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High-Level Multidrug-Resistance (MDR) Among Viridans Group Streptococci Isolated from Turkey: Report from the SENTRY Antimicrobial Surveillance Program

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Objectives: Viridans group streptococci (VGS) are composed of numerous species which are usually considered as harmless commensal of the oral cavity, gastrointestinal and female genital tract. However, VGS can cause invasive disease including endocarditis, deep abscesses, and bacteremia, and are especially problematic among neutropenic patients. The SENTRY Program has monitored VGS in Europe (EUR) since 1997 including centers in Turkey. After ten years of monitoring these sites, it was observed that VGS from Turkey were markedly more MDR compared to other EUR sites and this study documents the MDR patterns detected.
Methods: A total of 1,477 isolates of VGS were collected from EUR sites (1997-2006) of which 124 strains were from Turkey. Isolates were identified to species level by participants and referred to a central laboratory for confirmation using bile solubility, colony morphology and commercial kits when needed. The majority of VGS from Turkey were included in the *S. mitis* group. The susceptibility (S) profile was determined using broth microdilution methods in lysed-horse blood supplemented CAMHB according to CLSI recommendations/interpretations (2006). A subset of 16 strains that were high-level resistant (R) to penicillin (PEN) and mupirocin (MUP) were tested for clonality using PFGE.
Results: The table shows the R-profiles among 10 antimicrobials for strains collected in Turkey compared to centers in EUR. PEN non-S was 70.2% in Turkey (approx. 60% of these were non-S to ceftriaxone [CRO] or cefepime [FEP]) compared to VGS isolated in EUR at 22% PEN non-S; 30% non-S to CRO and FEP. Erythromycin (ERY)-R was also greater in Turkey (59.7%) compared to EUR (33.8%) with a constitutive clindamycin (CC)-R rate of 53 and 42%, respectively. R to levofloxacin (LEV) was similar, tetracycline (TET)-R was 20% higher, and strains R to quin/dalfo (Q/D) and MUP were rarely isolated outside Turkey. PFGE revealed clonally related strains within this population of unusually R patterns.

Table. MDR patterns of Turkish viridians group streptococci.

	Turkey (n = 124)		Europe and Israel (n = 1,353)	
VGS/Antimicrobial agent	% Intermediate[a]	% R[a]	% Intermediate[a]	% R[a]
PEN	35.5	34.7	17.2	4.5
CRO	5.5	33.9	3.3	3.0
FEP	10.5	28.2	3.9	3.1
ERY	6.5	53.2	3.7	30.1
CC	0.8	28.2	0.8	12.6
LEV	0.8	1.6	0.5	1.7
TET	3.2	51.6	2.1	33.2
Q/D	4.1	1.6	2.5	0.4
Imipenem	-	32.1[b]	-	2.6[b]
MUP	-	17.1[c]	-	0.9[c]

a. % based upon published CLSI S ranges (M100-S16).
b. % of isolates >=1 mg/L.
c. % of isolates >=16 mg/L.

Conclusions: This study documents significant variability among the S profile of Turkish VGS compared to strains from adjacent EUR nations. The percentage of strains from Turkey that were R to commonly prescribed antimicrobial agents such as beta-lactams and macrolides was alarming as was the R rate to TET, Q/D and MUP. Continued monitoring of VGS will be necessary because of the geographic variability in R for this increasingly important pathogen.

INTRODUCTION

Viridans group streptococci are generally considered to be avirulent, commensal bacteria when recovered from many clinical specimens including the oral cavity, and the gastrointestinal and genital tracts. They are also often observed as contaminants of blood cultures or as the cause of insignificant, transient bacteremia. However, members of this species group have also been recognized as significant human pathogens associated with severe infections including deep-seated abscesses, documented bacteremia and subacute bacterial endocarditis, particularly among immunocompromised hosts and patients with prosthetic heart valves.

The identification of viridans group streptococci to the species level has become problematic due to the recent increase in the number of recognized species and taxonomic changes that have occurred over the last two decades. This has caused decreased confidence in the species identifications provided by commercial systems commonly used by clinical microbiology laboratories. Regardless of the species identification, it is important to understand the overall antimicrobial susceptibility of the major groups of viridans streptococci.

The SENTRY Antimicrobial Surveillance Program has been monitoring the susceptibilities of viridans group streptococci in Europe since 1997. This study documents a significantly higher incidence of antimicrobial resistance among viridans group streptococci isolated from Turkey when compared to neighboring countries in Europe and Israel. This study also evaluated the differences in antimicrobial resistance among the five major groups of viridans group streptococci.

MATERIALS AND METHODS

During 1997-2006 (10 years), the SENTRY Program collected and tested 1,477 isolates of viridans group streptococci from Europe, Israel and Turkey (16 countries, 42 sites). Isolates were identified as viridans group streptococci or further speciated using local laboratory methods. The isolates were forwarded to a central laboratory (JMI Laboratories, North Liberty, IA, USA; Utrecht University, Utrecht, the Netherlands) for identification confirmation and reference susceptibility testing. Once received, isolate cultures with alpha-haemolytic colonies were tested for optochin susceptibility and bile solubility. When needed, further identifications were made using Vitek (bioMerieux, Hazelwood, MO, USA) and/or RapidID STR and the ERIC™ Electronic RapID Compendium identification system (Remel, Lenexa, KS, USA).

Isolates were tested for susceptibility using reference broth microdilution methods conforming to the Clinical Laboratory Standards Institute in lysed-horse blood (CLSI, M7-A7). Panels were provided by MicroScan (Dade Behring, Deerfield, IL, USA) or TREK Diagnostic Systems (Cleveland, OH, USA). Antimicrobials tested included penicillin (PEN), ceftriaxone (CRO), cefepime (FEP), erythromycin (ERY), clindamycin (CC), levofloxacin (LEV), tetracycline (TET), quinupristin/dalfopristin (Q/D), imipenem (IMP), mupirocin (MUP), gentamicin (GENT) and trimethoprim/sulfamethoxazole (T/S).

A subset of 16 strains that were high-level resistant to penicillin and mupirocin were tested for clonality using pulsed-field gel electrophoresis (PFGE). Etest strips (AB BIODISK, Solna, Sweden) were utilized to determine a broader range of MIC values for CRO, FEP, IMP and MUP tested against these isolates.

RESULTS

- *S. mitis* (19.9%), *S. oralis* (11.1%) and *S. bovis* (10.9%) were the most commonly isolated viridans group streptococcus species from medical centers in Europe, Israel and Turkey (Table 1). Eighteen percent of the isolates referred by the local laboratories were not identified to the species level.

- Table 2 shows the antimicrobial susceptibility profiles of the five groups of viridans group streptococci as defined by the ASM Manual of Clinical Microbiology. The most common species were within the *S. mitis* organism group which were much more resistant to penicillin (34.2%) compared to the other species groups (6.2 - 27.4%). Isolates in the *S. mitis* and *S. bovis* groups were less susceptible to macrolides, tetracyclines and fluoroquinolones.

Table 1. Rank order of viridans group streptococci identified from medical centers in Europe, Israel and Turkey during 1997-2006 SENTRY Program surveillance.

Species	Rank Order	%
S. mitis	1	19.9
viridans group streptococci[a]	2	18.1
S. oralis	3	11.1
S. bovis	4	10.9
S. anginosus	5	8.9
S. sanguinis	6	7.6
S. constellatus	7	6.0
S. salivarius	8	5.1
S. milleri	9	3.6
S. intermedius	10	3.5
S. parasanguinis	11	1.5
S. vestibularis	12	1.0
Other species[b]	-	2.8

a. Identified as alpha-haemolytic streptococci (not *S. pneumoniae*) or viridans group streptococci.
b. Includes *S. gordonii* (9), *S. mutans* (14), *S. acidominims* (5), *S. gallolyticus* (4), *S. equinus* (4), *S. uberis* (3), *S. porcinus* (1), *S. sobrinus* (1), and *S. canis* (1).

- The overall susceptibility of the viridans group streptococci from each country is listed in Table 3. Isolates from Turkey were much less susceptible to penicillin (29.8%), erythromycin (40.3%) and tetracycline (45.2%) compared to isolates collected from the other countries.

- Table 4 lists sixteen isolates of multidrug-resistant viridans group streptococci isolated from pediatric patients in a hospital in Turkey. These isolates were highly resistant to penicillin and other ß-lactams and showed unusual resistance (high- and low-level) to mupirocin. Among these isolates, molecular typing revealed three clonally related clusters of *S. mitis*, each with two or more strains (PFGE patterns C, E and F/F_1).

Table 2. Antimicrobial susceptibility profile among the five groups of viridans group streptococci isolated in Europe, Israel and Turkey during 1997-2006 SENTRY Program surveillance.

Species group (no. tested)	Antimicrobial agent (%S / %R)[a]						
	PEN	ERY	CC	TET	LEV	MUP	Q/D
S. mitis (579)[b]	65.8 / 9.0	55.5 / 38.3	86.2 / 12.4	63.7 / 34.1	97.5 / 2.0	96.8 / 3.2	98.4 / 0.4
S. anginosus (301)[c]	88.1 / 3.0	84.1 / 12.6	90.1 / 9.6	73.0 / 24.7	98.6 / 0.7	99.1 / 0.9	99.0 / 0.0
S. mutans (16)[d]	93.8 / 0.0	75.0 / 18.8	87.5 / 12.5	87.4 / 6.3	100.0 / 0.0	100.0 / 0.0	100.0 / 0.0
S. salivarius (84)[e]	72.6 / 1.2	73.8 / 26.2	94.0 / 6.0	73.5 / 25.3	98.8 / 1.2	100.0 / 0.0	100.0 / 0.0
S. bovis (164)[f]	93.9 / 1.2	54.9 / 44.5	63.4 / 36.0	27.8 / 70.3	95.6 / 3.1	99.1 / 0.9	82.3 / 3.1

a. Susceptibility percentages based upon the Clinical and Laboratory Standards Institute recommendations (CLSI, M100-S16).
b. Includes *S. mitis* (279), *S. sanguinis* (109), *S. parasanguinis* (20), *S. gordonii* (12) and *S. oralis* (159).
c. Includes *S. anginosus* (116), *S. constellatus* (87), *S. intermedius* (49) and isolates identified as "*S. milleri*" group (49).
d. Includes *S. mutans* (15) and *S. sobrinus* (1).
e. Includes *S. salivarius* (72) and *S. vestibularis* (12).
f. Includes *S. bovis* (157), *S. equinus* (4) and *S. gallolyticus* (3).

Table 3. Antimicrobial susceptibility among 1,477 strains of viridans group streptococci isolated from 16 countries in Europe, Israel and Turkey during 1997-2006.

Country (no. tested)	Antimicrobial Agent (% S)[a]					
	PEN	ERY	CC	LEV	TET	Q/D
Austria (14)	71.4	78.6	92.9	100.0	85.7	100.0
Belgium (54)	77.8	63.0	83.3	96.3	55.6	100.0
France (332)	75.3	57.4	79.5	98.2	61.0	95.8
Germany (190)	84.1	68.8	89.4	98.9	60.5	96.3
Greece (11)	90.9	81.8	90.9	100.0	63.6	100.0
Ireland (16)	68.8	68.8	100.0	93.8	75.0	100.0
Israel (76)	52.6	55.3	90.8	93.2	61.8	97.4
Italy (40)	70.0	47.5	67.5	97.5	67.6	97.5
Poland (9)	33.3	55.6	77.8	100.0	55.6	87.5
Portugal (11)	81.8	81.8	90.9	90.9	54.5	90.9
Spain (159)	72.3	61.0	79.1	97.9	55.1	95.0
Sweden (130)	91.5	84.6	96.1	97.7	74.4	99.2
Switzerland (174)	87.9	70.7	92.1	98.8	80.5	98.8
The Netherlands (15)	80.0	86.7	93.3	100.0	66.7	93.3
Turkey (124)	29.8	40.3	71.0	97.5	45.2	94.3
UK (122)	78.0	73.2	95.1	96.7	65.0	100.0

a. Susceptibility percentages based upon the Clinical and Laboratory Standards Institute recommendations (CLSI, M100-S16).

Table 4. Characterization of multidrug-resistant viridans group streptococci isolated from a pediatric hospital in Turkey.

			MIC (mg/L)											
Year	Isolate No.	Species	PEN	CRO	FEP	ERY	CC	GENT	LEV	MUP	Q/D	TET	T/S	IMP	PFGE
2000	7246A	*S. mitis*	8	8	16	0.5	≤0.06	4	1	32	1	>8	>1	2	A
2001	2613C	*S. mitis*	>32	>256	>256	>2	>2	>8	2	>1024	4	>8	≤0.5	>32	C
2001	2616C	*S. mitis*	>32	>256	>256	>2	>2	>8	2	>1024	4	>8	≤0.5	>32	C
2005	4859A	*S. mitis*	>32	>256	>256	>2	>2	>8	>4	>1024	2	>8	1	>32	D
2005	3059X	*S. mitis*	32	>256	>256	1	≤0.25	>8	1	>1024	0.5	>8	≤0.5	>32	E
2006	6093X	*S. mitis*	32	>256	>256	1	≤0.25	>8	1	>1024	1	>8	1	>32	E
2005	3065X	*S. mitis*	32	>256	>256	2	>2	>8	2	>1024	1	>8	≤0.5	>32	F
2005	3081X	*S. mitis*	32	>256	>256	0.5	>2	>8	2	>1024	1	>8	≤0.5	>32	F
2005	4592X	*S. mitis*	32	>256	>256	1	>2	>8	2	>1024	2	>8	≤0.5	>32	F
2006	677C	*S. mitis*	32	>256	>256	>2	>2	>8	2	>1024	0.5	>8	≤0.5	>32	F
2006	693C	*S. mitis*	32	>256	>256	2	>2	>8	1	>1024	1	>8	≤0.5	>32	F
2006	6076X	*S. mitis*	>32	>256	>256	2	>2	>8	2	>1024	2	>8	≤0.5	>32	F
2006	6114X	*S. mitis*	32	>256	>256	2	>2	>8	1	>1024	1	>8	≤0.5	>32	F_1
2006	6045X	*S. oralis*	32	>256	>256	>2	>2	8	>4	16	2	8	>2	>32	H
2006	6087X	*S. mitis*	32	>256	>256	1	≤0.25	>8	2	>1024	1	>8	1	>32	I
2006	6110X	*S. uberis*	8	64	64	>2	>2	>8	4	64	≤0.25	>8	≤0.5	4	J

CONCLUSIONS

- The *S. mitis* group was the most commonly isolated viridans group streptococci collected from patients in medical centers in Europe, Israel and Turkey; and was more resistant to commonly prescribed antimicrobial agents compared to the other species groups.

- Although identification of viridans group streptococci to the species level can be problematic, the overall susceptibility patterns of this pathogen group must be assessed by local laboratories due to the wide variation noted among countries monitored in this EU-area study.

- Viridans group streptococci isolated in Turkey appear to be consistently more resistant to ß-lactams and macrolides compared to other countries, although the patient population (pediatric) and clonally disseminated strains may have a significant role in the overall susceptibility profile.

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