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ARCHIVES OF CLINICAL MICROBIOLOGY

2012
Vol. 3 No. 3:3
doi: 10.3823/253

Antimicrobial Susceptibility Profiles of Gram-Negative Bacilli and Gram-Positive Cocci Isolated from Cancer Patients in Libya

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Abstract

Title: Antimicrobial Susceptibility Profiles of Gram-Negative Bacilli and Gram-Positive Cocci Isolated from Cancer Patients in Libya.

Background: Due to defects in their immunity cancer patients are prone to serious infections with substantial morbidity and mortality. However, there is lack of information on the microbial and antimicrobial susceptibility profiles of microorganisms associated with infections in cancer patients in Libya.

Methods and Findings: Organisms were isolated from sputum, throat swabs, skin infection swabs (SI), high vaginal swabs (HVS), urine and blood specimens from the cancer patients in the African Oncology Hospital (AOC) at the time an infection occurred. Isolated bacteria were identified to the species level and tested for their susceptibility to a variety of antimicrobial agents by an automated microbiology system. During the study period (January-December 2009) 256 organism (196 Gram-negative bacilli [GNB] and 60 Gram-positive cocci [GPC]) were isolated from cancer patients. The most common GNB identified were *Escherichia coli* (53%), *Klebsiella pneumoniae* (19.4%) and *Pseudomonas aeruginosa* (10.2%). We observed resistance rates between 36-73% among GNB to ampicillin, amoxycillin-clavulanic acid, piperacillin, tetracycline and trimethoprim-sulfamethoxazole. Fluoroquinolone resistance (FR) and extended-spectrum β-lactamases were detected in ≥ 21% and ≥ 10%, respectively, of GNB. Of the investigated GPC, 46.7% were identified as coagulase-negative staphylococci (CNS), 28.3% *Staphylococcus aureus*, 8.3% enterococci and 16.7% streptococci. Methicillin-resistance (MR) was detected in 35.3% of *S. aureus* and in 75% of CNS isolates. FR was detected in one group B *Streptococcus* (GBS) isolate and linezolid resistance in one enterococci (LRE) isolate.

Conclusion: Observed high rates of MRSA and MRCNS and emergence of FR-GBS and LRE in the population studied is of concern.

Key words: Antimicrobial resistance, Gram-negative bacilli, Gram-positive cocci, cancer, Libya

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Introduction

Due to defects in their immunity cancer patients particularly those with profound and prolonged neutropenia are prone to serious infections with substantial morbidity and mortality [1, 2]. Most infections in cancer patients are nosocomial in nature as a result of their prolonged and frequent contact with hospital environment [3]. In many institutions in developed countries, more Gram-positive bacteria, mainly staphylococci, than Gram-negative bacteria are isolated from cancer patients [4-6]. Use of indwelling catheters, oral mucositis, and prophylactic and empirical treatment directed mainly against Gram-negative bacteria are reasons, among others, that have been given for this phenomenon [7]. Emergence of antimicrobial resistance among staphylococci (e.g. methicillin-resistant *S. aureus* [MRSA]) and Gram-negative bacilli (e.g. fluoroquinolone-resistant *Escherichia coli* [FREC]) associated with infections in cancer patients is of particular concern in recent years. Reports on the microbial spectrum and antimicrobial resistance profiles of bacteria from cancer patients in the countries of North Africa are rare and such reports from Libya are lacking. The aim of this study was to determine the profile and susceptibility patterns of bacterial pathogens associated with infections in cancer patients attending the African Oncology Center (AOC) in Sabrata city, Libya.

Materials and Methods

Included in the present study 256 bacterial isolates (196 aerobic and facultative anaerobic Gram-negative bacilli [GNB] and 60 Gram-positive cocci [GPC]) isolated from patients with hematologic malignancies and solid tumors treated at the African Oncology Institute (AOC) in Sabrata, Libya between January and December 2009. The patients aged 1 to 97 years (mean=40.5 yrs) with female to male ratio 2:1. AOC is a 130 bed hospital that provides free medical care for cancer patients from all regions of Libya.

Organisms were isolated from sputum, throat swabs, skin infection swabs (SI), high vaginal swabs (HVS), urine and blood specimens from the patients at the time an infection occurred. All specimens were cultured on different media accordingly using standard bacteriological procedures [8]. Isolated organisms were identified to the species level and tested for their susceptibility to a variety of antimicrobial agents by the BD Phoenix Automated Microbiology System (PAMS, MSBD Biosciences, Sparks Md, USA) according to the manufacturer's instructions. The PAMS uses combination panels for identification (ID) and antimicrobial susceptibility testing (AST) of bacteria. These include the Phoenix™ NMIC/ID Panels intended for the *in vitro* rapid ID and AST by minimal inhibitory concentration (MIC) of Gram-negative aerobic and facultative anaerobic bacteria from pure culture belonging to the family *Enterobacteriaceae* and non-*Enterobacteriaceae*; the Phoenix™ PMIC/ID Panels intended for the *in vitro* rapid ID and AST of Gram-positive bacteria belonging to the genera *Staphylococcus, Enterococcus,* and other Gram-positive cocci and Gram-positive bacilli; and the Phoenix™ SMIC/ID Panels intended for the *in vitro* rapid ID and AST of bacteria belonging to the genera *Streptococcus*. PAMS provides AST results for antimicrobials as susceptible (S), intermediate susceptible (I) and resistant (R) and is interpreted according to CLSI criteria [9].

The following quality control strains were tested with each run according to the type of bacteria examined: *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603 and *Ps. aeruginosa* ATCC 27853 with GNB; *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 with *Staphylococcus* and *Enterococcus*; and *Strep. agalactiae* ATCC 13813 and *Strep. pneumoniae* ATCC 49619 with *Streptococcus*.

All oxacillin-resistant *S. aureus* (MRSA) and oxacillin-resistant coagulase-negative staphylococci (MRCNS) isolates were tested for *mecA* gene by PCR as previously reported [10]. Only the first isolate from each patient was included in this study. Patients were informed of the purpose of the study and their consent was obtained before specimen collection.

Results

Of the 196 GNB isolates examined 76% were from urine specimens. The predominant organisms were *Escherichia coli* (53%), *Klebsiella pneumoniae* (19.4%) and *Pseudomonas aeruginosa* (10.2%). **Table 1** shows the distribution of GNB isolated from different clinical specimens of cancer patients.

Table 1. Gram-negative bacilli isolated from different clinical specimens of cancer patients

Organism	No. (%) positive for:							
	Urine (*n* = 149)[1]	Blood (*n* = 6)	Pus (*n* = 7)	SI* (*n* = 20)	Sputum (*n* = 8)	HVS* (*n* = 2)	Throat (*n* = 4)	Total (*n* = 196)
Escherichia coli	92 (61.7)	2 (33.3)	2 (28.6)	6 (30)	1 (12.5)	1 (50)	0 (0.0)	104 (53)
Klebsiella pneumoniae	28 (18.8)	2 (33.3)	1 (14.2)	3 (15)	2 (25)	0 (0.0)	2 (50)	38 (19.4)
Klebsiella oxytoca	2 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0))
Enterobacter cloacae	6 (4.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	7 (3.6)
Proteus mirabilis	9 (6.0)	0 (0.0)	0 (0.0)	2 (10)	1 (12.5)	0 (0.0)	0 (0.0)	12 (6.1)
Morganella morganii	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Providencia stuartii	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (50)	0 (0.0)	1 (0.5)
Serratia marcescens	0 (0.0)	0 (0.0)	0 (0.0)	1 (5.0)	1 (12.5)	0 (0.0)	0 (0.0)	2 (1.0)
Citrobacter koseri	3 (2.0)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	1 (25)	5 (2.5)
Citrobacter freundii	1 (0.7)	0 (0.0)	0 (0.0)	1 (5.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)
Acinetobacter baumannii	0 (0.0)	1 (16.7)	1 (14.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)
Pseudomonas aeruginosa	7 (4.7)	1 (16.7)	3 (43)	5 (25)	3 (37.5)	0 (0.0)	1 (25)	20 (10.2)

* SI = skin infection swab, HVS=high vaginal swab.
[1] Number of organisms isolated from corresponding clinical specimen.

Table 2. Staphylococci isolated from different clinical specimens of cancer patients.

Organism	No. (%) positive for:				
	Urine (*n* = 6)[1]	Blood (*n* = 11)	SI* (*n* = 26)	Sputum (*n* = 2)	Total (*n* = 45)
Staphylococcus aureus	1 (16.7)	1 (9.1)	15 (57.7)	0 (0.0)	17 (37.9)
S. epidermidis	0 (0.0)	3 (27.3)	5 (19.3)	0 (0.0)	8 (17.8)
S. haemolyticus	1 (16.7)	3 (27.3)	0 (0.0)	2 (100)	6 (13.3)
S. hominis	2 (33.3)	3 (27.3)	1 (3.8)	0 (0.0)	6 (13.3)
S. warneri	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.2)
S. saprophyticus	1 (16.7)	1 (9.1)	2 (7.7)	0 (0.0)	4 (8.9)
S. lugdunensis	0 (0.0)	0 (0.0)	2 (7.7)	0 (0.0)	2 (4.4)
S. capitis	0 (0.0)	0 (0.0)	1 (3.8)	0 (0.0)	1 (2.2)

* S = skin infection swab
[1] Number of organisms isolated from corresponding clinical specimen.

Table 3. Antimicrobial susceptibility of Gram-negative bacilli isolated from cancer patients

Antibiotic	%											
	Escherichia coli (*n* = 104)			*Klebsiella* species (*n* = 40)			*Pseudomonas aeruginosa* (*n* = 20)			Other Gram-negative bacilli[2] (*n* = 32)		
	S	I	R	S	I	R	S	I	R	S	I	R
Ampicillin	40.4	1.9	57.7	2.5	0.0	97.5	NT[1]			18.8	3.1	78.1
Amoxycillin/clavulanic acid	62.5	21.2	16.3	40	10	50	0.0	0.0	100	56.2	0.0	43.8
Imipenem	97	1	2	95	0.0	5.0	90	0.0	10	93.8	6.2	0.0
Meropenem	100	0.0	0.0	100	0.0	0.0	95	0.0	5	90.6	3.1	6.3
Cephalothin	30.8	24	45.2	42.5	10	47.5	0.0	0.0	100	59.4	0.0	40.6
Cefoxitin	87.5	4.8	7.7	52.5	0.0	47.5	5	0.0	95	71.9	0.0	28.1
Cefuroxime	81.7	4.8	13.5	52.5	2.5	45	0.0	0.0	100	46.9	6.2	46.9
Cefotaxime	92.3	1	6.7	62.5	0.0	37.5	0.0	5	95	84.4	3.1	12.5
Cefepime	93.3	0.0	6.7	65	0.0	35	90	0.0	10	84.4	0.0	15.6
Ceftazidime	93.3	0.0	6.7	60	2.5	37.5	85	0.0	15	84.4	3.1	12.5
Aztreonam	91.3	0.0	8.7	60	2.5	37.5	75	10	15	81.2	0.0	18.8
Piperacillin	47.1	7.7	45.2	10	0.0	90	95	0.0	5	78.1	0.0	21.9
Piperacillin/tazobactam	94.2	2.9	2.9	87.5	7.5	5	95	0.0	5	96.9	0.0	3.1
Gentamicin	88.5	0.0	11.5	72.5	5	22.5	85	0.0	15	84.4	0.0	15.6
Amikacin	96.2	0.0	3.8	97.5	0.0	2.5	95	0.0	5	96.9	0.0	3.1
Ciprofloxacin	77.8	2	20.2	67.5	0.0	32.5	85	0.0	15	78.1	6.3	15.6
Norfloxacin	77.8	2	20.2	67.5	0.0	32.5	80	5	15	84.4	0.0	15.6
Nitrofurantoin	95.1	2	2.9	47.5	20	32.5	0.0	0.0	100	46.9	9.3	43.8
Tetracycline	56.7	0.0	43.3	67.5	0.0	32.5	10	0.0	90	50	0.0	50
Trimethoprim/sulfamethoxazole	71.2	0.0	28.8	67.5	0.0	32.5	5	0.0	95	68.7	0.0	31.3
Colistin	NT			NT			100	0.0	0.0	NT		

[1] NT = not tested. [2]Include species of the genera *Enterobacter*, *Morganella*, *Providencia*, *Serratia*, *Citrobacter*, and *Acinetobacter*.

Of the investigated GPC, 28 (46.7%) were identified as coagulase-negative staphylococci (CNS), 17 (28.3%) *S. aureus*, 5 (8.3%) enterococci and 10 (16.7%) streptococci. Speciation of CNS strains showed the predominance of *S. epidermidis*, *S. hominis* and *S. haemolyticus*. **Table 2** shows the distribution of staphylococci isolated from different clinical specimens of cancer patients.

Two (40%) of the enterococci isolates were from urine, 2 (40%) from SI and one (20%) from HVS specimens. All *Enterococcus* isolates were identified as *E. faecalis*. Most (40%) of the streptococci isolates were from HVS. Seven of the *Streptococcus* isolates were identified as *Strep. agalactiae* (also known as groups B *Streptococcus* [GBS]) and the remaining three as *Strep. acidominimus*.

High resistance rates were observed among total GNB to ampicillin (73.1%), amoxycillin-clavulanic acid (36.2%), piperacillin (46.4%), tetracycline (46.9%) and trimethoprim-sulfamethoxazole (36.7%). On the other hand, low resistance rates were detected for imipenem (3.1%), meropenem (1.5%), piperacillin/tazobactam (3.6%), amikacin (3.6%), and colistin

Table 4. Antimicrobial susceptibility of *Staphylococcus aureus* and coagulase-negative staphylococci (CNS) isolated from cancer patients.

Antibiotic	%					
	S. aureus (*n* = 17)			CNS (*n* = 28)		
	S	I	R	S	I	R
Penicillin	5.9	0.0	94.1	0.0	0.0	100
Ampicillin	5.9	0.0	94.1	0.0	0.0	100
Amoxycillin/clavulanic acid	64.7	0.0	35.3	25	0.0	75
Meropenem	64.7	0.0	35.3	25	0.0	75
Cefoxitin	64.7	0.0	35.3	25	0.0	75
Cefotaxime	41.2	23.5	35.3	25	0.0	75
Ceftriaxone	64.7	0.0	35.3	25	0.0	75
Oxacillin	64.7	0.0	35.3	25	0.0	75
Teicoplanin	94.1	0.0	5.9	85.7	3.6	10.7
Gentamicin	88.2	0.0	11.8	78.6	0.0	21.4
Ciprofloxacin	76.5	5.9	17.6	71.4	0.0	28.6
Quinupristin/dalfopristin	100	0.0	0.0	89.3	3.6	7.1
Linezolid	100	0.0	0.0	100	0.0	0.0
Clarithromycin	82.4	0.0	17.6	25	0.0	75
Erythromycin	82.4	0.0	17.6	21.4	3.6	75
Trimethoprim/sulfamethoxazole	82.4	0.0	17.6	53.6	0.0	46.4
Vancomycin	100	0.0	0.0	100	0.0	0.0

(2.6%). Of the three most common isolated GNB, *Ps. aeruginosa* strains showed extremely high rates of resistance to cephalothin (100%), cefoxitin (95%), cefuroxime (100%) and cefotxime (95%), tetracycline (90%) and trimethoprim/sulfamethoxazole (95%). MDR was observed in 95% of *Ps. aeruginosa* strains examined. Susceptibility of GNB isolated from cancer patients to antimicrobial agents is shown in **Table 3**.

Extended spectrum β-lactamases (ESBLs) were detected in 7 (6.7%) of *E. coli* and in 13 (32.5%) of *Klebsiella* spp. isolates. Of the ESBL-positive strains 14 (70%) were from urine, and 2 (10%) each from blood, pus and skin specimens.

The staphylococci examined showed high resistance rates to the β-lactam drugs ranging between 60 to100 percent. Oxacillin resistance was detected in 6 (35.3%) *S. aureus* (MRSA) and in 21 (75%) CNS (MRCNS). All oxacillin-resistant staphylococci were positive for the *mecA* gene by PCR. None of the staphylococci examined were resistant to linezolid or to vancomycin. **Table 4** shows the susceptibility of *S. aureus* and CNS isolated from cancer patients to antimicrobial agents.

Resistance to β-lactam drugs, gentamicin, and trimethoprim-sulfamethoxazole was observed in 100% and to erythromycin in 60% of *E. faecalis* isolates. In addition, one *E. faecalis* isolates (from SI) was also resistant to linezolid (LRE), teicoplanin, and ciprofloxacin.

All (100%) streptococci isolates were susceptible to meropenem, cefotaxime, ceftriaxone, cefepime, vancomycin and linezolid. Only one (10%) *Strep. agalactiae* isolate was resistant to penicillin, amoxycillin-clavulanic acid, ciprofloxacin, and clarithromycin. Furthermore, resistance to erythromycin and tetracycline was observed in 3 (30%) and 7 (70%) streptococci isolates, respectively.

Discussion

Identification and determination of antimicrobial susceptibility of bacterial pathogens can aid the clinician in selecting the appropriate antimicrobial agent (s) to treat his patients. Prabhash et al., in India, reported the predominance of *Pseu-*

domonas spp., followed by *Acinetobacter* spp. and *E. coli* among GNB isolated from blood stream infections (BSI) in cancer patients [11]. In the present study, *E. coli*, followed by *K. pneumoniae* and *Ps. aeruginosa* were the most common GNB identified. It should be noted that most of GNB strains examined were from urine specimens. This may explain the predominance of *E. coli* and *K. pneumoniae* amongst our GNB isolates. Ashour and El-Sharif, in Egypt, reported similar findings [12]. They found that GNB causing UTIs in both leukemic and solid-tumor patients were mainly *E. coli* and *K. pneumoniae*. Contrary to recent reports from developed countries, our findings and those reported by other investigators [11, 12] indicate that GNB are the predominant organisms associated with infections in cancer patients in developing countries. This observation may have important implications in selecting the appropriate treatment for such patients.

Infections due to GNB with high resistance rates to β-lactam and non-β-lactam drugs are common in cancer patients [13,14]. Similarly, our GNB isolates showed high resistance rates to different classes of antimicrobials, particularly among *Ps. aeruginosa* isolates with 95% of them being multidrug resistant. However, high susceptibility (< 4% resistance) rates were observed among the examined GNB isolates to imipenem, meropenem, piperacillin/tazobactam, amikacin. Nejad et al., in Iran, reported multidrug resistance in 37% and 33% of *E. coli* and *Klebsiella* spp. from cancer patients [14]. We observed lower rate of multidrug resistance for *E. coli* (29%) and similar rate for *Klebsiella* spp. (33%).

A study from neighboring Egypt reported high resistance rates to fluoroquinolones in *E. coli* (> 55%) and *K. pneumoniae* (> 30%) from cancer patients [12]. Resistance to fluoroquinolones (ciprofloxacin and norfloxacin) was observed in 20.2%, 32.5% and 15% of *E. coli*, *Klebsiella* spp. and *Ps. aeruginosa* from Libyan cancer patients, respectively. In many centers, fluoroquinolones are routinely used for prophylaxis in cancer patients [15]. Such clinical practice may have contributed to the observed high resistance rates against fluoroquinolones among GNB isolated from patients with cancer. Recently, Cattaneo et al. [13] reported very high rates of resistance (86.8%) to the fluoroquinolone levofloxacin among *E. coli* from patients with hematological malignancies. These investigators, using multivariate analysis, identified prophylaxis and neutropenia > 7 days as independent risk factors for fluoroquinolone resistance.

The emergence of resistance to β-lactam drugs due to the production of type 1 and extended-spectrum β-lactamases (ESBLs) among *E. coli* and other *Enterobacteriaceae* is of great concern [1]. Reports on the prevalence of ESBLs among GNB in cancer patients from the North Africa region are rare. In this investigation ESBLs were detected at 6.7% and 32.5% among *E. coli* and *Klebsiella* spp. isolates. Higher rates (> 80%) for ESBLs among GNB from cancer patients were reported from India [16].

In many cancer centers staphylococci, mainly the coagulase-negative (CNS), are the most commonly isolated bacteria, particularly from bacteremic patients [4, 17]. In the present study CNS were the most common staphylococci isolated from cancer patients in AOC with predominance of *S. epidermidis*. Others reported closely similar findings [18].

Several studies reported very high rates of methicillin resistance among *S. aureus* (> 81%) as well as CNS (> 90%) from cancer patients [4,18]. In the present investigation all oxacillin-resistant staphylococci were positive for the *mecA* gene by PCR. Although lower rates for MRSA (35%) and MRCNS (75%) were found among our patients compared with the previously mentioned studies, such rates remain high. The high MRSA rates could be the result of wide use of fluoroquinolones and other antimicrobials for treatment and prophylaxis of bacterial infections in cancer patients [19,20].

Recently, Ashour and El-Sharif investigated the antibiotic susceptibility profile of Gram-positive aerobic bacteria isolated from Egyptian cancer patients [18]. They reported that more than 15% of *S. aureus* were resistant to linezolid and vancomycin and 11% of CNS were vancomycin resistant. Fortunately, none of the staphylococci examined in the present study were resistant to linezolid or to vancomycin. Furthermore, teicoplanin and quinupristin/dalfopristin showed excellent activity against MRSA (94.1 ad 100%, respectively) and MRCNS (85.7 and 89.3%, respectively) and can be used for the treatment of these organisms in cancer patients at AOC.

Members of the genus *Enterococcus*, mainly the species *faecium* and *faecalis*, are important nosocomial pathogens. A recent report by the National Nosocomial Infections Surveillance (NNIS) System in the USA indicated that vancomycin-resistant enterococci (VRE) account for nearly 30% of all enterococci isolated from patients infected in ICUs [21]. None of our enterococci isolates were resistant to vancomycin. However, we detected one *E. faecalis* isolate resistant to linezolid (LRE). To our knowledge this is the first report of LRE from Libya.

The viridans streptococci (α-haemolytic streptococci) are the predominant streptococci isolated from patients with cancer [18, 22]. In the present study, only 3 of the 10 isolated streptococci were of the viridians group and identified as *Strep. acidominimus*. There are few reports on the association of

Strep. acidominimus with infection in cancer patients. Recently, Dalal and Urban reported the isolation of *Strep. acidominimus* from the blood sample of 76-year-old man with lung cancer, who presented to the emergency room with a 2-day history of productive cough and fever [23]. Group B *Streptococcus* usually causes no symptoms in healthy adults; however patients with malignancies are at increased risk of infection with this organism [24, 25].

In accordance with previous reports [25], none of our streptococci isolates were resistant to 3rd cephalosporins, vancomycin or linezolid. However, we found one (14.3%) *Strep. agalactiae* isolate resistant to penicillin and ciprofloxacin. Fluoroquinolone resistance in GBS (FR-GBS) is uncommon. Previous studies reported rates of FR-GBS ranging between 1.2 to 5% [26-28]. Recently, Wehbeh et al. reported FR-GBS isolated from 23 patients with multiple comorbid conditions, including, among others, carcinoma, and diabetes mellitus [27]. There are no reports from Libya on FR-GBS.

In conclusion, contrary to recent reports from developed countries GNB are the predominant organisms associated with infections in cancer patient in AOC. High resistance rates against most of the antimicrobials tested were observed among the organisms examined, particularly among *Ps. aeruginosa* and CNS isolates. Excellent activity was observed by amikacin and the carbapenems imipenem and meropenem against GNB and by linezolid, teicoplanin, quinupristin/dalfopristin and vancomycin against GPC. Observed high rates of MRSA and MRCNS and the emergence of LRE and FR-GBS in the population studied is of concern. In addition, a comprehensive infection control program should be implemented that include raising awareness of the importance of good hand hygiene among health care workers to help prevent the spread of MDR bacteria to cancer patients treated at AOC.

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