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Resistance Rates Among Selected Gram-Positive and -Negative Isolates from European Medical Centers: A Decade of SENTRY Program Surveillance (1997-2006)

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SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Objectives: To study the prevalence and resistance (R) trends among selected Gram-positive and -negative pathogens. The increase in R to antimicrobials can occur soon after introduction into clinical practice, evolve slowly, or be sporadic without a clear trend. Mupirocin (MUP)-R occurred soon after it was released onto the market (associated with increased use). A high level of quinupristin/dalfopristin (Q-D)-R in some countries has been said to be associated with the use of related agents in animal husbandry. Trimethoprim/sulfamethoxazole (T/S) is the drug of choice for the treatment of *S. maltophilia* (SM) and T/S-R SM are perceived as rare in occurrence. Carbapenemases and high-level class C chromosomal cephalosporinase production combined with altered outer membrane permeability are primarily responsible for carbapenem [CARB]-R among enteric bacilli which occur at varying frequency.

Methods: Countries in the European region (14-16) contributed isolates for reference broth microdilution testing using CLSI (2006) methods and interpretive criteria (2007). MUP was tested over a seven-year period (2000-2006) against *S. aureus* (SA) and coagulase-negative staphylococci (CoNS), and Q-D (1997-2006) against SA, CoNS and *E. faecium* (EFM). T/S was analyzed against 736 SM and CARB (imipenem [IPM]) against >30,000 enteric bacilli during 1997-2006.

Results: The highest % of Q-D non-susceptible SA/CoNS were recovered in France and Austria (10/3%) and Greece (2/4%) compared to other countries (<=1%). All monitored countries detected (Q-D)-non-susceptible EFM at a rate of 10->70%. Clonal dissemination influenced the rate of non-susceptible Q-D SA, CoNS and EFM during some monitored years. High MUP-R rates among MRSA were noted in Belgium, Ireland, Sweden and the UK (18.2-25.6%) and CoNS in Belgium, Ireland and Turkey (>=40%). T/S-R SM (4.5% overall) were detected in nearly all countries, but at very low prevalence and CARB-R among enterics was also rare in Europe with IPM-R at <0.1% (see Tables).

Conclusions: MRSA and CoNS were more resistant to Q-D and MUP compared to methicillin-S strains with R rates remaining stable or declining over the tested SENTRY Program time period. In contrast, (Q-D)-non-susceptible among EFM has steadily increased in Europe and is presently over 30%. Continued monitoring of regional pathogens is essential to determine the longitudinal efficacy of older and more recently introduced antimicrobial agents.

INTRODUCTION

Collectively, *Staphylococcus aureus*, coagulase-negative staphylococci and *Enterococcus* spp. are the Gram-positive pathogens responsible for the vast majority of patient infections. For decades, methicillin-resistant *S. aureus* (MRSA) have been serious hospital-acquired pathogens capable of multidrug-resistance that can become endemic in a medical center. More recently, community-acquired MRSA and glycopeptide-non-susceptible staphylococci and enterococci have emerged as threats to clinical practice. Mupriocin (topical) and quinupristin/dalfopristin (Q-D) were developed to provide activity against MRSA and other Gram-positive pathogens with multidrug-resistant phenotypes.

Gram-negative bacilli are also capable of circumventing the mechanisms of action of many antimicrobial agents. These include the production of inactivating enzymes such as ß-lactamase, target gene mutations, efflux pumps and altered outer membrane permeability. Some species such as *Stenotrophomonas maltophilia* have multiple intrinsic and acquired resistance mechanisms that make this pathogen very difficult to treat.

The SENTRY Antimicrobial Surveillance Program has monitored the prevalence and susceptibility rates among Gram-positive and –negative pathogens from various sources of infection for more than a decade. Differences in the pathogen prevalence and susceptibility related to geographic regions, patient populations as well as source of infection have been extensively documented and reported during this time period. In this study, the rates of antimicrobial resistance among selected Gram-positive and –negative species and antimicrobial agents will be evaluated to determine potential resistance trends that may have occurred over the ten monitored study years. This analysis will focus on the collection of patient infection isolates sampled in Europe, Israel and Turkey.

MATERIALS AND METHODS

During 1997-2006, a total of 30,163 strains of enteric bacilli, 16,696 *S. aureus*, 7,312 isolates of coagulase-negative staphylococci and 1,361 *Enterococcus faecium* were collected from medical centers in Europe, Israel and Turkey. During this same period, a total of 736 isolates of *S. maltophilia* were collected mainly from bloodstream (390 strains) and respiratory tract (286 strains) infections. Clinical isolates and quality control strains were tested by reference broth microdilution methods of the Clinical and Laboratory Standards Institute (CLSI, M7-A7) using validated panels (TREK Diagnostics, OH, USA). Susceptibility information was interpreted using the recommendations of the CLSI (M100-S17).

A collection of Enterobacteriaceae isolates (116 strains) with reduced susceptibility to imipenem or meropenem (MIC, ≥2 mg/L) were screened for the production of MßL and serine carbapenemases. This included using disk approximation tests with imipenem, meropenem and ceftazidime as substrates, and EDTA and 2-mercaptopropionic acid as enzyme inhibitors; and a disk potentiation test using clavulanic acid as the ß-lactamase inhibitor for serine carbapenemases. Isolates with positive MßL screen test results were evaluated by PCR using primers for bla_{IMP} and bla_{VIM} and those with negative PCR results for MßL genes were sequenced for IMI, KPC and Nmc-A genes.

S. maltophilia with resistance to trimethoprim/sulfamethoxazole were further characterized using Etest (AB BIODISK, Solna, Sweden) and molecular methods including plasmid isolation, southern hybridization, PCR and DNA sequencing analysis for the presence of *sul*1 and *sul*2 genes (Toleman et. al., 2007).

Isolates from the same medical center with similar antibiogram and/or molecular profiles were typed using the Riboprinter™ Microbial Characterization System (Qualicon Inc., Delaware, USA) and/or by pulsed-field gel electrophoresis using a CHEF DRII apparatus (Bio-Rad, CA, USA).

RESULTS

- Nearly all isolates of oxacillin-susceptible *S. aureus* were susceptible (>99%) to Q-D and mupirocin during all ten years compared to MRSA which had Q-D and mupirocin non-susceptibility rates of 1-6% and 6-15%, respectively (Table 1).
- No resistance to Q-D was noted among the oxacillin-susceptible CoNS strains while non-susceptible isolates were detected each year at rates of 0.5-3% among the oxacillin-resistant isolates (Table 1). Mupirocin resistance was generally two- to ten- fold higher among the oxacillin-resistant CoNS compared to oxacillin-susceptible strains.
- Resistance to Q-D among *E. faecium* remained between 20-25% until the last two surveillance years (2005-2006) which showed an escalated rate of 30% (Table 1).
- Table 2 shows that ≥99% of the Enterobacteriaceae isolates were susceptible to imipenem in Europe, Israel and Turkey. However, non-susceptible (≥8 mg/L) strains were found in nine of the monitored countries. Fifty percent of the isolates screened for MßL, (58 strains/6 sites/5 countries) had confirmed VIM-1 or IMP-1 (Turkey only) enzymes mostly in *Klebsiella* spp. and *Enterobacter* spp. (Table 3). All isolates with negative MßL results were also negative for IMI, KPC and Nmc-A genes.
- Ceftazidime and ticarcillin/clavulanate were only marginally active (50%) against the *S. maltophilia* isolates in Europe, Israel and Turkey (Table 4). However, levofloxacin (90.9%) and trimethoprim/sulfamethoxazole (T/S, 95.5%) had good activity against these strains.
- Further study of a subset of ten genetically unrelated T/S-resistant *S. maltophilia* isolates (Etest MIC, >32 mg/L) demonstrated that *sul* genes were present in all isolates (Table 4, footnote b) and that they were located on mobile elements including class 1 integrons and plasmids.

Table 1. Occurrences of resistance (R) to quinupristin/dalfopristin (Q-D) and mupirocin among staphylococci and *E. faecium* (1997-2006).

Organism/ Antimicrobial-R (no.)[b]	Year (% non-S)[a] 1997	1998	1999	2000	2001	2002	2003	2004	2005	2006
S. aureus										
OX-S/Q-D (11,967)	0.3 (1.0)[c]	0.1	0.0	0.3	0.2	<0.1	<0.1	0.3	0.2	0.2
OX-R/Q-D (4,813)	4.3	4.9	5.9	4.3	3.7	2.2	0.8	1.1	1.3	1.3
OX-S/MUP (9,399)	-	-	-	0.6	0.5	1.0	0.9	1.0	0.6	0.7
OX-R/MUP (3,903)	-	-	-	14.6	11.1	12.1	10.6	7.7	7.2	6.2
Coagulase-negative staphylococci										
OX-S/Q-D (1,822)	1.0[d]	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
OX-R/Q-D (5,517)	1.3	1.0	0.5	3.1	0.6	1.1	0.7	0.7	0.7	2.1
OX-S/MUP (1,259)	-	-	-	5.1	10.5	5.4	4.2	3.5	5.2	2.0
OX-R/MUP (3,984)	-	-	-	16.7	21.8	25.4	17.7	18.8	18.3	21.4
E. faecium/Q-D (1,361)	20.3	26.1	22.7 (50.0)[e]	18.7	25.5	26.3	23.6	27.4	29.5	30.2

a. Non-susceptibility % based upon the CLSI interpretive criteria for Q-D (M100-S17) and MUP at MIC values >=16 mg/L (high- and low-level rates combined).
b. OX-S = oxacillin-susceptible: OX-R = oxacillin-resistant; and MUP = mupirocin.
c. Clonal outbreak in France increased the prevalence to 1%.
d. Three isolates from different countries with intermediate MIC values (2 mg/L).
e. A large number of clonally disseminated strains in Austria, Germany, Italy, Portugal and Spain produced a higher percentage of non-susceptible isolates.

Table 2. Activity of imipenem tested against Enterobacteriaceae in 18 countries participating in the SENTRY Antimicrobial Surveillance Program in Europe, Israel and Turkey (1997-2006).

Country (no. strains)	Cumulative percentage at imipenem MIC (mg/L) ≤0.5	1	2	4	8	>8
Albania (41)	75.6	85.4	100.0	-	-	-
Austria (156)	82.7	91.7	98.1	100.0	-	-
Belgium (885)	88.0	94.6	98.4	99.7	99.9	100.0
France (6,142)	86.6	94.9	99.0	99.9	100.0	-
Germany (3,488)[a]	88.0	95.0	99.0	99.7	99.9	100.0
Greece (1,171)[a]	80.9	90.9	96.8	98.6	99.2	100.0
Ireland (678)	86.8	94.7	99.6	100.0	-	-
Israel (1,041)	85.3	94.5	99.0	99.3	99.9	100.0
Italy (2,662)[a]	87.9	94.6	98.0	99.6	99.9	100.0
Poland (1,257)	86.6	94.2	98.4	99.8	99.9	100.0
Portugal (362)	77.6	92.8	98.1	100.0	-	-
Russia (446)	97.1	98.4	100.0	-	-	-
Spain (4,636)[a]	88.8	94.8	99.1	99.9	100.0	-
Sweden (1,375)	92.4	97.3	99.6	100.0	-	-
Switzerland (1,602)	85.8	94.6	98.8	100.0	-	-
the Netherlands (381)	73.2	85.8	96.6	100.0	-	-
Turkey (2,314)[a]	90.7	96.1	99.1	99.7	99.8	100.0
United Kingdom (1,526)	87.4	95.4	99.6	100.0	-	-

a. Countries with confirmed metallo-ß-lactamase producing isolates.

Table 3. Geographic distribution of metallo-ß-lactamase producing strains among Enterobacteriaceae isolated in Europe (SENTRY Program 2000-2006).

Country (no.)	City	Organism (no.)	Metallo-ß-lactamase	Detection year
Italy (16)	Genoa, Catania	*E. cloacae* (2)	VIM-1	2004
	Genoa	*K. pneumoniae* (12)	VIM-1	2005-2006
		C. koseri (1)	VIM-1	2005
		K. ozaenae (1)	VIM-1	2006
Greece (19)	Athens	*K. pneumoniae* (16)	VIM-1	2001-2005
		E. aerogenes (2)	VIM-1	2005
		P. mirabilis (1)	VIM-1	2005
Turkey (14)	Ankara	*K. pneumoniae* (1)	VIM-1	2005
		E. cloacae (1)	VIM-1	2005
	Istanbul, Ankara	*E. cloacae* (12)	IMP-1	2003-2005
Germany (4)	Leipzig	*K. pneumoniae* (1)	VIM-1	2005
		E. cloacae (3)	VIM-1	2006
Spain (5)	Madrid	*K. pneumoniae* (3)	VIM-1	2005-2006
		E. cloacae (2)	VIM-1	2005

Table 4. Activity of antimicrobial agents tested against 736 isolates of *S. maltophilia* collected from 1997-2006 SENTRY Program surveillance in Europe, Israel and Turkey.

Antimicrobial agent	MIC (mg/L) 50%	90%	Range	% Susceptible[a]	% Resistant[a]
Ceftazidime	8	>16	≤2->16	51.2	36.6
Ticarcillin/clavulanate	≤16	128	≤16->128	53.8	15.8
Levofloxacin	1	2	≤0.5->4	90.9	4.5
Trimethoprim/sulfamethoxazole	≤0.5	1	≤0.5->2	95.5	4.5[b]

a. Criteria as published by the CLSI (M100-S17, 2007).
b. Thirty-three isolates with resistant MIC values were collected from centers in Belgium (one strain; *sul*2), France (3; *sul*2), Germany (4; *sul*1,*sul*2), Israel (1), Italy (3; *sul*1), Poland (1), Portugal (1), Spain (6; *sul*1, *sul*2), Switzerland (1), the Netherlands (3) and Turkey (9; *sul*1, *sul*2).

CONCLUSIONS

- In European medical centers, resistance to Q-D and mupirocin remains low among oxacillin-susceptible strains of *S. aureus* and the lowest rates were documented for these two agents during 2003-2006 for oxacillin-resistant strains.
- Mupirocin resistance among CoNS (oxacillin-resistant) and Q-D resistance among *E. faecium* remains problematic at rates of 20 and 30%, respectively.
- Although the presence of MßL-producing isolates of Enterobacteriaceae and of T/S-resistant *S. maltophilia* isolates in Europe remains rare, the fact that these resistance mechanisms are found within very mobile elements is of concern.
- The dissemination of clonally related strains was documented for Q-D-resistant staphylococci, Q-D-resistant *E. faecium* and MßL-producing Enterobacteriaceae and although T/S-resistant strains of *S. maltophilia* appear to be genetically unrelated, careful international surveillance of all of these MDR species remains necessary.

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