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ISOLATION, IDENTIFICATION AND ANTIMICROBIAL SUSCEPTIBILITY OF *BRUCELLA* BLOOD CULTURE ISOLATES

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Abstract: Isolation of slowly growing and fastidious *Brucella spp* strains from clinical specimens is difficult, because of varying factors, including species specificities, stadium of disease, and previous antibiotic treatment of the patients. The use of automated blood culture systems has overcome some cultivation problems. The automated identification system such as VITEK 2 compact allows more precise identification, as well.

Aim: To present our own experience in the isolation of *Brucella* species from blood cultures, by the Bact/Alert automated system, identification by the VITEK 2 compact system and antimicrobial susceptibility of isolated strains.

Material and Methods: Patients from various regions of Macedonia hospitalized in the University Infectious Diseases and Febrile Condition Clinic in Skopje. FAN blood culture bottles (aerobic and anaerobic) of the Bact/Alert system were used, inoculated with 5–10 ml of blood, incubated under continuous agitation and monitored for up to 5 days or until they became positive (in our cases for 2–3 days). Confirmations of all isolates were made by the VITEK 2 automated system on GN cards.

Results: During a period of three years, 113 blood cultures from patients with diagnosis of brucellosis hospitalized at the above-mentioned clinic were examined. A total of 16 blood cultures from different patients were positive (14.2%), showing Gram negative bacilli, oxidase positive small colonies on Columbia agar media. The isolates were identified as four biochemically different types of *B. mellitensis,* mainly within 8 hours. Susceptibility testing by the disk diffusion method on Muller Hinton agar showed sensitivity of all strains to cephalosporin, tetracycline, aminoglycoside and quinolone antibiotic groups.

Conclusion: With the BacT/Alert system *Brucella spp.* were isolated in 14.2% of suspected cases of brucellosis. Isolation was done within 2–3 days. Only *B. meliten-*

sis from the *Brucella* genus could be identified by the VITEK 2 system and some biochemical differences could be detected. The VITEK 2 system is not able to determine the susceptibility of *B. melitensis*. The Disk-diffusion method used in this study showed sensitivity to all tested antibiotics, although not recommended by CLSI for the *Brucella* genus.

Key words: *Brucella*, BacT/Alert blood culture system, VITEK 2 compact system.

Introduction

Brucella is a pathogen of global dispersal. Worldwide, brucellosis affects hundreds of thousands of people and animals each year. At the moment brucellosis is rare in Western Europe and North America since effective public health measures have been implemented. However, such countries are considering *Brucella* to be a potential bioterrorism threat leading to an increased interest in those countries [1]. Brucellosis caused by *B. melitensis* is endemic and specific for our country as well. During the period of 2007, 2008 and the first nine months of 2009, over 1000 cases of human brucellosis were registered in Macedonia (data from the of Public Health Institute, Skopje).

The disease has various clinical manifestations: a variable incubation period, an insidious or abrupt onset, and no pathognomonic symptoms or signs, septicaemia febrile illness or localised infection of bone, tissue, or organ systems in humans [2, 3].

The diagnosis of brucellosis is based on the isolation of bacteria from: blood, bone marrow and other lymphatic tissues or by serology. Isolation of *Brucella spp*. in the clinical laboratory is difficult and risky from the biosafety prerequisites. Primary culturing on a biphasic medium (Castaneda technique) and by white blood cells lysis for concentration of the bacteria have been recommended to improve the recovery of *Brucella spp*. from clinical specimens. All *Brucella spp* are slow-growing and fastidious [2, 4, 5]. The required incubation period lasts from several days to a few weeks [1, 2, 6]. Some factors, such as the nature of *Brucella spp*. and its fastidious growth, its intermittent and low concentration in blood, and previous use of antibiotics, reduce the recovery of *Brucella spp*. from blood cultures. In recent years, the use of automated blood culture systems has become widespread [2, 7]. Automated blood culture systems, such as the BacT/Alert, BACTEC system and other similar systems, seem to shorten the time of detection. These systems have some advantages, such as: early detection of microorganisms, a decrease in contamination risk and reduced labour. The BacT/Alert Microbial Detection System (Organon Technique) was introduced in 1990 as an automated colorimetric blood culture system consisting of standard aerobic (StAe) and anaerobic (Anae) blood culture bottles and

paediatric aerobic bottles (PeAe) containing sensors for detecting microbial growth. In the following years the manufacturer developed FAN-Ae and FAN-Anae (an aerobic and anaerobic medium with a brain–heart infusion base containing Ecosorb). Ecosorb is a proprietary substance of complex composition, containing adsorbent charcoal, Fuller's earth, and other components. FAN media were developed to enhance the recovery of fastidious organisms from blood, as well as to improve the detection of bacteraemia and fungaemia in patients receiving antimicrobial agents [1, 7].

Brucellae are intracellular parasites that infect host macrophage cells [8]. In consequence, specialized agents that are able to penetrate the macrophages and function within their cytoplasm are required for the treatment of brucellosis [7]. Tetracyclines, rifampicin, trimethoprim-sulphamethoxazole (SXT), streptomycin, and other aminoglycosides, separately or in combination, are most commonly used for brucellosis treatment. Fluoroquinolones, and macrolides may serve as an alternative drug choice. In 1986, the WHO released recommendations for the use of doxycycline, combined with either rifampicin or streptomycin for treating human brucellosis. This recommendation is still in force [7, 9, 10].

The aim of this study is to present our own experience in the isolation of *Brucella spp*. from blood cultures, using the Bact/Alert automated system and identifying the isolated strains by the VITEK 2 system as well as the susceptibility of the isolates to some antibiotics.

Material and method

Blood cultures of patients from various regions of Macedonia, hospitalized at the Clinic of Infectious Diseases and Febrile Condition and other University Clinics in Skopje were analyzed. FAN blood culture bottles (aerobic and anaerobic) for the BacT/Alert automated sensor-metric system were used, inoculated with 5–10 ml of patients' blood at the hospital departments. All bottles were incubated under continuous agitation and monitored for up to 5 days or until they became positive, depending on diagnosis. The increased amounts of CO2, produced by the bacterial growth diffuses through a semi-permeable membrane in the base of the culture bottle and reacts with water-generating hydrogen ions. The pH decrease in the bottle results in the colour change of a built-in sensor. Reflectance values from the sensor of each culture bottle are monitored and analysed with a complex algorithm which allows differentiation of microbial from background CO2, produced by other components in the blood. Results for any bottle are presented as individual plots of the reflectance units as a function of incubation time and growth. From the flagged positive bottle a Gram stain was performed, with the subculture on Columbia agar medium, incubated

at 37°C. Conventional microbiological procedures such as colony morphology and standard biochemical tests were made for all the isolates. All the procedures were carried out routinely in conventional laboratory conditions, and safety cabinets were not used. Additional incubation and subcultures on other media (such as *Haemophilus* agar medium Oxoid, UK) were made from the positively flagged bottles from which Columbia agar plates were sterile. Gram negative short bacilli from oxidise and catalase positive small colonies on subcultures of the Columbia agar media were tested for urease and H_2S production. Isolated strains on Columbia agar were identified by the VITEK 2 system, using GN cards.

Susceptibility testing for all isolates was done by a disk-diffusion assay on Mueller–Hinton agar in a rich CO_2 atmosphere and by VITEK 2 GNS cards. The following antibiotic disks (Oxoid, UK) were used: Beta lactames-amoxicillin/clavulanic acid (20 μg/10 μg), piperacillin (75 μg), piperacillin/tazobactam (75 μg/10 μg), imipenem (10 μg), ceftriaxone (30 μg), cefotaxime (30 μg), ceftazidime (30 μg), cefepime (30 μg); Aminoglygosides – gentamicin (15 μg), rifampicin (30 μg), amikacin (30 μg); Fluoroquinolones – pefloxacin (5 μg), ofloxacin (5 μg), ciprofloxacin (5 μg), rifampicin (30 μg); Tetracycline – tetracycline (30 μg); and trimethoprim/sulfamethoxazole (1.25 μg/23.75 μg).

Results

During the period of 2007, 2008 and the first nine months of 2009, a total of 26 984 blood cultures (aerobic, anaerobic or both) were examined at the Institute of Microbiology and Parasitology, Medical Faculty, Skopje using the Bact/Alert system. In the same period a total of 162 patients were hospitalized at the Clinic of Infectious Diseases and Febrile Condition with diagnosis of brucellosis and 113 blood cultures from that group of patients were examined.

Gram negative short bacilli and oxidise positive small colonies on subcultures of the Columbia agar media were detected in 16 blood cultures (14.2%). All the isolates were from aerobic FAN bottles. The blood cultures all originated from different patients (Fig. 1). The incubation period for these bottles ranged from 2 to 3 days, mean time of 2.8 days. A curve of growth shows a slighty and slow increase compared to other Gram positive bacteria and Gram negative enterobactericeae.

All 16 isolates were finally identified by the VITEK 2 compact system. GN cards were used. According to the activities shown and 39 biochemical reactions as well as one external test (oxidaze test) the strains were identified as *Brucella melitensis.* The identification in the VITEK 2 compact system was done in approximately 8 hours for most of the strains [10]; the shortest identification time was 6 hours (for 3 strains) and the longest 10.25 hours (for 3 strains).

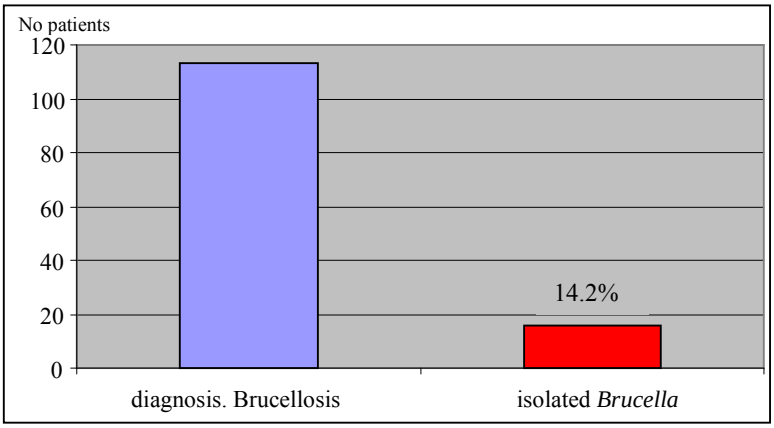

Figure 1 – Isolated strains of Brucella from a total of 113 patients with diagnosis of brucellosis
Слика 1 – Изолирани соеви на Brucella од вкупно 113 пациенти со дијагноза бруцелоза

Summarised results of the biochemical reactions of identified *B. melitensis* strains by VITEK 2 system were presented as bionumbers. All strains could be grouped in 4 biochemical types. Proline Arylamidase, Tyrosine Arylamidase and Urease reactions were positive in all the isolates; Glycine Arylamidase positive in 3 strains, Alpha-Glucosidase in 2 strains and L-Lactate alkalinisation in only one strain. All other tests were negative (Appendix 1).

The results from the disk diffusion method of susceptibility testing showed high level sensitivity to all examined antibiotics: amoxycillin, amoxycillin/clavulanic acid, piperacillin, piperacillin/tazobactam, imipenem, ceftriaxone, cefotaxime, ceftazidime, cefepime, gentamicin, amikacin, rifampicin, pefloxacin, ofloxacin, ciprofloxacin, trimethoprim/sulfamethoxazole and tetracycline. The VITEK 2 system was not able to show the antibiotic susceptibility of the *Brucella* strains with any of the available cards.

Discussion

On the basis of traditional methods and experience, incubation of the cultures for the isolation of *Brucella spp.* has been advocated to continue for 30 days with the aim of maximizing the recovery of these fastidious organisms [2, 4, 11]. Automated blood culture systems provide advantages in the detection of *Brucella spp.* [5, 6, 12]. The mean detection time for *B. melitensis* was 4.5 days

using the BacT/Alert system, and 5 days using the *Brucella* broth method ($P > 0.05$) [13, 14, 15]. Bosch *et al*. [16] observed no significant differences between the BacT/Alert and the *Brucella* broth culture methods with respect to growth time of *B. melitensis*. Prolonged incubation time and periodic performance of subcultures are required to maximize detection of the organism by the BacT/Alert system.

There are a lot of studies on the detection of *Brucella spp*. with automated blood culture systems, but the experience with the BacT/Alert blood culture system for the recovery of *Brucella spp*. is limited [12, 17]. Casas *et al*. [18], using the BacT/Alert system, recovered one of five *Brucella* isolates within 3 days, and the others by subsequent subculture. They suggest that the BacT/Alert system did not solve the problem of the diagnosis of brucellosis, and subcultures were required. T. Hanscheid *et al.* [12] compared the VITAL system with the tryptose broth medium, and found that the latter was more sensitive. They recommended prolongation of incubation times and subculture, as well.

There are no published studies on factors affecting the growth of *Brucella spp*. in the BacT/Alert system. Factors such as sodium polyanethol sulfonate (SPS), used in BACTEC NR 730 automated systems, inhibitsthe growth of bacteria via their harmful effect on the bacterial membrane [19]. Gamazo *et al*. [13] also suggest that the pH of the medium is unsuitable for the growth of *B. melitensis* and, most important, the carbon dioxide release could be undetectable because of the peculiarities of *Brucella* metabolism. Therefore, they claimed that the BACTEC NR automated system is unsuitable for *Brucella spp*.

Low bacterial concentration in brucella bacteremia is also a problem to be solved by blood culture methods. Several studies were performed on the effect of the concentration of an organism on the time for detection of a positive result in the BacT/Alert system. A critical number of bacteria must be reached to generate CO_2 at a rate which is recognized by the BacT/Alert and a longer time was required for the lower initial concentrations of the organism. An inverse linear relationship was demonstrated between the log of the initial concentration of the organism and the time to detection of a positive result [19]. The mean time for detection was usually 48 h, with a standard deviation of ± 1.0 h [4, 6, 7, 10]. Zimmerman *et al*. [20], using the BACTEC NR 730 automated blood culture system, found that if the concentration was 5–500CFU/mL, *B. melitensis* growth in the automated system would be within 2 days, whereas 5–7 days would be required for detection if the concentration was lower than 5 CFU/mL. Solomon and Jackson [19] detected *B. melitensis* after an incubation period of only 2.8 days, and reported that the growth time of *Brucella* was 48h with a 10CFU/mL concentration of bacteria. Casas *et al.* [18] also detected the organism in 2–3 days in five cases. Gedikoglu *et al.* [21] recovered 30 *B.*

melitensis isolates with the automated system in 4 days of incubation, while Yagupsky *et al.* [22] recovered 15 of 22 *Brucella* isolates in 3 days and Bannatyne *et al*. [23] recovered 93% of 97 isolates in 5 days. All positive blood cultures from our patients were detected by BacT/Alert after a mean incubation period of only 2.8 days (range, 2.7 to 2.9 days). Decreased time for detection has also been observed with a number of other pathogenic organisms (*Staphylococcus aureus, E. coli*, etc) [24].

In contrast to the early and sustained increase in CO2, in production observed in a laboratory with clinical isolates of *Escherichia coli* and *Staphylococcus aureus*, the increase with *B. melitensis* occurred more slowly and was the lowest in magnitude and the briefest in duration. Nevertheless, this pattern was recognized by the BacT/Alert as positive. Despite the subsequent return to the baseline rate of CO2 production, *B. melitensis* remained viable, as demonstrated by terminal subculture [4]. In a lot of studies [4, 7, 20, 25] there is information that when different concentrations of the organism were incubated in the BacT/Alert, the general appearance of the curves of reflectance units versus time were similar in size and shape but differed in the times at which they were recognized as positive by the instrument. The effectiveness of the BacT/Alert in detecting growth in blood cultures seeded with various fastidious microorganisms has been reported [1, 6, 7, 18].

In a study on 6 patients infected with *B. melitensis*, 15 (79%) of 19 blood cultures were positive within 4 to 8 days of inoculation, using the radiometric system [4].

Automated blood systems could be used for the cultivation of microorganisms from primarily sterile specimens. Involvement of the skeletal system and especially septic arthritis occurs in up to 40% of patients with brucellosis [2, 7, 8]. Clinical diagnosis of the disease, however, is frequently difficult because brucellosis may mimic other clinical conditions such as rheumatic disorders [2, 7, 26]. The results of a recent study [6] suggest that the aerobic Peds Plus BACTEC blood culture bottles may be a convenient tool for culturing brucellae from synovial fluid of patients with arthritis. So, the rapid detection of brucellae with the automated system may lead to an earlier diagnosis of *Brucella* arthritis and improve clinical case management.

The identification of bacteria with the VITEK 2 system is mainly done within several hours, from 3–18 hours, faster for the *Enterobacteriacea* family than other fastidious and non-fermentative bacterial genera [27]. The VITEK 2 system identifies only *Brucella melitensis* strains and not the other bacteria from the *Brucella spp.* Nevertheless, all the oxidase positive strains isolated from blood cultures which were not pseudomonades were identified by VITEK 2 in the examined period, so we are sure that other *Brucella* strains were not isolated from the blood cultures. The identification based on 48 biochemical and physio-

logical test reactions is useful for determining the differences in the isolated strains. In our study there were four types of *Brucella melitensis* identified by the VITEK 2 system. Minor differences were detected in Glycine Arylamidase, Alpha-Glucosidase and L-Lactate alkalinisation (GlyA+, AGAL+ and ILATk+) reactions.

Brucella spp. are highly infectious pathogens and level 3 biosafety precautions must be observed during the susceptibility testing procedure [8]. The monitoring of bacterial growth in automated systems is performed by technology that avoids the creation of dangerous aerosols, which is very important for laboratory safety, especially when working with dangerous and transmissible organisms, such as *Brucellae* [2, 6, 23, 26].

Brucella organisms have a capacity for survival inside the macrophages conditions, both the undulant course of the disease and its tendency to relapse [4, 26, 28]. It is very difficult to obtain antibiotics able to eradicate the microorganism from macrophages [6, 14]. The type of antibiotic used for the treatment of brucellosis influences the relapse rate to a large extent. Relapses, at a rate of about 10 percent, usually occur in the first year after infection, but they are caused by inadequate treatment in most cases. Antibiotic-resistant *Brucella* strains are rarely a cause of therapy failure. However, strains resistant to the main antimicrobial agents may emerge and lead to treatment failure [16, 28]. It is clear that synergistic combinations of antibiotics with marked intracellular activity achieve the best results [2, 13, 28]. Nevertheless, the factors determining why some patients relapse and others do not are not well understood. There are no studies up to now investigating whether development of resistance during treatment or even minor differences in antibiotic susceptibility of the initial strains are significant in explaining the clinical outcome. Treatment failure in brucellosis is related to such factors as inappropriate dose, short-term administration, insufficient intracellular penetration of the drug, and poor patient compliance, rather than drug resistance [16]; however, antimicrobial susceptibility testing may be recommended in cases of life-threatening organ involvement (i.e. brucella endocarditis and meningitis) and in the event of treatment failure and relapse [1, 16].

Routine *in vitro* antimicrobial susceptibility testing of *Brucella spp*. is not generally recommended [4, 30, 31, 32]. Such testing carries the risk of intralaboratory infection among laboratory personnel and requires biological safety level 3 precautions [3, 28, 30, 33]. Examination of *in vitro* efficacy of antibiotics against *Brucella spp.* has usually been based on the MIC determination by micro broth dilution, agar dilution, and E test methods. The Disc diffusion method has not been recommended [31, 32]. There is no standardized method for susceptibility testing recommended by CLSI for these microorganisms. *Brucella* agar, Muller-Hinton agar, and Muller-Hinton broth supplemented with 1% Po-

lyvitex, or combined 1% Polyvitex and 1% haemoglobin, and Muller-Hinton agar supplemented with 5% sheep blood agar are the media used for antibiotic susceptibility testing of *Brucella*. Despite all this, we decided to do a disk diffusion test for our isolates, and not the dilution tests because of the lower safety risk in laboratory manipulations in conditions not completed to biosafety level 3 in our laboratory during that period, and also because the VITEK 2 system was not able to determine antimicrobial susceptibility by any of the cards. *Brucella* was one of those bacteria which were not included in the software for antimicrobial susceptibility testing. After getting the obtained results with the identification and susceptibility testing cards we noticed this in the VITEK 2 system Product information.

Conclusion

1. The BacT/Alert automated system with FAN aerobic bottles was able to recover *B. melitensis* from 14.2% blood cultures of suspected human cases of brucellosis. The isolations were done within 2–3 days. The fastidious and highly contagious bacteria are able to grow in the BacT/Alert automated system and bearing this in mind it is very important to use good laboratory safety prerequisites.

2. The VITEK 2 compact system identifies the oxidase positive gram negative rod as *B. melitensis* according to 48 biochemical and physiological tests. Only *B. melitensis* from the genus *Brucella* could be identified by the VITEK 2 system. Some biochemical differences could be detected in the strains of *B. melitensis.*

3. The recommended antibiotic susceptibility methods for testing of *Brucella* strains are micro broth dilution, agar dilution, and E test methods for MIC values. The VITEK 2 system is not able to determine the susceptibility of *B. melitensis* strains. Disk-diffusion tests in this study were done to detect the susceptibility of our isolated strains.

4. All laboratory manipulation for each blood culture bottle must be performed carefully and safely, according to the WHO Laboratory Biosafety Manual, because of the ability of the automated systems to isolate bacteria such as *Brucella* with a low infectious dose and easily aerosolized.

5. All isolates should be confirmed by the World reference laboratory by standard tests or by PCRs.

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Appendix 1 – Додаток 1

Biochemical test included in GN card of VITEK 2 system and Brucella melitensis strains
Биохемиски тестови вклучени во GN картичка на VITEK 2 систем и соеви на Brucella melitensis

	REAKCIJA	**10 strains**	**3 strains**	**2 strains**	**1 strain**
2	Ala-Phe-Pro-ARYLAMIDASE			(+)	(+)
3	ADONITOL				
4	L-Pyrrolydonyl-ARYLAMIDASE				
5	L-ARABITOL				
7	D-CELLOBIOSE				
9	BETA-GALACTOSIDASE				
10	H2S PRODUCTION				
11	BETA-N-ACETYL-GLUCOSAMINIDASE				
12	Glutamyl Arylamilase pNA				
13	D-GLUKOSE				
14	GAMMA-GLUTAMYL-TRANSFERASE				
15	FERMENTATION/GLUKOSE				
17	BETA-GLUKOSIDASE				
18	D-MALTOSE				
19	D-MANNITOL				
20	D-MANNOSE				
21	BETA-XYLOSIDASE				
22	BETA-Alanine arlylamidase pNA				
23	**L-Proline ARYLAMIDASE**	**(+)**	**(+)**	**(+)**	**(+)**
26	LIPASE				
27	PALATINOSE				
29	**Tyrosine ARYLAMILASE**	**(+)**	**(+)**	**(+)**	**(+)**
31	**UREASE**	**(+)**	**(+)**	**(+)**	**(+)**
32	D-SORBITOL				
33	SACCHAROSE/SUCROSE				
34	D-TAGATOSE				
35	D-TREHALOSE				
36	CITRATE (SODIUM)				
37	MALONATE				
39	5-KETO-D-GLUCONATE				
40	**L-LACTATE alkalinisation**				(+)
41	**ALPHA-GLUCOSIDASE**			(+)	(+)
42	SUCCUNATE alkalinisation				
43	Beta-N-ACETYL-GALACTOSAMINIDASE				

	REAKCIJA	**10 strains**	**3 strains**	**2 strains**	**1 strain**
44	ALPHA-GALACTOSIDASE				
45	POSPHATASE				
46	**Glycine ARYLAMIDASE**	(+)		(+)	(+)
47	ORNITHINE DECARBOXYLASE				
48	LYSINE DECARBOXYLASE				
52	DECARBOXYLASE BASE				
53	L-HISTIDINE assimilation				
56	COURMARATE				
57	BETA-GLUCORONIDASE				
58	O/129 RESISTANCE (comp. vibrio.)				
59	Glu-Gly-Arg-ARYLAMIDASE				
61	L-MALATEassimilation				
62	ELLMAN				
64	L-LACTATE assimilation				

Резиме

ИЗОЛАЦИЈА, ИДЕНТИФИКАЦИЈА И АНТИМИКРОБНА ОСЕТЛИВОСТ НА ИЗОЛАТИТЕ НА *BRUCELLA MELITENSIS*

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Апстракт: Изолацијата на бактериите од родот *Brucella* од клинички примероци е тешка и зависи од повеќе фактори кои вклучуваат: специфичност на родот (бавно растечки и чувствителни бактерии), различните стадиуми на болеста која ја предизвикуваат како и од претходниот антибиотски третман на пациентите. Со употреба на автоматизирани системи за обработка на хемокултури се надминуваат голем број проблеми поврзани со нивната култивација и изолација. Автоматизираните апарати, како што е ВИТЕК 2 системот, овозможуваат нивна прецизна идентификација.

Цел: да се прикаже нашето искуство со изолацијата на *Brucella spp.* од хемокултури со помош на автоматизираниот Бакт/Алерт (Bact/Alert) систем и идентификацијата со ВИТЕК 2 компакт системот.

Материјал и методи: Испитани се хемокултури од пациенти кои потекнуваат од различни региони на Македонија, хоспитализирани на Универзитетската клиника за инфективни болести и фебрилни состојби во Скопје. Инокулираните FAN аеробни и анаеробни шишиња со 5–10 мл крв од пациентот, инкубирани се во Bact/Alert апаратот, со континуирано мониторирање и агитирање, во период од 5 дена или сè додека да станат позитивни (во нашиот случај за 2–3 дена). Потврдата на сите изолати е направена со ВИТЕК 2 системот со помош на ГН (GN) картички. Осетливоста е испитана со диск дифузионен метод на Милер-Хинтонов агар.

Резултати: Во период од три години испитани се 113 хемокултури од пациенти со дијагноза бруцелоза. Позитивни беа вкупно 16 хемокултури од различни пациенти. Грам-негативни бацили во препаратот и ситни оксидаза позитивни колонии пораснати на Колумбија агар беа идентифицирани со ВИТЕК 2 системот за 8 часа како *Brucella melitensis* и групирани во четири биохемиски типови. Сите соеви покажаа осетливост кон тестираните антибиотски групи: цефалоспорини, тетрациклини, аминогликозиди и кинолони.

Заклучок: Бакт/Алерт и ВИТЕК 2 компакт апаратите се покажаа успешни во дијагностицирањето на суспектни случаи на бруцелоза кај 14,2% од пациентите. Изолацијата на соевите траеше само 2–3 дена. ВИТЕК 2 системот може да идентификува само *Brucella melitensis*. Со него можат да бидат детектирани неколку биохемиски различни типови. ВИТЕК 2 системот не е способен да ја детерминира осетливоста на соевите на *Brucella melitensis*. Во

оваа студија осетливоста е испитана со диск дифузониот метод и добиените резултати упатуваат на осетливост на соевите кон сите групи антибиотици.

Клучни зборови: *Brucella*, Bact/Alert систем за хемокултури, ВИТЕК 2 компакт систем.

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