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Etiological factors of bloodstream infections in neutropenic patients

Hanna Połowniak-Pracka, Anna Fuksiewicz, Agnieszka Magdziak, Edyta Zbiciak

Introduction. The purpose of the study was the microbiological analysis of bloodstream infections in patients with neutropenia. Proper epidemiological evaluation should be the base for modifying the outlines of empiric therapy and antibiotic prophylaxis.
Material and methods. Between 1997-2000, 4365 blood cultures obtained from patients hospitalised in the Maria Skłodowska-Curie Memorial Center and Institute of Oncology were analysed in the Department of Clinical Microbiology. Among these – 640 cultures were collected from neutropenic patients. Blood culture techniques: bacterial cultures were performed with the VITAL automated system (bioMérieux), whereas fungi cultures – with the BACTEC 9050 automated system (Becton-Dickinson). Identification of strains: isolated strains were identified by differentiation and identification tests (bioMérieux, Oxoid, Murex, Sanofi-Pasteur) and tests provided by the Center of Microbiological Research and Vaccines in Cracow.
Results. The proportion of positive blood cultures from neutropenic patients to the number of all blood cultures obtained from this group of patients amounted to 29.2%. The proportion of positive blood cultures from the remaining group of patients without symptoms of neutropenia was 10.8%. A total of 152 strains of microorganisms recognised as an etiological factor of infection (reduced isolates) were isolated from neutropenic patients. The most common isolates of gram-positive bacteria were cocci of Staphylococcus species, mainly S. epidermidis (19.1%), S. aureus (11.1%) and S. haemolyticus (6.6%). A high proportion of Staphylococcus isolates were methicillin-resistant. Gram-negative infections were most frequently caused by gram-negative bacilli of Enterobacteriaceae family. The most common isolates were E.coli (12.5%). Nonfermenting bacilli were most frequently represented by Pseudomonas aeruginosa (3.9%). Anaerobic bacteria and yeasts were rarely isolated from neutropenic patients.
Conclusions. 1. Microbiologically confirmed bloodstream infections are observed three times as often in neutropenic patients than in the group of cancer patients without neutropenia. 2. Gram-positive flora, especially cocci of Staphylococcus species, play the most important part in neutropenic patient infections, as well as in the group of other cancer patients. 3. Gram-negative bacilli are mainly represented by Enterobacteriaceae family in infections in both groups of patients. The most common isolates were E. coli. Among nonfermenting bacilli the most common isolates belonged to the P. aeruginosa species. 4. In neutropenic patients infections are usually caused by opportunistic flora. "True" pathogens eg. Streptococcus pyogenes or strains of Salmonella species were rarely isolated. 5. In neutropenic patients we could observe an increasing tendency to develop infections caused by strains of species, in which, potentially, numerous mechanisms of resistance to antibiotics may become active.

Czynniki etiologiczne zakażeń krwi u chorych z neutropenią

Wprowadzenie. Celem pracy była analiza mikrobiologiczna zakażeń krwi u chorych z neutropenią. Prawidłowa ocena epidemiologiczna powinna być podstawą modyfikacji schematów terapii empirycznej i profilaktyki antybiotykowej.
Materiał i metody. Materiał kliniczny. W latach 1997-2000 w Zakładzie Mikrobiologii Klinicznej wykonano 4365 posiewów krwi pobranej od osób hospitalizowanych w Centrum Onkologii-Instytucie im. Marii Skłodowskiej-Curie w Warszawie, w tym 640 próbek pochodziło od pacjentów z neutropenią. Posiew krwi: hodowlę w kierunku bakterii prowadzono w systemie automatycznym VITAL (bioMérieux), natomiast w kierunku grzybów w systemie automatycznym BACTEC 9050 (Becton- Dickinson). Identyfikacja szczepów: do identyfikacji wyhodowanych szczepów stosowane były testy różnicujące i identyfikacyjne firmy bioMérieux, Oxoid, Murex, Sanofi-Pasteur oraz testy Centrum Badań Mikrobiologicznych i Autoszczepionek w Krakowie.
Wyniki. Odsetek dodatnich posiewów krwi u pacjentów z neutropenią w odniesieniu do liczby posiewów wykonanych dla tej grupy chorych wynosił 29,2%. Odsetek dodatnich posiewów krwi w pozostałej grupie pacjentów, nie wykazujących objawów neutropenii, wynosił 10,8%. Z krwi pobranej od pacjentów z neutropenią wyhodowano 152 szczepy drobnoustrojów, uznanych za

Department of Clinical Microbiology
The Maria Skłodowska-Curie Memorial Cancer Center
and Institute of Oncology, Warsaw, Poland

czynnik etiologiczny zakażenia (izolaty zredukowane). W grupie bakterii Gram-dodatnich najczęściej izolowano ziarniaki z rodzaju Staphylococcus, głównie S. epidermidis (19,1%), S. aureus (11,1%), S. haemolyticus (6,6%). Wśród wyhodowanych gronkowców obserwowano wysoki odsetek szczepów metycylinooopornych. Zakażenia krwi o etiologii Gram-ujemnej w większości przypadków spowodowane były pałeczkami z rodziny Enterobacteriaceae. Najczęściej hodowano szczepy z gatunku E. coli (12,5%). Pałeczki niefermentujące najliczniej reprezentowane były przez szczepy z rodzaju Pseudomonas aeruginosa (3,9%). Do rzadko izolowanych z krwi pobranej od pacjentów z neutropenią należały bakterie beztlenowe i grzyby drożdżopodobne. Wnioski. 1. U pacjentów z neutropenią w porównaniu z grupą pozostałych chorych z chorobą nowotworową obserwowano trzykrotnie częściej potwierdzone mikrobiologicznie zakażenie w łożysku naczyniowym. 2. W zakażeniach krwi chorych z neutropenią, podobnie jak w grupie pozostałych osób z chorobą nowotworową, największą rolę odgrywa flora Gram-dodatnia, głównie ziarniaki z rodzaju Staphylococcus. 3. Pałeczki Gram-ujemne w zakażeniach krwi w obydwu grupach chorych reprezentowane są głównie przez drobnoustroje z rodziny Enterobacteriaceae. Najczęściej izolowano szczepy E. coli. Spośród pałeczek niefermentujących najczęściej hodowano szczepy P. aeruginosa. 4. Większość zakażeń krwi u pacjentów z neutropenią spowodowana była florą oportunistyczną. „Prawdziwe" patogeny np. Streptococcus pyogenes czy szczepy z rodzaju Salmonella izolowane były sporadycznie. 5. U chorych z neutropenią obserwowano narastającą tendencję do wystąpienia zakażeń wywołanych szczepami należącymi do gatunków, u których potencjalnie może dochodzić do uruchomienia licznych mechanizmów oporności na antybiotyki.

Key words: neutropenia, bloodstream infections
Słowa kluczowe: neutropenia, zakażenia krwi

Introduction

Cancer is a major factor contributing to the development of infections. The occurrence of chemotherapy-induced neutropenia considerably increases the risk of profound, life-threatening infections [1, 2]. Bloodstream infections are common complications in cancer patients with neutropenia. In over 60% of neutropenic patients fever is observed, and in 20% of the cases this fever is associated with bacteremia [3].

The changes observed over the last years in the epidemiology of infections, including blood infections in neutropenic patients [3-6], make proper evaluation of a current epidemiological situation in a given area necessary. Common occurrence of gram-positive cocci, still common occurrence of gram-negative bacilli and the increasing probability of the occurrence of new pathogens such as *Stenotrophomonas maltophilia, Burkholderia cepacia, Leuconostoc sp.* and others should be a base for modifications in the outlines of empiric therapy, as well as antibiotic prophylaxis [1, 7-11].

Material and methods

Clinical material. Blood samples were collected from adult patients hospitalised in The Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Warsaw (CO) between 1997 and 2000. During this period in the Department of Clinical Microbiology in CO (ZMK) 4365 blood cultures from 396 patients with symptoms of infection were performed, 640 of the specimen were collected from 108 neutropenic patients (neutrophil count of $0.5x10^9$/l) with fever.

Blood culture techniques. Blood specimens (5-10 ml) were inoculated on culture media securing the growth of aerobic and anaerobic bacteria and pathogenic fungi. The cultures for bacteria were performed by VITAL automated system (bioMérieux) on VITAL AER and VITAL ANA culture media, whereas the cultures for fungi were conducted by BACTEC 9050 automated system (Becton-Dickinson) on Bactec-Mycosis-IC/F medium. The duration of cultures varied depending on the rate of growth of isolated microorganisms. Negative blood cultures for bacteria were incubated for 7 days, whereas for fungi the incubation lasted for 14 days.

Positive blood cultures testing. Cultures from VITAL AER medium were inoculated onto Columbia agar plates with 5% sheep blood, chocolate agar, MacConkey solid medium and Chapman medium. Inoculated plates were incubated in aerobic conditions and atmosphere of 5% CO_2 at 37°C for 24-48 h. Cultures from VITAL ANA medium were inoculated onto Columbia agar plates containing 5% sheep blood and vitamin K. Plates were incubated in strictly anaerobic conditions produced by the use of Genebox anaer generators (bioMérieux) at 37°C for 48-72 h. In each case aerobic control on Columbia agar medium containing 5% sheep blood was performed.

Identification of strains. Identification of isolated species was made by use of bioMérieux differentiation and identification tests (API tests, ID tests for automated system ATB, GPI and GNI identification cards for automated system Vitek), Murex, Oxoid and Sanofi-Pasteur tests and tests provided by The Center of Microbiological Research and Vaccines in Cracow.

Methicillin resistance testing. Resistance to methicillin in Staphylococcus strains was determined by a screening method on Mueller-Hinton medium containing 6 mg/ml oxacillin and 4% added NaCl, by disc-diffusion method using 1 μg oxacillin and by automated system VITEK by use of GPS-10, SG and SD cards.

ESBL production testing. Beta-lactamases (ESBL) production in gram-negative bacilli of *Enterobacteriaceae* family was determined by double disc method according to the NCCLS guidelines [12] as well as by automated system VITEK (GNS-650 card).

Results

Of the total number of blood cultures made in ZMK CO between 1997-2000, 13.5% were positive (number of positive cultures 589) – Figure 1. 560 strains of aerobic and anaerobic bacteria and pathogenic fungi (reduced isolates) were isolated from clinical material. The occurrence of gram-positive bacteria in bloodstream infec-

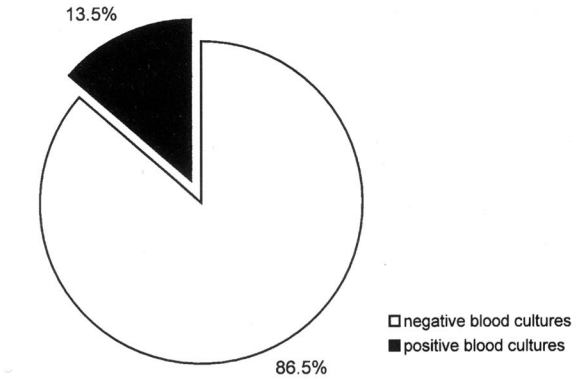

Figure 1. Blood cultures performed in the Department of Clinical Microbiology CO (Cancer Center) between 1997-2000

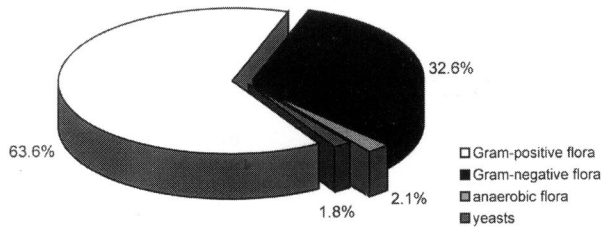

Figure 2. Frequency of occurrence of respective groups of organisms in bloodstream infections in patients hospitalised in CO between 1997-2000

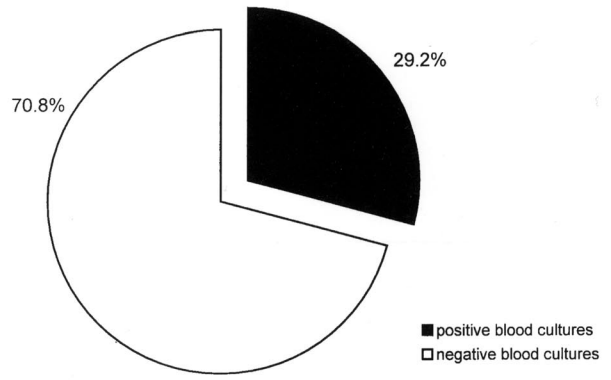

Figure 3. Blood cultures from neutropoenic patients

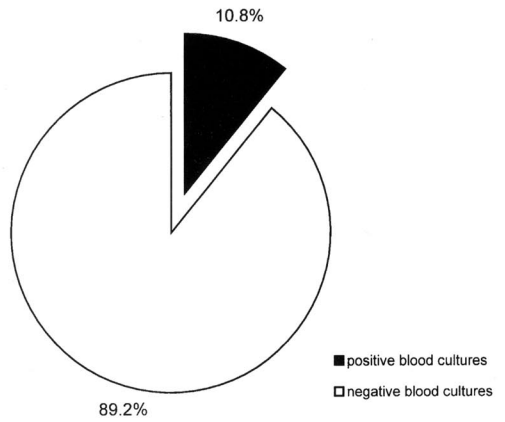

Figure 4. Blood cultures from patients without neutropenia

tions in all patients was 63.6%, of gram-negative bacilli from *Enterobacteriaceae* family and nonfermentors – 32.6%, of anaerobic bacteria – 2.1% and of yeasts – 1.8% – Figure 2.

Among all the blood cultures from neutropenic patients 29.2% were positive (number of positive blood cultures – 187) – Figure 3. In comparison, the proportion of positive blood cultures in the remaining group of cancer patients without neutropenia amounted to 10.8% – Figure 4.

A total of 152 strains recognised as etiological factors of infection (reduced isolates) were isolated from neutropenic patients. The percentage of organisms of respective groups involved in infections is shown in Figure 5. In the cases of bloodstream infections in neutropenic patients gram-positive flora was considerably more frequently encountered (57.7%) than gram-negative bacilli (36.9%) or anaerobic flora (4.1%). Only two strains of yeasts (1.4%) were isolated from neutropenic patients.

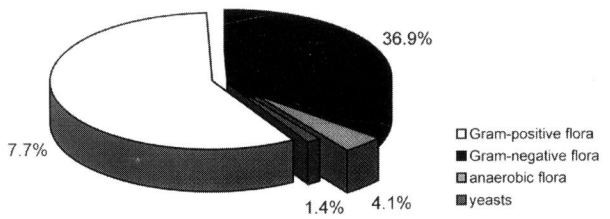

Figure 5. Frequency of occurrence of respective groups of organisms in bloodstream infections in neutropoenic patients

Table 1 shows the species of microbes responsible for bloodstream infections in neutropenic patients. The most common gram-positive isolates were *Staphylococcus* species, mainly *S. epidermidis* (19.1%), *S. aureus* (11.1%) and *S. haemolyticus* (6.6%). Most of Staphylococcus isolates were methicillin-resistant strains – 90% of *S. epidermidis isolates*, 41.2% of *S. aureus isolates* and 80% of *S. haemolyticus* isolates. *Enterococcus* species accounted for 2.7% of infections, with most of them caused by *E. faecium*. Two cases of isolating *Listeria monocytogenes* are worth mentioning.

Gram-negative infections were, in a majority of cases, caused by gram-negative bacilli of *Enterobacteriaceae* family. The most common isolates were *Escherichia coli* (12.5%). *Klebsiella pneumoniae* was isolated in 3.9% of cases (all the strains produced ESBL), *Enterobacter cloacae* in 3.3%. Strains of other Enterobacteriaceae species were rarely isolated. Considerable proportion of nonfermenting bacilli was observed in infections in neutropenic patients. These were most frequently found to be *Pseudomonas aeruginosa* (3.9%). The incidence of *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* isolates is clinically significant due to their multiresistance.

Among the least common isolates from neutropenic patients were anaerobic bacteria and yeasts.

Table I. Microorganisms isolated from neutropenic patients with fever (clinical material collected from CO patients between 1997-2000)

Organism	No of isolates	%
Gram-positive flora		
Staphylococcus epidermidis*	29	19.1
Staphylococcus aureus **	17	11.1
Staphylococcus haemolyticus ***	10	6.6
Other S. coagulase-negative ****	11	7.2
Enterococcus faecium	3	2.0
Enterococcus faecalis	1	0.6
Streptococcus pyogenes	1	0.6
Streptococcus of "oralis" type	10	6.6
Corynebacterium sp.	4	2.6
Listeria monocytogenes	2	1.3
Gram-negative flora		
Escherichia coli	19	12.5
Klebsiella pneumoniae ESBL	6	3.9
Klebsiella oxytoca	1	0.6
Klebsiella ozaenae	1	0.6
Enterobacter cloacae	5	3.3
Pantoea sp.	1	0.6
Citrobacter sp.	2	1.3
Salmonella sp.	1	0.6
Serratia plymuthica	2	1.3
Serratia liquefaciens	1	0.6
Pasteurella sp.	1	0.6
Pseudomonas aeruginosa	6	3.9
Stenotrophomonas maltophilia	2	1.3
Aeromonas hydrophila	2	1.3
Acinetobacter baumannii	3	2.0
Acinetobacter haemolyticus	1	0.6
Acinetobacter junii	1	0.6
Alcaligenes faecalis	1	0.6
Anaerobic flora		
Bacteroides fragilis	1	0.6
Fusobacterium sp.	2	1.3
Propionibacterium acnes	1	0.6
Clostridium perfringens	1	0.6
Gemella haemolysans	1	0.6
Yeasts		
Candida parapsilosis	1	0.6
Malassezia furfur	1	0.6
Total	152	100.0%
	Number of neutropenic patients – 108	

* Percentage of MR S. epidermidis – 90.0%
** Percentage of MR S. aureus – 41.2%
*** Percentage of MR S. haemolyticus – 80.0%
**** Percentage of MR S. coagulase – negative – 90.9%

Figures 6, 7, 8 and 9 show the number of strains of respective species isolated from neutropenic patients compared with the number of strains of the species isolated from all specimens of clinical material.

The percentages of most commonly isolated pathogens from neutropenic patients and cancer patients without neutropenia were compared (Figure 10) In the group of neutropenic patients there is a tendency towards the increasing frequency of the occurrence of gram-negative bacilli such as *E. coli* and *K. pneumoniae* and other potentially multiresistant microorganisms.

Discussion

The analysis of blood culture results from neutropenic cancer patients shows a triple increase in the number of positive blood cultures, as compared with the group of cancer patients without neutropenia [13]. The patterns of distribution of the occurrence of disease-producing flora in bloodstream infections in neutropenic patients and in the other patients are similar. These infections are predominantly caused by gram-positive cocci of *Staphylococcus* species. An alarmingly large number of the strains are methicillin-resistant [1, 9, 14]. Paradoxically, the high frequency of occurrence of gram-positive cocci is related to the developments in anti-cancer treatment. The introduction of invasive diagnostic and therapeutic methods, permanent vascular lines, extensive surgical interventions and radiotherapy allow for the translocation of physiological flora of the skin and contribute to the development of infections [15, 16].

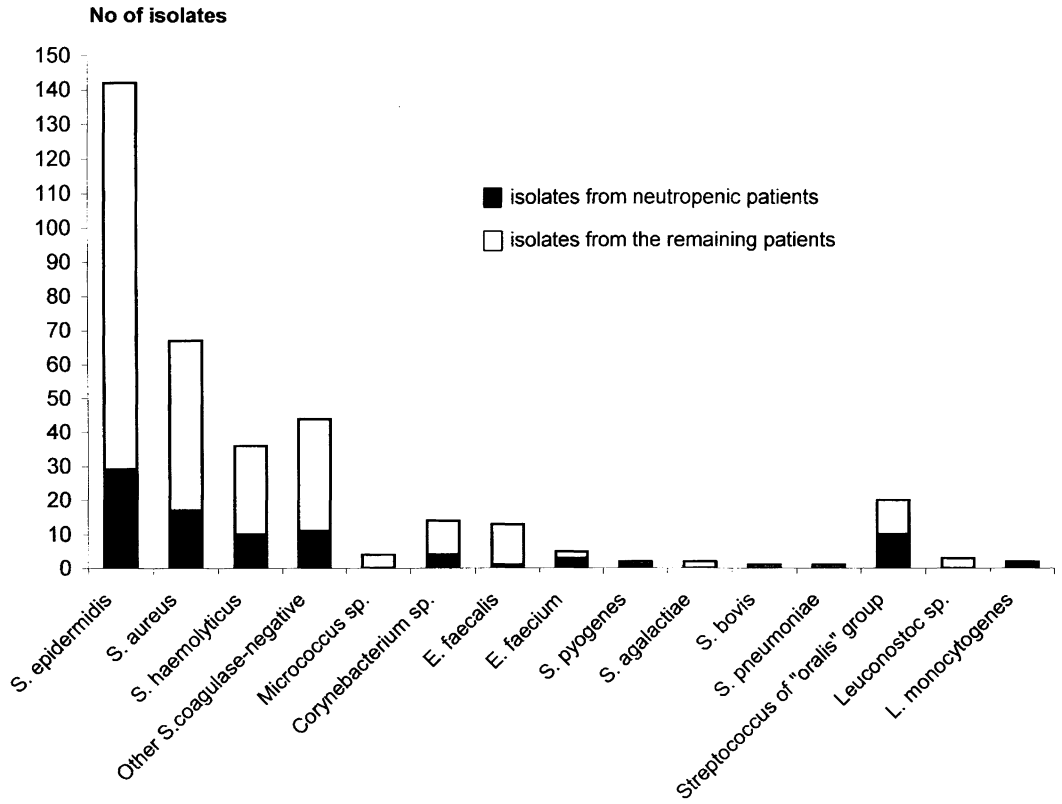

Figure 6. Gram-positive bacteria isolated from CO patients between 1997-2000

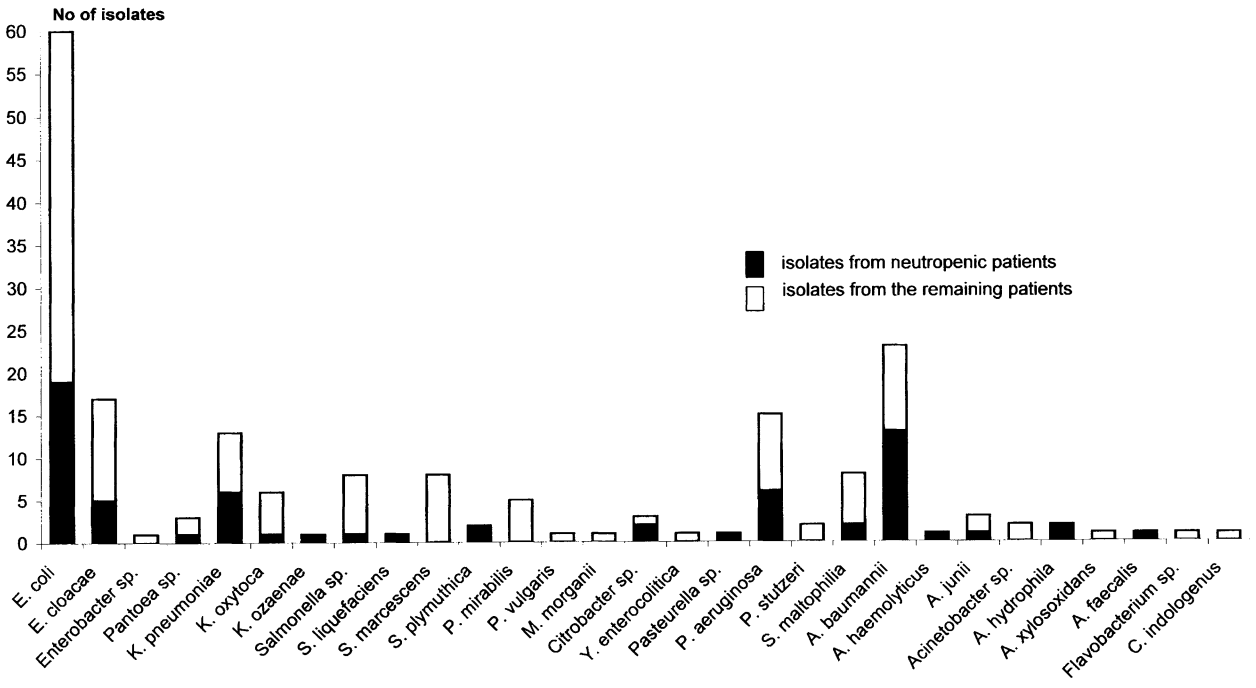

Figure 7. Gram-negative bacilli isolated from CO patients between 1997-2000

The source of bloodstream infections, especially in neutropenic patients, can be the alimentary tract or oral cavity. This connection is confirmed by the high frequency of infections with gram-negative bacilli of *Enterobacteriaceae* family in neutropenic patients observed in the Centre of Oncology and other clinical centres [7, 17, 18]. Due to their severe course and high mortality rate these infections are of particular clinical significance. The threat of gram-negative infection is intensified by the selective role of antibiotics most extensively used in empiric therapy, such as third-generation cephalosporins or piperacillins/ureidopenicillins. This frequently leads to the

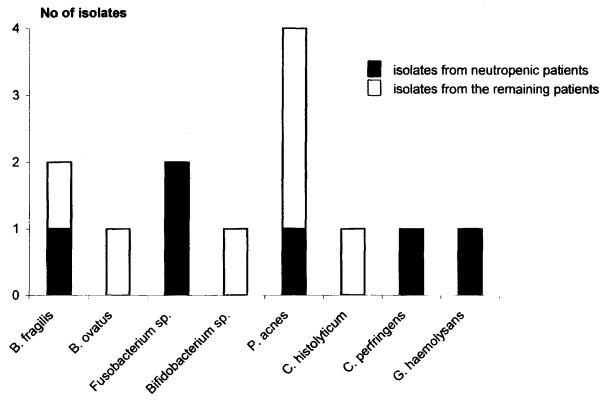

Figure 8. Anaerobic bacteria isolated from CO patients between 1997-2000

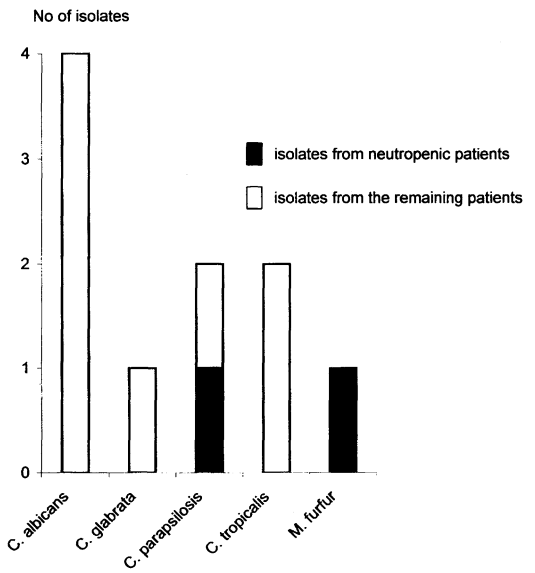

Figure 9. Yeasts isolated from CO patients between 1997-2000

selection of multiresistant strains which, in the absence of activity from the immune system, generate serious therapeutic problems.

While comparing bacterial flora isolated from infected blood of neutropenic patients with flora isolated from the remaining cancer patients, attention must be drawn to the incidence of single isolates or the absence of typical pathogen isolates, such as *Streptococcus pyogenes*, *Streptococcus pneumoniae*, or bacilli of *Salmonella* species. The majority of microorganisms frequently associated with infections in neutropenic patients belong to opportunistic flora, originating from the skin, the oral cavity or the alimentary tract. In most cases these are endogenous infections.

Bloodstream infections as the cause of fever in cancer patients with neutropenia require further systematic surveillance.

Conclusions

1. Microbiologically confirmed bloodstream infections are observed three times more frequently in neutropenic patients than in the group of cancer patients without neutropenia.
2. Gram-positive flora, especially cocci of *Staphylococcus* species, play the most important part in infections in neutropenic patients, as well as among other cancer patients.
3. In infections in both groups of patients Gram-negative bacilli are mainly represented by *Enterobacteriaceae* family. The most common isolates were *E. coli*. Among nonfermenting bacilli the most common isolates belonged to P. aeruginosa species.
4. In neutropenic patients most infections are caused by opportunistic flora. "True" pathogens eg. *Streptococcus*

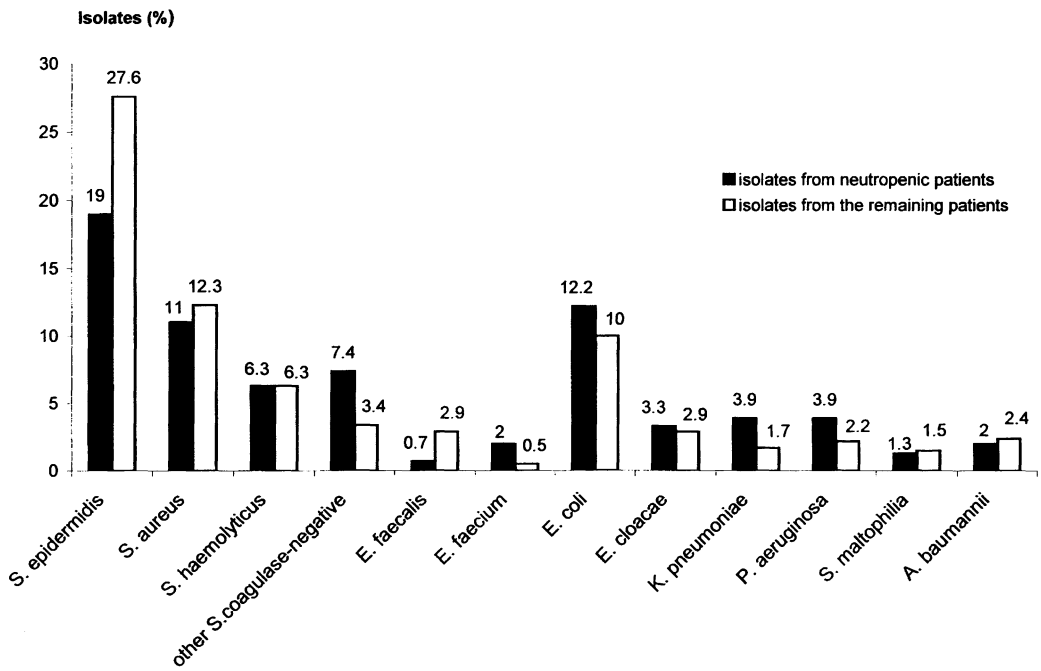

Figure 10. Comparison of occurrence of organisms isolated from neutropoenic patients and the remaining cancer patients

pyogenes or strains of *Salmonella* species were rarely isolated.

5. In neutropenic patients we can observe an increasing tendency to develop infections caused by strains of species, in which, potentially, numerous mechanisms of resistance to antibiotics may become active.

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Hanna Połowniak-Pracka M.D.
Department of Clinical Microbiology
The Maria Skłodowska-Curie Memorial Cancer Center
and Institute of Oncology
Roentgena 5, 02-781 Warsaw, Poland

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