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Staphylococcus species and their Methicillin-Resistance in 7424 Blood Cultures for Suspected Bloodstream Infections

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Abstract
Objectives: The aim of this study was to evaluate the distribution of *Staphylococcus* species in bloodstream infections and to assess their susceptibility to methicillin. *Material and Methods:* Between January 1st 2008 - December 31st 2010, 7424 blood culture sets were submitted to the Laboratory Department of the Hospital for Clinical Infectious Diseases in Cluj-Napoca, Romania. The blood cultures were performed using BacT/Alert until January 2010 and BacT/Alert 3D automated system (bioMérieux) after that date. The blood culture bottles were incubated at 37°C in a continuously monitoring system for up to 7 days. The strain identifications were performed by conventional methods, ApiStaph galleries and Vitek 2 Compact system. Susceptibility to methicillin was determined by disk diffusion method with cefoxitin disk and by using Vitek 2 Compact system. *Results:* From the total number of performed blood cultures, 568 were positive with *Staphylococcus* species. From 168 bacteriemic episodes 103 were with *Staphylococcus aureus*. Among 65 coagulase-negative staphylococci isolates, *Staphylococcus epidermidis* was the most frequently isolated species (34), followed by *Staphylococcus hominis* (15), *Staphylococcus haemolyticus* (8), *Staphylococcus saprophyticus* (3), *Staphylococcus cohnii* (1), *Staphylococcus auricularis* (1), and 3 strains that were not identified at species level. Methicillin resistance was encountered in 53.40% of *Staphylococcus aureus* strains and in 80% of coagulase-negative staphylococci. *Conclusions:* An important percentage of blood cultures were contaminated with *Staphylococcus* species. The main species identified in true bacteriemia cases were *Staphylococcus aureus* and *Staphylococcus epidermidis*. The percentage of methicillin-resistance, proved to be high not only for coagulase-negative staphylococci but also for *Staphylococcus aureus*.

Keywords: *Staphylococcus*; Bloodstream infection; Methicillin resistance.

Introduction

Blood culture is an important tool to detect bacteriemia despite of numerous false positive results. It remains the "gold standard" to detect bacteriemia [1,2]. The leading cause for false positive results is represented by blood culture contamination, mainly due to patients' skin flora microorganisms introduced during specimen collection. This is the case especially for coagulase-negative staphylococci who are considered the most common isolates from bloodstream samples and the predominant blood culture contaminants [2-5].

Among *Staphylococcus* species, *Staphylococcus aureus* almost always (>90%) represents the source of true infection, unlike coagulase-negative staphylococci (CoNS) which are predominantly blood culture contaminants, but with increasing clinical importance, especially in patients with indwelling medical devices such as central and peripheral catheters, valvular prostheses, artificial heart valves, pace-makers, orthopaedic prostheses, other infections involving biofilm formation on implanted biomaterials, patients with endocarditis and immunocompromised hosts [2,5-9].

The first question when a blood culture appears to be positive should always be whether it's a true or a false bacteriemia? Many studies have been performed, clinical and microbiological guidelines have been proposed aiming to assess positive blood cultures. They suggested clues to identify contaminants and to discern among true bacteriemia, pseudobacteriemia and contamination: the microorganism itself, the number of positive blood culture sets, the number of positive bottles in the sets, growth of the same microorganism as found in the blood if harvested from another normally sterile site, time to growth, quantity of growth, clinical signs of bacteriemia-hyperthermia or hypothermia, hypotension, other laboratory data like leukocyte count with left shift, inflammation markers [2,6,7]. Laboratory criteria suggestive for true bacteriemia comprise a 48 hours-growth, multiple positive blood cultures for the same microorganism and the microorganism itself, whereas prolonged time to positivity, isolation of polimicrobial skin microorganisms, growth during antibiotic treatment and negative subsequent blood cultures suggest contamination [4,10].

Situations where coagulase-negative staphylococci are isolated only from a single blood culture must be assessed with precaution, since these situations may occur in missing bacteriemic episodes. Another potential pitfall is represented by polimicrobial bacteriemia, since multiple microorganisms usually suggest contamination [11].

To reduce contamination rate some measures were proposed: collection from different venipuncture sites preceded by the use of specific antiseptics, avoidance to collect from indwelling intravenous catheters, use of the double-needle technique [12]. Because the most common source of contamination is the skin flora, it is important that all health institutions establish a protocol for skin antisepsis before venipuncture. There are many studies that compare different antiseptic products, but all of them recommend to combine an alcohol (isopropylic/ethylic) with an iodine containing compound (iodine tincture /povidone-iodine), with chlorine peroxide or 0.5% chlorhexidine, instead of the sole use of ethanol 70% [2,13].

Potential altering of clinical significance through contaminated blood cultures has a negative impact on patient management [1]. Accurate identification of contaminants reduces unnecessary costs derived from increased hospitalisation time, antibiotherapy and additional laboratory testing [12,14,15].

Methicillin resistance among *Staphylococcus* species is a really concerning problem in many countries, especially in the MRSA case that is one of the most important cause of antibiotic resistant healthcare infections worldwide, infections that may result in prolonged hospital stay and higher mortality rates [16].

The aim of our study was to evaluate the clinical significance and distribution of *Staphylococcus* species in blood samples from patients with suspected bloodstream infections and to assess microbial susceptibility to methicillin in confirmed bloodstream infections.

Material and Method

Data Collection

Between January 1st 2008 - December 31st 2010, 7424 blood culture sets were submitted to the Laboratory Department of the Hospital for Clinical Infectious Diseases in Cluj-Napoca, Romania. The specimens came from inpatients of medical, surgical, intensive care units and pediatric departments referring to the above mentioned laboratory.

Considering laboratory criteria correlated with clinical findings as well as the presence of predisposing factors like hemodialysis, intravascular catheters, valvular prostheses, artificial heart

valves, by-pass, immunocompromised hosts, all isolates were included into a category: true bacteriemia, unknown clinical significance or contaminated blood cultures. True bacteriemia was considered in (i) cases with two or more positive blood culture sets, growth in 48 hours from collection time, (ii) patients with clinical evidence of infection with or without predisposing factors where just one set of blood culture was collected and was found positive. Repeated isolates from the same patient were excluded if isolation was within 7 days, as it was considered the same bacteriemic episode [10,17]. Positive blood cultures (PBC) were appreciated as contaminated for patients who did not have a clinical course consistent with sepsis with or without predisposing factors, and when the microorganism was isolated after prolonged incubation (more than 48 hours), or just one blood culture set from two or more sets was positive and/or the absence of isolated microorganisms in subsequent cultures. Situations were just one set of blood culture was collected and insufficient data was available to assess clinical significance were considered with unknown clinical significance.

Blood Cultures Procedure

The blood culture sets were performed using BacT/Alert until January 2010, and BacT/Alert 3D automated system (bioMérieux) after that date. Following antisepsis of the bottle membrane with ethanol 70% the blood specimen was inoculated into BacT/Alert bottles in the manner of the producer's recommendations: up to 10 ml for FAN and standard aerobic and anaerobic bottles, up to 4 ml per pediatric bottles. FAN bottles were used for patients who received antibiotics before collection of blood. The blood culture bottles were incubated at 37°C in a continuously monitoring system BacT/Alert and/or BacT/Alert 3D for up to 7 days.

Organism Identification and Susceptibility Testing

The PBC were subcultured on solid selective and nonselective media. Additionally, a Gram stain smear was prepared for each positive bottle. The strain identifications was performed by conventional methods, ApiStaph galleries (bioMérieux) and Vitek 2 Compact system (bioMérieux). Susceptibility to methicillin was determined using Vitek 2 Compact system- while minimal inhibitory concentrations (MICs) were determined between two breakpoints for oxacillin and by Kirby-Bauer disk diffusion method with cefoxitin disc (BioRad) according to CLSI guidelines.

Statistical Methods

Description of data was performed as absolute and relative frequencies, by calculating the distribution percentages and their 95% CI for the following parameters: age category of patient, *Staphylococcus* species, methicillin resistance of staphylococcus strains. The corresponding graphic representation of data was performed using MS Excel.

Results

From the total of 7424 blood culture sets, 1351 (18.20±0.88%) tested positive, and among these, 597 blood culture sets (44.19±2.65%) were positive with *Staphylococcus* species. Some sets contained two different *Staphylococcus* species. The most commonly isolated microorganisms were CoNS (456), *S. aureus* (168), *Streptococcus spp.* (42), and *Escherichia coli* (136) (Figure 1).

Among 455 patients exhibiting positive blood cultures (PBC) with *Staphylococcus* species, 120 (26.37±4.05%) were from pediatric patients (age ≤ 16 years) and 335 from adults (73.63±4.05%). In terms of collected blood culture sets, from 335 adult patients, 129 (38.51±5.21%) had one set collected and 206 (61.49±5.21%) had two or more blood culture sets collected. From 120 pediatric patients, 100 (83.33±6.67%) had one set collected and 20 (16.67±6.67%) had two or more sets collected. In 321 cases blood cultures turned positive within 48 hours (70.55±4.19%, n=455), respectively 137 isolates from 224 contaminants (61.16±6.38%) grew within 48 hours. True bacteriemia was found in 168 cases, 23 pediatric patients and 145 adults. From 224 contaminants, 63 cases were found in pediatric patients and 161 in adults. In many cases it proved impossible to

assess clinical significance, for 28.33±8.06% of pediatric patients and 8.66±3.01% of adults (Table 1).

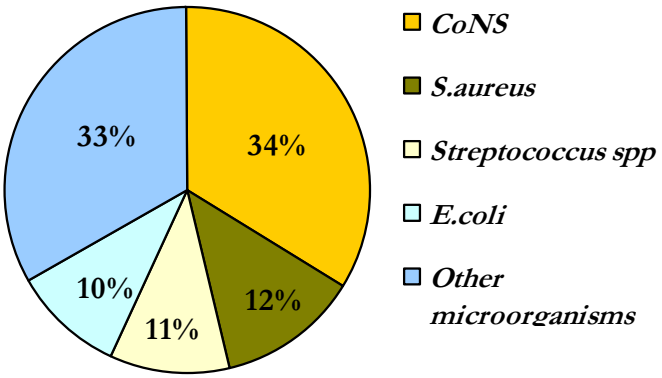

Figure 1. The distribution of the main microorganisms isolated from blood cultures.

Table 1. Distribution of isolated strains by their clinical significance in adult and pediatric patients

Clinical significance	Total (n=455)		Pediatrics (n=120)		Adults (n=335)	
	no	% 95% CI	no	% 95% CI	no	% 95% CI
Clinically significant	168	36.92±4.43	23	19.17±7.04	145	43.28±5.31
Unknown clinical significance	63	13.85±3.17	34	28.33±8.06	29	8.66±3.01
Contaminants	224	49.23±4.59	63	52.5±8.93	161	48.06±5.35

The most commonly isolated species were *S. hominis* (29.23±4.18%), *S. epidermidis* (27.03±4.08%), and *S. aureus* (23.30±3.88%). 7.03±2.35% of isolates were not identified at species level. *Staphylococcus* species distribution in adults and pediatric patients is shown in Table 2.

Table 2. Distribution of isolated strains in adult and pediatric patients

Species	Total (n=455)		Pediatrics (n=120)		Adults (n=335)	
	no	% 95% CI	no	% 95% CI	no	% 95% CI
S. aureus	106	23.30±3.88	14	11.67±5.74	92	27.46±4.78
S. epidermidis	123	27.03±4.08	34	28.33±8.06	89	26.57±4.73
S. hominis	133	29.23±4.18	38	31.67±8.32	95	28.36±4.83
S. haemolyticus	34	7.47±2.42	16	13.33±6.08	18	5.37±2.41
S. saprophyticus	8	1.76±1.21	1	0.83±1.62	7	2.09±1.53
S. cohnii	5	1.10±0.96	1	0.83±1.62	4	1.19±1.16
S. capitis	3	0.66±0.74	1	0.83±1.62	2	0.60±0.83
S. chromogenes	1	0.22±0.43	-	-	1	0.30±0.59
S. simulans	1	0.22±0.43	-	-	1	0.30±0.59
S. auricularis	2	0.44±0.61	-	-	2	0.60±0.83
S. warneri	7	1.54±1.13	4	3.33±3.21	3	0.89±1.01
CoNS	32	7.03±2.35	11	9.17±±5.16	21	6.27±2.6

In a majority of cases (97.17±3.16%), *S. aureus* proved to be clinically significant, except for two cases which were assessed as contaminants, and one case with unknown clinical significance. In 48.79±4.59% (n=455) of cases CoNS proved to be contaminants. From 349 CoNS strains, 63.61±5.05% were contaminants, 18.62±4.08% were clinically significant, and 17.77±4.01% had unknown clinical significance (Figure 2).

For adult patients, among 145 bacteriemic episodes, 62.07±7.9% involved *S. aureus*, 18.62±6.34% *S. epidermidis*, 10.34±4.96% *S. hominis*, 4.83±3.49% *S. haemolyticus*, 1.38±1.9% *S. saprophyticus*, and 0.69±1.35% *S. auricularis*. Also in adult patients, we observed that among 161 blood cultures contaminated with staphylococci, *S. hominis* was the most frequently isolated

(44.10±7.67%), followed by *S. epidermidis* 32.30±7.22%. In unknown clinical significance situations *S. epidermidis*, *S. hominis* and *S. haemolyticus* predominated (Table 3).

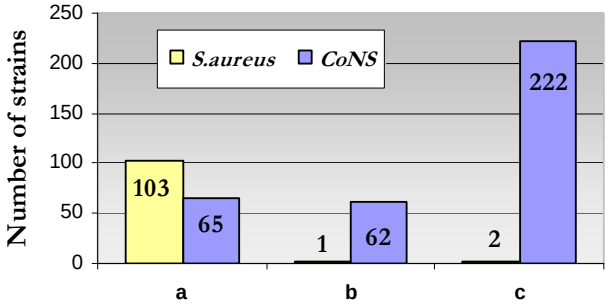

Figure 2. Distribution of *S.aureus* and CoNS strains by their clinical significance: (a) clinically significant. (b) unknown clinical significance. (c) contaminated blood cultures.

Table 3. Distribution of *Staphylococcus* spp. strains, by their clinical significance in adult patients.

Species	Clinically significant (n=145)		Uncertain clinical significance (n=29)		Contaminant (n=161)	
	no	% 95% CI	no	% 95% CI	no	% 95% CI
S. aureus	90	62.07±7.9	1	3.45±6.64	1	0.62±1.21
S. epidermidis	27	18.62±6.34	10	34.48±17.3	52	32.30±7.22
S. hominis	15	10.34±4.96	9	31.03±16.84	71	44.10±7.67
S. haemolyticus	7	4.83±3.49	6	20.69±14.74	5	3.11±2.68
S. saprophyticus	2	1.38±1.9	-	-	5	3.11±2.68
S. cohnii	-	-	-	-	4	2.48±2.4
S. capitis	-	-	-	-	2	1.24±1.71
S. chromogenes	-	-	-	-	1	0.62±1.21
S. simulans	-	-	1	3.45±6.64	-	-
S. auricularis	1	0.69±1.35	-	-	1	0.62±1.21
S. warneri	-	-	1	3.45±6.64	2	1.24±1.71
CoNS	3	2.07±2.32	1	3.45±6.64	17	10.56±4.75

Most episodes of bacteriemia caused by staphylococci in pediatric patients have been shown to be due to *S. aureus* (n=13) and *S. epidermidis* (n=7). Similar to adult patients, we observed that *S. hominis* (38.09±11.99%) predominated as contaminant, followed by *S. epidermidis* (26.98±10.96%) and *S. haemolyticus* (14.29±8.64%), and that in unknown clinical significance situations *S. hominis*, *S. epidermidis* and *S. haemolyticus* predominated (Table 4).

Table 4. Distribution of *Staphylococcus* spp. strains, by their clinical significance in pediatric patients

Species	Clinically significant (n=23)		Uncertain clinical significance (n=34)		Contaminant (n=63)	
	no	% 95% CI	no	% 95% CI	no	% 95% CI
S. aureus	13	56.52±20.26	-	-	1	1.59±3.09
S. epidermidis	7	30.43±18.8	10	29.41±15.32	17	26.98±10.96
S. hominis	-	-	14	41.18±16.54	24	38.09±11.99
S. haemolyticus	1	4.35±8.34	6	17.65±12.82	9	14.29±8.64
S. saprophyticus	1	4.35±8.34	-	-	-	-
S. capitis	-	-	-	-	1	1.59±3.09
S. cohnii	1	4.35±8.34	-	-	-	-
S. warneri	-	-	1	2.94±5.68	3	4.76±5.26
CoNS	-	-	3	8.82±9.53	8	12.70±8.22

In most polimicrobial cases where *Staphylococcus* species were isolated, staphylococci proved to be contaminants. The *Staphylococcus* species most often observed in these situations where: *S. hominis*, *S. epidermidis*. Just in four cases association between *Staphylococcus* species and other bacterial genera proved to be polimicrobial cases with clinical significance: one case with *S.aureus* and *Proteus mirabilis*, two cases with *S. epidermidis* and *Acinetobacter baumanni*, and one case with *Candida albicans* and CoNS unidentified at species level.

From 103 true bacteriemia cases with *S.aureus*, 46.60±9.63% were MSSA, and 53.40±9.62% MRSA. CoNS proved to be clinically significant in 65 cases, 80.00±9.72% of which were MR and 20.00±9.72% MS (Figure 3). Table 5 shows the distribution of methicillin sensitive and methicillin resistant strains in adult and pediatric patients. The number of MR strains by years is represented in Figure 4.

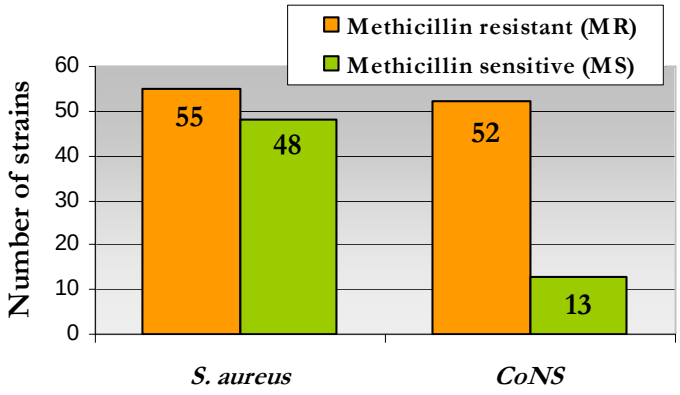

Figure 3. Distribution of MR and MS strains among *Staphylococcus* species.

Table 5. Number of MS and MR strains in adult and pediatric patients

Species	Adult		Pediatrics	
	MS	MR	MS	MR
S.aureus	42	48	6	7
S epidermidis	6	21	1	6
S. hominis	1	14	-	-
S.haemolyticus	-	7	-	1
S. saprophyticus	2	-	1	-
S.auricularis	1	-	-	-
S.cohnii	-	-	-	1
CoNS	1	2	-	-

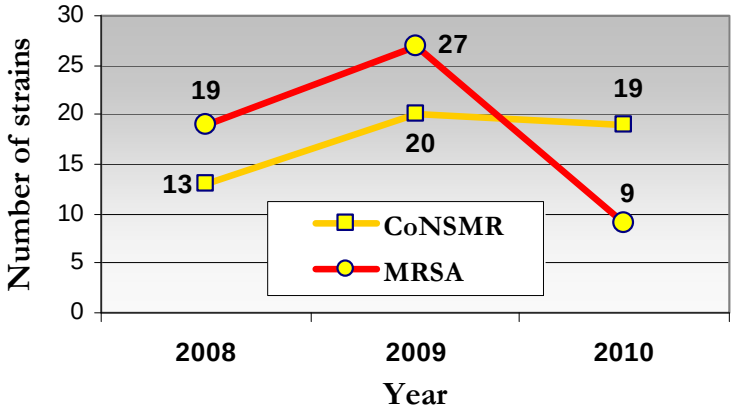

Figure 4. Number of methicillin resistant (MR) strains by years

Discussion

The aim of our study has been reached by evaluating clinical significance and distribution of *Staphylococcus* species in blood samples from patients with suspected bloodstream infections and by assessing microbial susceptibility to methicillin in confirmed bloodstream infections.

We obtained a slightly higher positive isolation rate than has been reported by other studies [6,18]. Many studies showed that *Staphylococcus* species and *E.coli* were the most frequently isolated microorganisms in PBC [1,19-21]. In a SETRY Program study conducted in North America over a period of five years (1997-2002), the occurrence rates of bloodstream infection (BSI) pathogens from among 42,857 episodes has been reported as follows: the most frequently isolated was *S. aureus* (22.9-28.7%), followed by E.coli (16.7-18.6%) and CoNS (9.3-12.9%) [20]. The European Antimicrobial Resistance Surveillance Network (EARS-Net) and the former EARSS, in a study conducted between 2002 and 2009 in 22 countries, reported that BSI caused by *S. aureus* increased by 34% [19].

Unlike CoNS, *S. aureus* almost always represented the true cause of infection [7,12,18,21]. In fact, CoNS were detected in a relatively high percentage of PBC, but in a majority of cases proved being of contaminant nature. As in practice the clinical significance is often difficult to assess, this may explain why a relatively high proportion of these cases were classified as having uncertain clinical significance, especially in pediatric patients where, in many cases, just one set of blood cultures had been collected [3,10,11]. Some authors showed a high prevalence of contaminants among CoNS (over 85%) because they automatically assessed as contaminants the isolates from patients with just a single positive CoNS blood culture [1,14].

In our study, we appreciated CoNS as contaminants in only 63.61±5.05% cases and 17.77±4.01 % as having unknown clinical significance. In terms of CoNS contaminated blood cultures, very similar results have been obtained in the Q-Probes study involving 640 institutions, by Richter et al, and by Mirret, Weinstein et al [3,12,14]. Compared to other studies [6], we noticed a slightly higher percentage of CoNS as true pathogens. Considering the fact that about more than two-thirds of *Staphylococcus* PBC and more than half of *Staphylococcus* contaminated blood cultures had a time of positivity within 48 hours, in many cases we couldn't use this criterion in assessing clinical significance of isolated strains, thus, as also shown by other studies [4,10], the time of growth should not be regarded as a reliable clue for assessing true bacteriemia.

In agreement with the findings of other authors, among CoNS, *S. epidermidis* was all-to-frequently isolated in true bacteriemia cases, not only in adult patients but also in pediatric patients [6,9,22,23].

Clinically significant species other than *S. epidermidis* found in our study have been: *S. hominis*, *S. haemolyticus*, *S. saprophyticus*, *S. cohnii* and *S. auricularis*. *Staphylococcus* species documented as true pathogens in cases of bacteriemia, although occurring with much lower frequency, are: *S. haemolyticus*, *S. saprophyticus*, *S. homnis*, *S. lugdunensis*, *S. capitis*, *S. cohnii*, *S. schleiferi*, *S. simulans*, *S .warneri*

EARS-Net and the former EARSS presented for the period between 2002 and 2009 that the proportion of MRSA in BSI decreased from 21.5% to 19.7% [19]. For Romania, the antimicrobial resistance surveillance in the European Annual Report of the EARS-Net 2009 showed a proportion of MRSA of 35.6% in invasive *S.aureus* isolates and a decreasing trend of MRSA from 2006 to 2009. The report specifies that not all inland laboratories were consistently supported by data reporting for all four years [16].

A study of nosocomial BSI conducted in 49 US hospitals between 1995-2002 showed that 41% of 1699 *S. aureus* isolates were MRSA and 75% of 4946 CoNS isolates were MR [24]. A study conducted in Turkey between 1999-2006 showed that, among 200 CoNS isolates from true bacteriemia cases, 67.5% were MR [23].

Compared to results reported by previous studies, we obtained a higher rate of methicillin resistance among *Staphylococcus* species isolated from BSI. Our study showed more than half of *S. aureus* to be MR and 80±9.72% of CoNS to be MR.

Conclusions

In true bacteriemia cases caused by *Staphylococcus* species, *S. aureus* counted nearly two thirds of all isolates. Amongst CoNS we found a not-to-neglect percentage of true pathogens, almost half of them being *S. epidermidis*. The same *Staphylococcus* species may be found both as true pathogens and as contaminants. A high frequency of methicillin resistance among *Staphylococcus* strains isolated from BSI has been observed in our study. For pediatric patients it was more difficult to assess clinical significance of isolates, since only one blood culture set has been sampled in many cases. Both for adult and pediatric patients, many blood cultures were contaminated with *Staphylococcus* species of the normal skin flora. This finding highly suggests the need for all health institutions to implement protocols for correct blood culture collection in order to reduce contamination rates. It is highly desirable that well-trained technicians collect blood cultures, using a set of appropriate antiseptic products for skin antisepsis before venipuncture. This would reduce the negative impact of blood sample contamination in the clinical management of patients.

Conflict of Interest

The authors declare that they have no conflict of interest.

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