocomial pathogens, especialy in compromised patients with such risk factors as mechanical ventilation, surgery and prolonged stays in the intensive care units – ICU. This microorganism spread easily in the environment of infected or colonized patients and can persist in that units for many days, a factor that may explain their possible clonal propensity for causing extended outbreaks.

This paper was conducted in order to assess the epidemiology of A.baumannii in a big multiprofile hospital as well as to present the significance of some risk factors associated with the possible source of an outbreak.

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MATERIALS AND METHODS

Bacterial isolates

Nineteen non duplicate A.baumannii strains, isolated in October 2007 from different clinical samples and samples, from the environment in ICU at Military Medical Academy /MMA/, Sofia, were investigated **(Table 1)**. The samples were taken according to the programme for control and prevention of nosocomial infections /NI / in the hospital. The identification of the strains was done by automated system VITEC TWO v. 4.1. /Biomerieux/ France and by the schema, developed by us (2). The resistance of A.baumannii strains to antimicrobial drugs was done by automated system VITEC TWO v. 4.1. /Biomerieux/ and disk-diffusion method of (3) according to the recommendations of CLS I 2007. (4)

Table 1. *Origin of A.baumannii strains*

№	*Patient name*	*Lab. N*	*Unit*	*Sample*
1	**Bed 1/5 (after disinfection)**	**11199**	**ICU**	**Hospital environment**
2	**Nebulizer 1/4**	**11200**	**ICU**	**Hospital environment**
3	**Nebulizer 1/3**	**11192**	**ICU**	**Hospital environment**
4	**M.C.N.**	**11955**	**ICU**	**Bronchial/traheal**
5	**I.N.**	**11912**	**Nephrology**	**Urine**
6	**T.V.K.**	**11965**	**ICU**	**Bronchial/traheal**
7	**V.B.D.**	**11957**	**ICU**	**Bronchial/traheal**
8	**S.T.I.**	**11959**	**ICU**	**Bronchial/traheal**
9	**N.M.K.**	**12013**	**ICU**	**Bronchial/traheal**
10	**K.T.G.**	**12016**	**Bile - liver surgery**	**Pleural fluid**
11	**M.A.K.**	**11840**	**Thoracic Surgery**	**drainage**
12	**L.N.I.**	**11746**	**ICU**	**Bronchial/traheal**
13	**V.B.D.**	**12102**	**ICU**	**Bronchial/traheal**
14	**I.V.B.**	**11406**	**ICU**	**Bronchial/traheal**
15	**C.B.I.**	**11487**	**ICU**	**woud**
16	**I.A.I.**	**11408**	**ICU**	**Bronchial/traheal**
17	**R.P.M.**	**11412**	**ICU**	**Bronchial/traheal**
18	**S.D.S.**	**11596**	**ICU**	**Bronchial/traheal**
19	**M.I.V.**	**8859**	**ICU**	**Bronchial/traheal**

ICU – Intensive Care Unit
1 / 4 - room / bed
1 / 3 - room / bed

Polymerase chain reaction /PCR/

DNA for PCR analysis was extracted from the strains by boiling one to three colonies in 100 µl of strile destillate water for 15 min and after that the test tubes were transferred in ice for 1 min. The next step is centrifugation 20 s. at 12000 g. A 2 µl volume of this crude DNA extract was used for each PCR. Reactions were carried out in 25 µl volume, containing 18 µl sterile destilate water, 5 µl of primer DAF 4/ in concentration 5pmol/ µl, 2µl DNA extract and Ready-To-Go PCR beads (0.2ml/tubes/plate 96) Illustra TM/GE Healthcare /USA/ with 2.5units of PuReTaq DNA polymerase. The primer DAF4: 5´-CGG CAG CGC C-3´ was synthesed by MWE- Germany. The PCR was done in thermal cycler of Applied Biosystems 9700 under the following conditions: (5)

For DAF4 primer

Temperature	**Time**	**Cycles**
94°C	2 min.	1 cycle
94°C 50°C 72°C	40 s. 40 s. 40 s.	45 cycles
72°C	5 min.	1 cycle

Products /25 μl/ were analysed by electrophoresis on agarose 1%/w/v gels and stained with ethidium bromide and the bands were examined on a UV transilluminator.

Background

Military Medical Academy /MMA/ in Sofia, Bulgaria is a community hospital with 800 beds. The hospital is a one of the national centers for trauma, respiratory disease, liver transplantation patients treatment. Antibiotic prescription includes all groups of antibiotics together with carbapenems, quionolones, third and forth generations of cephalosporins. Because of that that the MMA is a multiprofile hospital /with several surgery units, two ICU/ it can be a pattern for the etiological structure of nosocomial infections investigation as well as the tendency in the resistance' development in Bulgaria, nevertheless the variety, detected in different regions in the world, countries, hospital to hospital in the same country, reported in this respect.

RESULTS AND DISCUSSION

Hospital outbreaks caused by problematic microorganisms, like multidrug-resistant Acinetobacter baumannii, resulting in increased morbidity and mortality, especially in intensive care units /ICU/, surgical wards in a big hospital complexes, have been reported worldwide (6). Also, there are many reports, showing that persistent hospital environmental contamination with A.baumannii strains, may play an important role in the nosocomial dissemination of these organisms (7, 8). According to Allen, K. et al 1987 (7) an epidemic strain of multiresistant Acinetobacter spp has been shown to survive up to 6 days after inoculation on to dry filter paper, a duration similar to that found with S.aureus, which persisted for 7 days, but significantly greater than the survival times for E.coli and Pseufomonas spp., both of which persisted for 24h or less. Our data for 2007 show that A.baumannii strains takes the first place in the etiological structure of bacterial infections in ICU with 21%. Most of these isolates originated from samples of respiratory system – sputum, bronhial and tracheal secretions. In this sense, predominant number of A.baumannii strains, isolated from different patients in ICU at MMA for the last two years, was the main reason to conduct this study. The data, obtained by us show that 3 / NN 1, 2 and 3 / of the A.baumannii strains were isolated from hospital environment – two of them from nebulizers in ICU **(Fig. 1 and 2)** and one from bed'mattress at the same unit. The other 16 strains originated from clinical samples, predominantly from bronchial/tracheal secretions **(Table 1)**. Very similar data were reported also by Cunha,B. et al 1980 (9), Sheretz,R. et al 1985 (10) and Bergogne-Berezin, E. et al 1996 (1) All of the strains were multiresistant to different groups of antimicrobials, including penicillins / ampicillin/sulbactam, piperacillin/tazobactam/, third generation of cefalosporines, quinolones, carbapenems – both imipenem and meropenem, aminoglicosides. The strains were sensitive only to colistin and tobramycin. All of them were identified by VITEC TWO v. 4.1. /Biomerieux/ system as Acinetobacter baumannii, that means, all of the strains were phenotipicaly equal. Using randomly amplified polymorphic DNA /RAPD/ PCR with DAF4 primer typing we tried to establish the source of transmission and possible clonal relationship between the strains as well. DAF4 has been used as a single primer to determine the relatedness of A. baumannii strains (5, 11). The results, obtained by this way show 7 discrete clusters /A- G/ of strains formed according to their genotype. **(Table 2, Fig. 3-4)**. Group B includes 9 A.baumannii strains. Two of the strains / NN 2,3 / are isolated from nebulizers of oxygen providing system in ICU, six strains from tracheal secretions also from patients in ICU and one strain / N 11 / from drainage in patient originating from chest surgery. The generated PCR fingerprinting demonstrates that the strains investigated are most likely clonal related and that probably an epidemic strains can spread between the patients in different units in the hospital. PCR typing also turn out an important tool for establishing the source / nebulizers / and mode of transmission– by oxygen providing system in ICU of the epidemic strain. The sequencing of the strains and the dendogram, obtained by JL Gala and L.Irenge /preliminary information/ show that the Acinetobacter baumannii strains do not belong to the same clone as they display differences in their 16-23S spacer sequences, but their identity is in the frame between 91 and 100%. **(Fig. 5)**

Fig. 1. Oxygen providing system

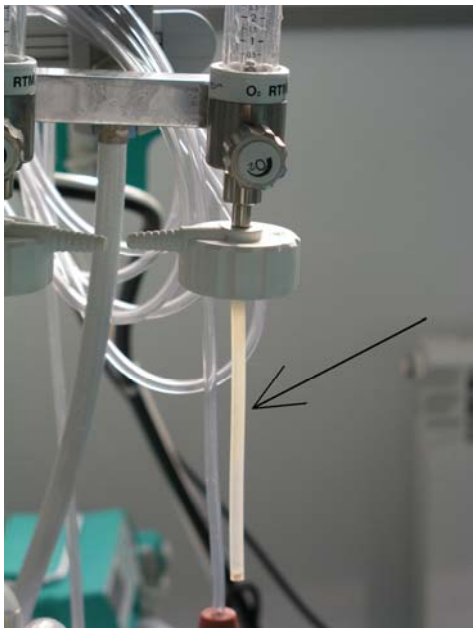

Fig. 2. Nebulizer

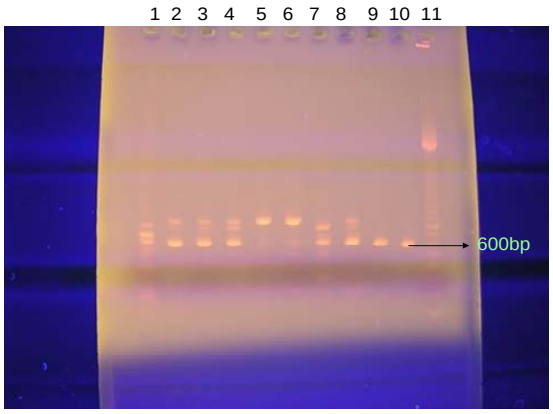

Fig. 3. RAPD-PCR with DAF4 primer
1 -10 (NN of the strains)
11 – 100bp Leader

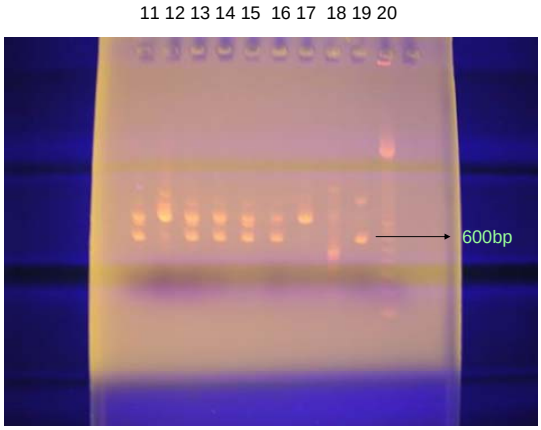

Fig. 4. RAPD-PCR with DAF4 primer
11-19 (NN of the strains)
20 – 100bp Leader

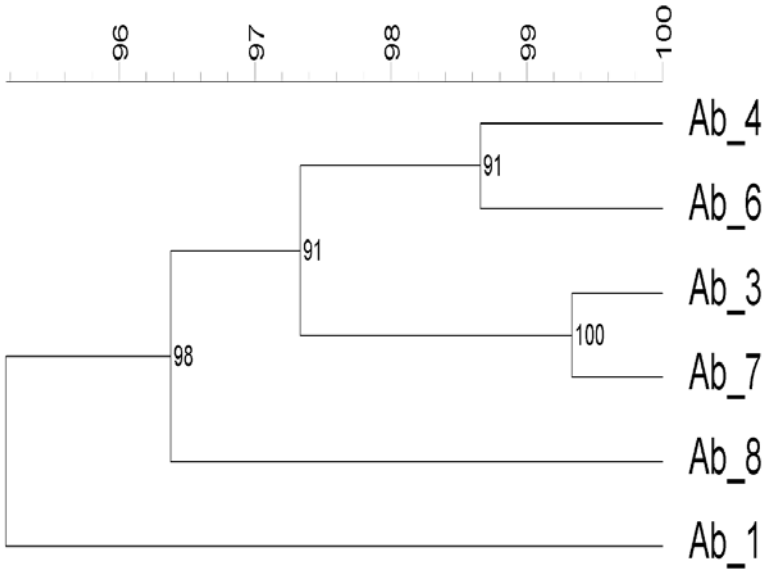

Fig. 5. Dendogram of A.baumannii strains NN 1,3,4,6,7,8 belonging to cluster B

Table 2. *Clusters of A.baumannii strains according to their PCR fingerprinting with DAF4 primer*

Strains №	**Clusters DAF 4**
1,7	**A**
2,3,4,8,11,13,14,15,16	**B**
5,6	**C**
9,10	**D**
12,17	**E**
18	**F**
19	**G**

We can conclude by this way that A.baumannii have unique characteristics among Gram-negative bacteria and now they are renowned for their ability to survive in the hospital environment for different periods of time and environmental contamination represents an important reservoir for their dissemination in patients.

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