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Frequency of Occurrence and Antimicrobial Susceptibility of Bacterial Isolates Causing Bloodstream Infections in European Medical Centers: Report from 9 Years of the SENTRY Antimicrobial Surveillance Program in Europe (1997-2005)

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: To evaluate the frequency of occurrence and antimicrobial susceptibility (S) of pathogens causing bloodstream infection (BSI) in Europe.

Methods: The first 20 unique and clinical relevant BSI isolates from 42 medical centers (10-31/year) were sent to a monitor each month. The isolates were tested for S (>30 antimicrobials) by broth microdilution methods and results interpreted according to the 2006 CLSI M100-S16 document. Selected resistance (R) mechanisms were characterized.

Results: 42,411 isolates (51.6% Gram-negative bacilli [GNB]) were processed. The rank order for all years was (no. of isolates/%of total): *E. coli* (9,586/22.7%) > *S. aureus* (8,354/19.7%) > coagulase-negative staphylococci (5,638/13.3%) > *Enterococcus* spp. (3,203/7.6%) > *Klebsiella* spp. (3,099/7.3%) > *P. aeruginosa* (PSA; 2,642/6.2%). This rank order and the frequencies were very stable through the years. The antimicrobial S rates of the key GNB are summarized in the table.

Organism (no. tested)	MIC_{50}/% S			
	Cefepime	Ciprofloxacin	Gentamicin	Imipenem
E. coli (9,586)	≤0.12/98.4	>4/86.7	2/94.2	≤0.5/>99.9
Klebsiella spp. (3,099)	8/91.9	1/90.0	>8/85.0	≤0.5/99.7
PSA (2,642)	>16/75.4	>4/70.8	>8/71.2	>8/79.6
Enterobacter spp. (1,805)	4/96.2	>4/82.0	8/88.9	1/99.3
Acinetobacter spp. (ASP; 1,093)	>16/48.2	>4/41.1	>8/42.1	>8/74.1

ESBL phenotypes were observed in 4.8% of *E. coli*, 24.2% of *K. pneumoniae* and 9.1% of *P. mirabilis*. Metallo-beta-lactamase (MBL) production was detected among Enterobacteriaceae (ENT) from Greece, Italy, Spain and Turkey. S rates for key antimicrobials against *S. aureus* were: 71.9% for oxacillin (OXA), 68.9% for ciprofloxacin, 47.7% for erythromycin, 81.3% for clindamycin, 98.1% for TMP/SMX, >99% for quinupristin/dalfopristin, teicoplanin, vancomycin (VAN), linezolid (LNZ) and daptomycin (DAP). VAN R was observed in 1.3% of *E. faecalis* and 10.7% of *E. faecium* with escalating rates in recent years, and no R to DAP or LNZ was detected among enterococci. Among *S. pneumoniae*, S and R to penicillin were 74.5 and 12.4%, respectively, while 99.6% of strains were S to levofloxacin and gatifloxacin.

Conclusions: The main R problems detected among BSI strains collected in Europe by the SENTRY Program were: OXA R *S. aureus*, ESBL-producing ENT, multi-drug resistant PSA and ACB, and MBL-producing ENT and PSA.

INTRODUCTION

The treatment of patients with significant bloodstream infection (BSI) is becoming more complicated in an era of increasing antimicrobial resistance among frequently occurring pathogens. Furthermore, the increased complexity of patients requiring hospitalization and widespread use of indwelling devices or invasive diagnostic procedures have created higher risks for BSI. Despite advanced diagnostic tests and preventative technologies, morbidity due to BSI remains relatively high. However, mortality can be reduced significantly when appropriate empiric antimicrobial therapy is initiated rapidly. In this context, antimicrobial surveillance studies (global and local) provide important information regarding the prevalence of pathogens responsible for BSI and antimicrobial resistance rate trends.

The SENTRY Antimicrobial Surveillance Program has monitored (1997 to present) BSI pathogens, among other objectives, on a worldwide scale. This report provides a comprehensive evaluation of BSI isolates collected by the SENTRY Program in Europe during the first nine years (1997–2005) of the program.

MATERIALS AND METHODS

Study Design. Participant medical centers are requested to send the first 20 unique and clinically relevant BSI isolates to a monitoring center (JMI Laboratories, North Liberty, IA, USA) each month. Forty-two medical centers have participated in the program, with the number of centers varying from 10 to 31 each year.

Bacterial isolates. A total of 42,411 isolates from BSI were processed during the study period. All isolates were identified by the participant laboratories and confirmed by the monitoring facility by biochemical tests or the Vitek system (bioMerieux, Hazelwood, MO), when necessary.

Susceptibility testing. The isolates were susceptibility tested by Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS) reference broth microdilution methods against more than 30 antimicrobial agents. Validated dry-form microdilution panels and broth for inoculation were provided by Trek Diagnostics (Cleveland, OH, USA). Data was analyzed using CLSI (2006) categorical interpretive criteria. Quality control tests and colony counts were routinely performed with *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 and 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Streptococcus pneumoniae* ATCC 49619.

Screening for carbapenemases. Enterobacteriaceae isolates with reduced susceptibility to imipenem and meropenem (MIC, ≥ 2 mg/L) were screened using disk approximation and PCR techniques. PCR amplicons for the carbapenemase genes were sequenced using a Sanger-based dideoxy sequencing strategy involving the incorporation of fluorescent-dye-labeled terminators into the sequencing reaction products.

RESULTS

- *E. coli* was the most frequently isolated pathogen from BSI in the European medical centers evaluated, followed by *S. aureus*, CoNS, *Enterococcus* spp. and *Klebsiella* spp. These five pathogens accounted for >70% of bacterial strains isolated (Table 1). The frequency of occurrence remained very stable during the study period (data not shown).
- Among *E. coli*, the highest rate of resistance among shown agents was observed for levofloxacin (9.9%), while 4.8% of strains showed an ESBL phenotype (Table 2). Two isolates with reduced susceptibility to imipenem (MIC, 8 mg/L) originated from Israel.
- The prevalence of ESBL phenotypes was relatively high among *Klebsiella* spp. (21.7%; Table 2). Resistance to gentamicin was also high among this species group (13.3%) and isolates with decreased susceptibility to imipenem were observed in Germany, Greece, Italy, Spain and Turkey.

Table 1. Frequency of occurrence of pathogens isolated from bloodstream infections in Europe as monitored by the SENTRY Program, 1997-2005.

Organism	No. of isolates	% of total
1. *E. coli*	9,586	22.6
2. *S. aureus*	8,354	19.7
3. Coagulase-negative staphylococci	5,638	13.3
4. *Enterococcus* spp.	3,203	7.6
5. *Klebsiella* spp.	3,099	7.3
6. *P. aeruginosa*	2,642	6.2
7. *Enterobacter* spp.	1,805	4.3
8. *S. pneumoniae*	1,225	2.9
9. *Acinetobacter* spp.	1,093	2.6
10. viridans group streptococci	899	2.1
11. ß-haemolytic streptococci	858	2.0
12. *P. mirabilis*	803	1.9

- Only 69.1% of *Enterobacter* spp. strains were susceptible to ceftazidime (Table 2). In contrast, cefepime was highly active against this pathogen (only 2.0% resistance). *Enterobacter* spp. isolates with decreased susceptibility to imipenem (MIC ≥ 8 mg/L) were detected in France, Belgium, Germany, Greece, Italy, Israel and Turkey.
- An ESBL phenotype was observed in 9.5% of *P. mirabilis,* while 13.4% of strains showed resistance to gentamicin (Table 2).
- *P. aeruginosa* showed high rates of resistance to most antimicrobials tested, mainly levofloxacin (26.4%), gentamicin (25.4%) and ceftazidime (19.5%). Cefepime (13.2% resistance) and imipenem (12.1% resistance) showed similar in vitro activity; while amikacin (87.9% susceptible) and polymyxin B (99.2% susceptibility) were the most active compounds against this pathogen (Table 2). Metallo-ß-lactamase producing *P. aeruginosa* has been observed in France (VIM-2), Germany (VIM-1 and GIM-1), Greece (VIM-1), Italy (VIM-1 and IMP-13), and Poland (VIM-2).
- Only imipenem (74.1% susceptible) and polymyxin B (97.8% susceptible) showed reasonable activity against *Acinetobacter* spp. (Table 2).
- Overall, 28.1% of *S. aureus* showed resistance to oxacillin, with high rates of co-resistance to erythromycin and levofloxacin. In contrast, trimethoprim/sulfamethoxazole was highly active against this pathogen (98.1% susceptible), including oxacillin-resistant strains. Linezolid was the most active compound against *S. aureus* with only one non-susceptible BSI strain (MIC, 8 mg/L) detected in the first nine years of the SENTRY Program (Table 3).
- Vancomycin resistance was observed in 3.6% of *Enterococcus* spp., and 70% of vancomycin-resistant strains (VRE) were also resistant to teicoplanin (VanA phenotype; Table 3). VRE (*E. faecium*) bacteremias in Europe have steadily increased to 17.6% (2005 results) since 1999 (data not shown). Linezolid was the most active compound against *Enterococcus* spp. with only 11 strains (0.4%) at the intermediate level (MIC at 4 mg/L) and no fully resistant (MIC > 8 mg/L) strains being detected (Table 3).
- Among *S. pneumoniae* isolated from BSI, 74.5% of strains were susceptible to penicillin (12.5% resistant), while cefepime (97.7% susceptible) and ceftriaxone (95.5% susceptible) showed near identical in vitro activity. The resistance rate to levofloxacin was only 0.3% (Table 3).
- Metallo-ß-lactamase (VIM and IMP types) producing Enterobacteriaceae strains were detected in Germany, Greece, Italy, Spain and Turkey (Table 4).

Table 4. Metallo-ß-lactamase producing Enterobacteriaceae strains isolated from BSI in European Medical Centers (SENTRY Program, 1997-2005).

Organism	No. of isolates	Year of isolation	City	Country	MIC range (mg/L)		MßL
					Imipenem	Meropenem	
Citrobacter koseri	1	2005	Genoa	Italy	8	1	VIM-like[a]
Enterobacter aerogenes	2	2005	Athens	Greece	>8	>8	VIM-1
Enterobacter cloacae	1	2004	Genoa	Italy	8	8	VIM-1
	1	2005	Madrid	Spain	4	4	VIM-1
	5	2003	Istanbul	Turkey	2-4	4->8	IMP-1
	1	2003	Ankara	Turkey	4	4	IMP-1
Klebsiella pneumoniae	1	2005	Leipzig	Germany	>8	8	VIM-1
	16	2003-2005	Athens	Greece	4->8	2->8	VIM-1
	9	2005	Genoa	Italy	4->8	0.5->8	VIM-like[a]
	1	2005	Madrid	Spain	2	2	VIM-like[a]
	1	2005	Ankara	Turkey	8	0.5	IMP-like[a]

a. Sequence analysis in progress.

Table 2. Activity of antimicrobial agents tested against Gram-negative bacilli.

Organism/antimicrobial agents (no. tested)	MIC_{50}	MIC_{90}	Range	%susceptible/ resistant[a]
Escherichia coli (9,586)				
Cefepime	≤0.12	≤0.12	≤0.12->16	98.4 / 1.2
Ceftazidime	≤1	≤1	≤1->16	97.5 / 1.5 (4.7)[b]
Ceftriaxone	≤0.25	≤0.25	≤0.25->32	97.2 / 2.2 (4.0)[b]
Aztreonam	≤0.12	0.25	≤0.12->16	97.3 / 2.1 (4.8)[b]
Piperacillin/tazobactam	2	4	≤0.12->256	96.0 / 1.8
Imipenem	≤0.5	≤0.5	≤0.5-8	>99.9 / 0.0
Levofloxacin	≤0.03	4	≤0.03->4	87.1 / 9.9
Gentamicin	≤2	≤2	≤2->16	94.2 / 5.1
Klebsiella spp. (3,099)				
Cefepime	≤0.12	8	≤0.12->16	91.9 / 5.8
Ceftazidime	≤1	>16	≤1->16	84.4 / 13.3 (20.5)[b]
Ceftriaxone	≤0.25	>32	≤0.32->32	83.5 / 10.8 (21.7)[b]
Aztreonam	≤0.12	>16	≤0.12->16	81.5 / 16.8 (21.5)[b]
Piperacillin/tazobactam	2	>64	0.25->64	83.6 / 11.6
Imipenem	≤0.5	≤0.5	≤0.5->8	99.7 / 0.2
Levofloxacin	0.06	2	≤0.03->4	91.7 / 5.7
Gentamicin	≤2	>8	≤2->8	85.0 / 13.3
Enterobacter spp. (1,805)				
Cefepime	≤0.12	4	≤0.12->16	96.2 / 2.0
Ceftazidime	≤1	>16	≤1->16	69.1 / 26.6
Ceftriaxone	≤0.25	>32	≤0.25->32	71.3 / 17.1
Aztreonam	≤0.12	>16	≤0.12->16	71.4 / 20.7
Piperacillin/tazobactam	4	>64	0.25->64	72.9 / 10.9
Imipenem	≤0.5	1	≤0.5->8	99.3 / 0.3
Levofloxacin	0.06	>4	≤0.03->4	83.7 / 13.7
Gentamicin	≤2	8	≤2->16	88.9 / 9.4
Proteus mirabilis (803)				
Cefepime	≤0.12	0.5	≤0.12->16	95.5 / 3.3
Ceftazidime	≤1	≤1	≤1->16	95.8 / 3.2 (8.5)[b]
Ceftriaxone	≤0.25	0.5	≤0.25->32	93.0 / 4.2 (9.1)[b]
Aztreonam	≤0.12	0.25	≤0.12->16	97.8 / 1.3 (6.6)[b]
Piperacillin/tazobactam	≤0.5	1	≤0.5->64	98.6 / 0.3
Imipenem	1	2	≤0.06->8	99.6 / 0.1
Levofloxacin	0.06	4	≤0.03->4	89.2 / 7.9
Gentamicin	≤2	>8	≤2->8	84.1 / 13.4
Pseudomonas aeruginosa (2,642)				
Cefepime	4	>16	≤0.12->16	75.4 / 13.2
Ceftazidime	4	>16	≤1->16	75.8 / 19.5
Piperacillin/tazobactam	8	>64	≤0.5->64	81.6 / 18.4
Imipenem	1	>8	≤0.5->8	79.6 / 12.1
Levofloxacin	≤0.5	>4	≤0.5->4	69.9 / 26.2
Gentamicin	2	>8	≤0.12->8	71.2 / 25.4
Amikacin	4	32	≤2->32	87.9 / 8.2
Polymyxin B	≤1	2	≤1->8	99.2 / -[c]
Acinetobacter spp. (1,093)				
Cefepime	16	>16	≤0.12->16	48.2 / 36.5
Ceftazidime	>16	>16	≤0.5->16	40.6 / 50.4
Piperacillin/tazobactam	64	>64	≤0.12->64	42.7 / 46.1
Ampicillin/sulbactam	16	>32	≤0.25->32	46.9 / 38.1
Imipenem	1	>8	≤0.5->8	74.1 / 21.8
Levofloxacin	4	>4	≤0.03->4	46.8 / 38.9
Gentamicin	>8	>8	≤2->8	42.1 / 51.7
Amikacin	16	>32	≤0.25->32	52.6 / 44.6
Polymyxin B	≤1	≤1	≤1->8	97.8 / -[c]

a. Criteria as published by the CLSI [2006].
b. Percentage of ESBL phenotype in parentheses.
c. - = breakpoint has not been established by the CLSI [2006].

Table 3. Activity of antimicrobial agents tested against Gram-positive cocci.

Organism/antimicrobial agents (no. tested)	MIC_{50}	MIC_{90}	Range	%susceptible/ resistant[a]
Staphylococcus aureus (8,354)				
Oxacillin	0.5	>8	≤0.25->8	71.9 / 28.1
Cefepime	2	>16	≤0.12->16	76.4 / 20.8[b]
Erythromycin	0.5	>8	≤0.06->8	66.2 / 31.7
Clindamycin	0.12	>8	≤0.06->8	81.3 / 18.4
Levofloxacin	0.25	>4	≤0.03->4	69.6 / 29.0
Trimethoprim/sulfamethoxazole	≤0.5	≤0.5	≤0.5->2	98.1 / 1.9
Quinupristin/dalfopristin	0.25	0.5	≤0.25->8	99.0 / 0.6
Linezolid	2	2	≤0.06-8	>99.9[c] / -[d]
Vancomycin	1	1	≤0.12-4	99.8 / 0.0
Coagulase-negative staphylococci (5,638)				
Oxacillin	>2	>2	≤0.25->2	23.8 / 76.2
Cefepime	4	>16	≤0.12->16	73.2 / 16.4[b]
Erythromycin	>8	>8	≤0.06->8	36.6 / 62.7
Clindamycin	0.12	>8	≤0.06->8	64.6 / 34.6
Levofloxacin	2	>4	≤0.03->4	46.4 / 45.9
Trimethoprim/sulfamethoxazole	1	>2	≤0.5->2	73.4 / 26.6
Quinupristin/dalfopristin	≤0.25	0.5	≤0.25->8	99.1 / 0.3
Linezolid	1	1	≤0.06-4	100.0 / -
Vancomycin	2	2	≤0.12-4	100.0 / 0.0
Enterococcus spp. (3,203)				
Ampicillin	≤2	>16	≤2->16	77.3 / 22.7
Gentamicin (High level)	≤500	>1000	≤500->1000	67.3 / 32.7
Teicoplanin	≤2	≤2	≤2->16	97.0 / 2.5
Vancomycin	1	2	0.25->16	95.5 / 3.6
Quinupristin/dalfopristin	>2	>2	≤0.06->2	22.5 / 65.3
Linezolid	2	2	≤0.06-4	99.6 / 0.0
Streptococcus pneumoniae (1,225)				
Penicillin	≤0.016	2	≤0.016->4	74.5 / 12.4
Cefepime	≤0.12	1	≤0.12-4	97.7 / 0.1
Ceftriaxone	≤0.25	1	≤0.25-2	97.5 / 0.0
Erythromycin	≤0.06	>8	≤0.06->8	76.3 / 22.9
Clindamycin	≤0.06	>8	≤0.06->8	83.5 / 16.0
Trimethoprim/sulfamethoxazole	≤0.5	>2	≤0.5->2	75.0 / 15.6
Tetracycline	≤2	>8	≤2->8	79.8 / 19.7
Levofloxacin	1	1	≤0.03->4	99.6 / 0.3
Linezolid	1	1	≤0.06-2	100.0 / -
Vancomycin	0.25	0.5	≤0.12-1	100.0 / -
Viridans group streptococci (899)				
Penicillin	0.06	2	≤0.016->4	69.8 / 7.2
Cefepime	0.24	1	≤0.06->16	90.3 / 4.5
Erythromycin	≤0.25	4	≤0.25->32	66.3 / 28.5
Clindamycin	≤0.06	4	≤0.06->8	87.8 / 10.9
Tetracycline	≤2	>8	≤2->8	66.4 / 31.7
Levofloxacin	1	1	≤0.03->4	98.3 / 1.2
Linezolid	1	1	≤0.06-2	100.0 / -
Vancomycin	0.5	1	≤0.12-1	100.0 / -
ß-haemolytic streptococci (858)				
Penicillin	≤0.016	0.06	≤0.016-0.25	99.5 / -
Cefepime	≤0.12	<0.12	≤0.12-1	99.4 / -
Erythromycin	≤0.06	2	≤0.06->32	87.1 / 12.5
Clindamycin	≤0.25	≤0.25	≤0.25->8	94.5 / 5.0
Tetracycline	4	16	≤2->16	49.5 / 49.7
Levofloxacin	0.5	1	≤0.03->4	99.6 / 0.4
Linezolid	1	1	≤0.06-2	100.0 / -
Vancomycin	0.5	0.5	≤0.12-1	100.0 / -

a. Criteria as published by the CLSI [2006].
b. ß-lactam susceptibility should be directed by the oxacillin test results.
c. Only one non-susceptible strain.
d. - = breakpoint has not been established by the CLSI [2006].

CONCLUSIONS

- The main antimicrobial resistance problems detected among bacteria isolated from BSI in European medical centers surveyed by the SENTRY Program in the 1997 – 2005 period were:
  - Fluoroquinolone resistance among Enterobacteriaceae
  - ESBL phenotype among *Klebsiella* spp. (21.7%) and *P. mirabilis* (9.1%)
  - Emergence of metallo-ß-lactamase-producing strains among Enterobacteriaceae species, mainly *K. pneumoniae* and *Enterobacter cloacae*
  - *P. aeruginosa* and *Acinetobacter* spp. isolates only susceptible to polymyxins
- Large longitudinal surveillance networks can provide robust data that can be very useful for guiding empiric therapy, infection control policies in participant medical centers and antimicrobial drug development. The SENTRY Program has contributed substantially to the detection of resistance mechanisms emerging in European medical centers.

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