IDENTIFICATION OF STAPHYLOCOCCAL ISOLATES FROM BLOOD CULTURES AND INDICATING THE B-LACTAM RESISTANCE PHENOTYPE USING VITEK® 2 COMPACT AUTOMATED SYSTEM

M. COLCIERU [1], E. JAKAB[2], O. POPESCU[3]

[1]C.F. General Hospital Sibiu, [2,3]Babeş-Bolyai University, Cluj-Napoca

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Abstract: *Blood cultures are one of the most important investigation methods in medical bacteriology. Rapid identification of pathogens and determination of the relevant antibiotic sensitivity profiles are an important step for effective treatment with appropriate antimicrobial agent. Using blood cultures the prognosis of patients can be improved, reducing the acquisition of the antibiotic resistance determinants by bacteria and lowering the total cost of healthcare. Systemic staphylococcal infections caused by community-acquired and the hospital-acquired strains are one of the major causes of mortality worldwide (1). In the last twenty years the increased number of invasive procedures, the widespread use of broad-spectrum antimicrobial agents leads to the emergence of coagulase-negative staphylococci, Staphylococcus epidermidis in particular (2). In our days automated bacteriological systems allows rapid detection of microbial growth in blood cultures. The BACTEC® system (Becton Dickinson Instruments Systems, Sparks, USA) detects directly the CO_2 production taking serial samples from the produced gas in the culture bottle (3). The aims of this study are the identification of staphylococcal isolates from blood cultures and the detection of its β-lactam resistance phenotypes with VITEK® 2 COMPACT automated system (bioMerieux, Inc., Hazelwood, USA.) using samples collected during a nearly two-year period in the bacteriology laboratory of the Emergency County Hospital, Sibiu, Romania.*

Cuvinte cheie: *hemocultură, stafilococ, fenotip*

Rezumat: *Hemocultura constituie una din cele mai importante investigaţii în laboratorul de bacteriologie medicală. Identificarea rapidă a patogenilor cu precizarea speciei şi determinarea profilului de sensibilitate la antibiotice este importantă, bolnavii putând fi trataţi eficient cu agentul antimicrobian corespunzător. Prognosticul pacienţilor poate fi îmbunătăţit, câştigarea rezistenţei de către patogen poate fi redusă, iar costul îngrijirii va fi mai redus. Infecţiile sistemice cu stafilococi reprezintă o cauză majoră a mortalităţii atât datorită tulpinilor dobândite în comunitate cât şi celor intraspitaliceşti.(1) În ultimii douăzeci de ani creşterea numărului de proceduri invazive, folosirea antibioticelor cu spectru larg, a permis emergenţa stafilococilor coagulazo-negativi şi în special Staphylococcus epidermidis.(2) La ora actuală există sisteme automate ce permit detecţia rapidă a creşterii bacteriene în hemoculturi. Sistemul BACTEC® (Becton Dickinson Instruments Systems, Sparks, SUA) permite detecţia directă a producerii CO_2 prin prelevare seriată de eşantioane de gaz din flaconul de cultură.(3) Studiul de faţă îşi propune să prezinte identificarea tulpinilor stafilococice din hemoculturi şi totodată precizarea fenotipurilor de rezistenţă la β-lactami utilizând sistemul automat VITEK® 2 COMPACT (bioMerieux Inc., Hazelwood, SUA) pe o perioadă de aproximativ 2 ani, date obţinute în laboratorul de bacteriologie al Spitalului Clinic Judeţean de Urgenţă Sibiu.*

INTRODUCTION

Blood cultures are one of the most important investigation methods in medical bacteriology. Rapid identification of pathogens and determination of the relevant antibiotic sensitivity profiles are an important step for effective treatment with appropriate antimicrobial agent. Using blood cultures the prognosis of patients can be improved, reducing the acquisition of the antibiotic resistance determinants by bacteria and lowering the total cost of healthcare.

PURPOSE OF THE STUDY

The aims of this study are the identification of staphylococcal isolates from blood cultures and the detection of its β-lactam resistance phenotypes with VITEK® 2 COMPACT automated system (*bioMerieux, Inc., Hazelwood, USA.*).

MATERIAL AND METHOD

Biological samples

The blood samples taken from patients in sterile conditions were inoculated in special bottles containing nutrient medium for blood cultures.

Bacterial strains

The strains used in this study were collected in the bacteriology laboratory of the Emergency County Hospital, Sibiu, and were obtained from patients hospitalized in different departments of the hospital. Study period extends from November 2007 to August 2009. Positive cultures detected with BACTEC® 9050 equipment were processed further using VITEK® 2 COMPACT automated system. Only data related to the *Staphylococcus* genus were processed further.

[1]Corresponding Author: M. Colcieru, General C.F. Hospital of Sibiu, 20, C-tin Noica street, Sibiu, România, e-mail:_mcolcieru@yahoo.com @yahoo.com, tel. +40-0723 809490

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Medical devices

The BACTEC® 9050 equipment is designed for rapid detection of bacteria and fungi in clinical blood cultures. If microorganisms are present in the culture tube, they metabolize nutrients from the culture medium, releasing CO_2. The sensor of the equipment contains a dye that reacts with the produced CO_2, modulating the amount of light that can be absorbed by the fluorescent material of the sensor. Photosensitive detectors measure the fluorescence corresponding to the amount of CO_2 released by the bacteria. Cycle test is performed every ten minutes. Positive cultures are immediately marked by an indicator light, and an alarm, being displayed on the screen.

The VITEK® 2 COMPACT automated system performs within few hours the identification and antibiotic susceptibility testing of the germs. Colorimetric comparison of reading cards allows identification of bacterial strains; the turbidimetric method is used for antibiotic susceptibility testing. In the presence of antimicrobial agents the system evaluates the growth pattern for each microorganism, comparing them with a control growth-well. It use several parameters based on observed growth characteristics to ensure the adequate data for MIC (minimum inhibitory concentration) determination. MIC results must be associated with the identification data for a correct diagnostic (4).

In the initial phases of the study AST-P535 cards were used to determine antibiotic resistance; oxacillin MIC determination values are between 0.50 µg/ml and 8.00 µg/ml. Besides the MIC test, oxacillin screening predicts the nature of the strain (sensitive or resistant). The results MIC tests were correlated with those obtained using agar-screen method.

Lately cefoxitin containing AST-P554 cards were used to predict *mecA*-mediated oxacillin-resistance. This method is based on cefoxitin disk screening tests. The combined results of cefoxitin screening and oxacilin tests were used to determine the oxacillin resistance. Table 1 presents oxacillin MIC values of VITEK® 2 COMPACT cards used in case of staphylococcal strains (5).

Table no. 1. Oxacillin MIC values of VITEK® 2 COMPACT cards used in case of staphylococcal strains

Strain	Oxacillin MIC (µg/ml)	Interpretation
S. aureus	≤ 2	S
	≥ 4	R
Coagulase-negative *Staphylococci*	≤ 0,25	S
	≥ 0,5	R

RESULTS

During this study a total of 62 *Staphylococcus* strains were isolated: 24 strains (38.70%) were isolated from male patients and the rest of 38 strains (61.29%) from women.

The calculated average age was 55.77 years. We can affirm that elderly people are more susceptible for staphylococcal infections, possibly due to gradual decrease of immunity with age.

Initial diagnoses of patients identified with staphylococcal infections are: febrile syndrome (n=22), pneumopathy (n=11), peritonitis (n=10), endocarditis (n=4) and urinary tract infections (n=3). The remaining 12 strains were isolated from patients suffering from other disorders, like: thrombophlebitis, various abscesses, tumours, varicose ulcers, psoriasis, etc. (Figure 1).

The total of 62 strains were identified using VITEK® 2 COMPACT automated system (Table 2).

A total of 7 MRSA (methicillin-resistant *S. aureus*) strains and 36 coagulase-negative oxacillin resistant staphylococcal strains were detected; 69.35% of the total isolated strains were resistant to oxacillin.

Figure no. 1. Etiological distribution of the isolated staphylococcal strains

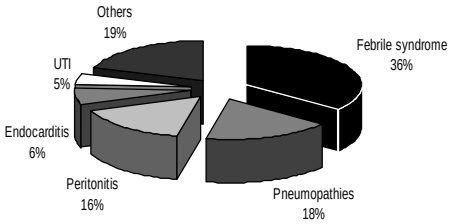

Table no. 2. The identified staphylococcal strains and their share relative to total number of isolated strains

Nr. crt.	Species	No. of strains	Share %
1	*S. epidermidis*	24	38.71
2	*S. aureus*	17	27.42
3	*S. haemolyticus*	8	12.90
4	*S. hominis*	7	11.29
5	*S. warneri*	2	3.23
6	*S. saprophyticus*	1	1.61
7	*S. capitis*	1	1.61
8	*S. auricularis*	1	1.61
9	*S. cohnii*	1	1.61

There are several β-lactam resistance mechanisms. The most common is the macromolecular target change mechanism. In this case the bacteria produce a modified type of penicillin binding protein, called PBP 2a (PBP 2'), which has a low affinity to all β-lactam antibiotics. This modified PBP is encoded by the chromosomal *mecA* gene, which is a part of a supplementary DNA fragment integrated into the bacterial DNA (6).

Currently are described three non-*mecA* related oxacillin-resistance mechanisms. The borderline resistant *S. aureus* (BORSA) strains are β-lactamase overproducers and they lack intrinsic resistance mechanisms (did not produce PBP 2a).

In the absence of *mecA* gene some strains produce a predicted methicillinase enzyme which could hydrolyse methicillin, but its gene were not yet described.

Modification of other penicillin binding proteins leads to modificated *S. aureus* (MODSA) strains. These strains have low level resistance to oxacilin and did not produce β-lactamase (7).

Table 3. presents the acquired β-lactam-resistance phenotypes of staphylococci.

Advanced Expert System (AES) were used for analysis data management and also for validation of results. Using this system, antibiotic resistance patterns were obtained indicating specific phenotypes.

Table 4 presents the β-lactam-resistance phenotypes of the tested 62 strains. Wild-type phenotype is defined as the phenotype of the strains prior to any mutation of chromosome or acquisition of DNA, that might affect susceptibility to antibiotics. The results show that from a total of 24 *S. epidermidis* strains, 21 contained modified PBP and two of them acquired penicillinase enzyme. Only one strain shows the wild phenotype. A total of 17 *S. aureus* strains were found: seven

strains contained modified PBP, nine of them acquired penicillinase and one showed the wild phenotype. Seven modified PBP producing *S. haemolyticus* strains were identified and only one showed the wild phenotype. In case of *S. hominis* six strains contained modified PBP and only one the wild phenotype.

Table no. 3. Acquired β-lactam-resistance phenotypes of staphylococci (S – sensitive, R - resistant)

Mechanism	Penicillin G, penicillin A, carboxipenicillins, ureidopenicillins	Antibiotic combined with β-lactamase inhibitor	Penicillin M	Cephalosporins, carbapenems
Wild	S	S	S	S
Penicillinase	R	S	S	S
PBP modification, *mecA* gene	R	R	R	R
BORSA (rarely)	R	S/R	R	S
MODSA (rarely)	S	S	R	S

Table no. 4. Phenotypes of resistance to β-lactams for the 62 identified strains

Strains (no. strains)	Phenotype of resistance to β-lactams (no. strains)
S. epidermidis (24)	PBP modification(21), Acquired penicillinase (2), Wild (1)
S. aureus (17)	PBP modification (7), Acquired penicillinase (9), Wild (1)
S. haemolyticus (8)	PBP modification (7), Wild (1)
S. hominis (7)	PBP modification (6), Acquired penicillinase (1)
S. warneri (2)	Acquired penicillinase
S. saprophyticus (1)	Acquired penicillinase
S. capitis (1)	Acquired penicillinase (1)
S. auricularis (1)	PBP modification (1)
S. cohnii (1)	PBP modification (1)

CONCLUSIONS

People with relatively advanced age are more susceptible staphylococcal infections due to gradual decrease of immunity with age. This kind of infections is frequently associated with febrile syndrome.

The most frequently isolated strain was *S. epidermidis*, followed by *S. aureus*. Share of oxacillin resistant strains was 69.35%, while the sensitive strains were represented by 30.65% of the isolates. We found an unexpected ratio between oxacillin-resistant coagulase-negative staphylococcus strains (36) and MRSA strains (7).

Predominant phenotype were the modified PBP, followed by acquisition of penicillinase and only a few strains showed the wild type phenotype.

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REFERENCES

1. Diekema DJ, Pfaller MA, Schimtz FJ, Smayevsky J, Bell J, Jones RN, Beach M. Survey of infections due to Staphylococcus species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe and Western Pacific region for the Sentry Antimicrobial Surveillance program, 1997–1999. Clin. Infect. Dis. 2001; 32:S114–S132.
2. Kloos WE, Bannerman TL. Update on clinical significance of coagulase-negative staphylococci. Clin. Microbiol. Rev. 1994; 7:117–140.
3. Diederen BM, Zieltjens M, Wetten H, Buiting AG. Identification and susceptibility testing of Staphylococcus aureus by direct inoculation from positive BACTEC blood culture bottles. Clin Microbiol Infect. 2006; 12:84-86.
4. Ligozzi M, Bernini C, Bonora MG, De Fatima M, Zuliani J, Fontana R. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J. Clin. Microbiol. 2002; 40:1681–1686.
5. Informaţii referitoare la produsul VITEK® 2 Sistems. bioMérieux. 2009.
6. Buiuc D, Neguţ M. Tratat de microbiologie clinică. Editura Medicală. Bucureşti. 2008. pp. 472-474.
7. Jehl F, Chomarat M, Weber M, Gerard A. De l'antibiogramme à la prescription. Édition bioMérieux. 2003. pp. 80-81