

Checking For Incidence of Extended Spectrum Beta-Lactamase (ESBL) Producing Enterobacteriaceae, Vancomycin Resistant *Enterococcus Faecium* (VREF) and Methicillin Resistant *Staphylococcus Aureus* (MRSA) in 26 Centers from 12 European Countries: The Pan-European Antimicrobial Resistance Using Local Surveillance (PEARLS) Study

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Revised Abstract

Background: Resistant isolates found in the hospital environment continue to increase and are of great concern in the treatment of serious infections. This study is part of an ongoing study examining resistance determinants in common pathogens. The current incidence of ESBL producers in selected Enterobacteriaceae, VREF and MRSA were measured in 26 investigative sites from 12 European countries. The information gathered from the PEARLS study will be useful in determining selective pressures that influence the increasing incidence of resistance. **Methods:** Each site collected consecutive isolates consisting of no more than 50 *Enterococcus faecium*, 100 *Enterobacter* species, 75 *Escherichia coli*, 75 *Klebsiella pneumoniae*, 25 *Staphylococcus aureus*. All isolates were tested in a central reference laboratory using broth microdilution following manufacturer's instructions and NCCLS guidelines. ESBL producers were screened for cephalosporin MICs ≥ 1 mcg/ml for cefotaxime, ceftazidime or ceftriaxone and confirmed using NCCLS guidelines. **Results:** There were 6,217 isolates available for analysis. The overall incidence of VREF and MRSA was 9.2% and 34.6%, respectively. *Klebsiella pneumoniae* (16.3%) was the highest producer of ESBL followed by *Escherichia coli* (4.0%). The total ESBL rate for the Enterobacteriaceae (*Klebsiella pneumoniae* plus *Escherichia coli*) was 9.6%. ESBL production rates (*Klebsiella pneumoniae* plus *Escherichia coli*) were observed in the following countries: Greece, 35.5%; Italy, 18.7%; South Africa, 17.3%; Turkey, 11.7%; Portugal, 9.8%; Spain, 6.9%; Germany, 4.3%; Austria, 3.9%; Switzerland, 3.6%; Belgium, 3.0%; The Netherlands, 2.1%; France, 0.0%. The highest occurrences of VREF were in Portugal (58.6%) and Austria (16.3%). No occurrences of VREF were seen in Belgium, France, Greece, South Africa and Turkey. MRSA rates varied from country to country with a high of 88.5% in Portugal to a low of 2.0% in The Netherlands. **Conclusion:** Resistant rates are affected by many factors including patient population, prior antimicrobial therapy, prolonged hospitalization, formulary changes and regional differences, among other factors. The rates of these three determinants of resistance are consistent with current literature for VREF, MRSA and ESBL in Europe but vary greatly by country. These data will serve as a baseline for determining the effects of selective pressures that influence resistance rates over time in these centers.

Introduction

The increasing occurrence of infections with antibiotic-resistant microorganisms has required the development of flexible and timely surveillance systems for monitoring these problems. The Pan-European Antimicrobial Resistance Using Local Surveillance (PEARLS) study is an ongoing surveillance to examine the resistance determinants and patterns of common pathogens. We attempt to set a baseline for the current incidence of ESBL producers in selected Enterobacteriaceae, VREF and MRSA in Europe. These data will be used to identify selective pressures and determinants affecting the incidence of drug resistance.

Materials and Methods

- Isolates were collected between Feb. 2001 and Dec. 2001 from 26 study centers in 12 European countries.
- All isolates were derived from blood, respiratory tract, urine (no more than 30% of all isolates), skin, wound, fluids, and other defined sources. Only one isolate per patient was accepted.
- Organism collection, transport, confirmation of organism identification, antimicrobial susceptibility testing and ESBL determination, as well as construction and management of a centralized database, was coordinated by International Health Management Associates, Inc. (IHMA, Rolling Meadows, IL)

Antimicrobial Susceptibility Testing

- MICs were determined by the NCCLS recommended broth microdilution testing method.¹ The microbroth dilution panels used in this study were purchased from Microscan (Dade Behring Inc. Sacramento, CA, USA.)
- Quality Control of Microscan panels included the following ATCC strains: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *Enterococcus faecalis* ATCC 29212.
- Escherichia coli* and *Klebsiella pneumoniae* were screened and confirmed for ESBL activity according to NCCLS guidelines² (table 2A, M100-S11)
- Preliminary ESBL activity was determined by screening cefotaxime, ceftazidime, and ceftriaxone with MICs ≥ 1 using microbroth dilution panels.
- ESBL activity was confirmed by testing the following antibiotic disks: cefotaxime (30 mg), cefotaxime/clavulanic acid (30/10mg), and ceftazidime (30mg), ceftazidime/clavulanic acid (30/10mg). Antibiotic disks were manufactured by Oxoid Inc. Ogdensburg, New York. Mueller-Hinton agar used in testing was manufactured by Remel Inc. Lenexa, Kansas.
- An organism is interpreted as containing an ESBL if there is an increase of ≥ 5 mm in the inhibition zone of the combination disc when compared to that of the cephalosporin alone: cefotaxime/clavulanic acid – cefotaxime ≥ 5 mm or ceftazidime/clavulanic acid – ceftazidime ≥ 5 mm.
- Quality control of antibiotic disks followed manufacturers guidelines (Oxoid) using the following ATCC strains: *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 35922.

Results

Results are shown in the following Tables and Graphs.

Table 1. Incidence (%) of Extended Spectrum Beta-Lactamase (ESBL) Producing Enterobacteriaceae (*E. coli/K. pneumoniae*), Vancomycin-Resistant *Enterococcus Faecium* (VREF) and Methicillin-Resistant *Staphylococcus Aureus* (MRSA) from 6,217 isolates collected in 26 Centers from 12 European Countries.

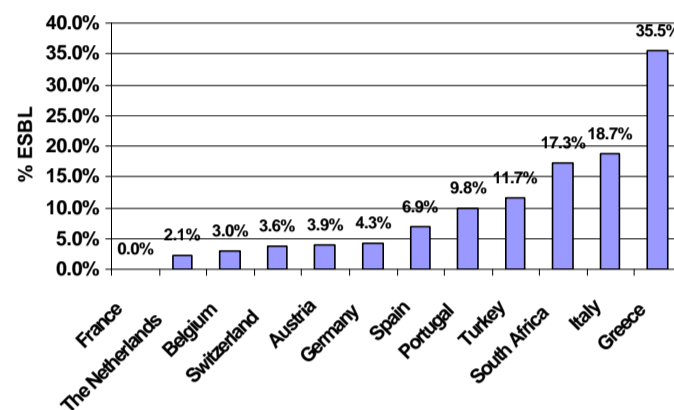
Organism	Phenotype	n	Total N	% Total
<i>Escherichia coli</i>	ESBL ¹	71	1760	4.0
<i>Klebsiella pneumoniae</i>	ESBL ¹	241	1483	16.3
<i>Enterococcus faecium</i>	VREF	58	629	9.2
<i>Staphylococcus aureus</i>	MRSA	225	650	34.6

¹ Using the antimicrobial disk Phenotypic Confirmatory Tests for ESBL's as described for *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Escherichia coli* in NCCLS documents (M100-S10, Table 2A).

Table 2. Incidence (%) of Extended Spectrum Beta-Lactamase (ESBL) in 3,243 *Escherichia coli* and *Klebsiella pneumoniae* Isolates Categorized by Country

Country	Organism	n	Total N	% ESBL
Austria	<i>Escherichia coli</i>	3	153	2
	<i>Klebsiella pneumoniae</i>	7	106	6.6
Belgium	<i>Escherichia coli</i>	0	110	0
	<i>Klebsiella pneumoniae</i>	6	89	6.7
France	<i>Escherichia coli</i>	0	99	0
	<i>Klebsiella pneumoniae</i>	0	80	0
Germany	<i>Escherichia coli</i>	3	196	1.5
	<i>Klebsiella pneumoniae</i>	13	179	7.3
Greece	<i>Escherichia coli</i>	19	81	23.5
	<i>Klebsiella pneumoniae</i>	31	60	51.7
Italy	<i>Escherichia coli</i>	30	281	10.7
	<i>Klebsiella pneumoniae</i>	76	285	26.7
Portugal	<i>Escherichia coli</i>	5	161	3.1
	<i>Klebsiella pneumoniae</i>	23	126	18.3
South Africa	<i>Escherichia coli</i>	4	131	3.1
	<i>Klebsiella pneumoniae</i>	39	118	33.1
Spain	<i>Escherichia coli</i>	3	285	1.1
	<i>Klebsiella pneumoniae</i>	33	240	13.8
Switzerland	<i>Escherichia coli</i>	0	74	0
	<i>Klebsiella pneumoniae</i>	4	38	10.5
The Netherlands	<i>Escherichia coli</i>	4	152	2.6
	<i>Klebsiella pneumoniae</i>	2	139	1.4
Turkey	<i>Escherichia coli</i>	0	37	0
	<i>Klebsiella pneumoniae</i>	7	23	30.4

Figure 1. Overall Incidence (%) of ESBL Producers (*E. coli/K. pneumoniae*) Graphed in Ascending Order By Country¹



¹ ESBL rates combined for both *E. coli* and *K. pneumoniae*.

Table 3. Incidence (%) of Vancomycin-Resistant *Enterococcus faecium* (VREF) in 627 Isolates Categorized by Country

Country	n	Total N	% VREF
Austria	8	49	16.3
Belgium	0	14	0
France	0	54	0
Germany	3	72	4.2
Greece	0	8	0
Italy	7	187	3.7
Portugal	34	58	58.6
South Africa	0	18	0
Spain	3	101	3
Switzerland	0	7	0
The Netherlands	3	52	5.8
Turkey	0	7	0

Figure 2. Overall Incidence (%) of Vancomycin-Resistant *Enterococcus faecium* (VREF) Graphed in Ascending Order By Country

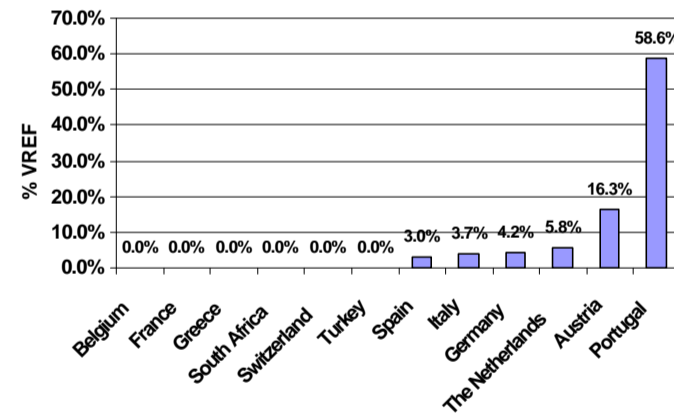
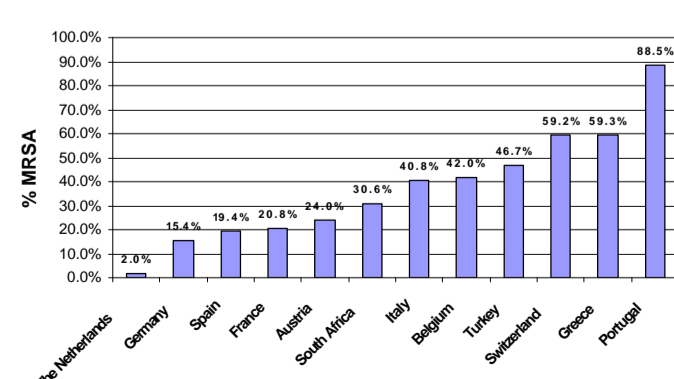


Table 4. Incidence (%) of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in 650 *Staphylococcus aureus* Categorized by Country

Country	n	Total N	% Total
Austria	12	50	24
Belgium	21	50	42
France	5	24	20.8
Germany	12	78	15.4
Greece	16	27	59.3
Italy	40	98	40.8
Portugal	46	52	88.5
South Africa	15	49	30.6
Spain	21	108	19.4
Switzerland	29	49	59.2
The Netherlands	1	50	2
Turkey	7	15	46.7

Figure 3. Overall Incidence (%) of Methicillin-Resistant *Staphylococcus aureus* (MRSA) Graphed in Ascending Order By Country



Discussion

The incidence of ESBL producing Enterobacteriaceae, VRE and MRSA vary widely one region to the next and even from hospital to hospital within the same region. Current reports place the incidence of ESBL

producing *Klebsiella* in Europe between 23% and 25% from 1994 to 1998 [3, 4]. While our initial screening for ESBL (cefotaxime, ceftazidime, or ceftriaxone MICs ≥ 1 mcg/ml) concurred with identical results of 25%, further testing using the NCCLS recommended phenotypic confirmatory test (cefotaxime or ceftazidime with and without clavulanic acid) identified the ESBL rate for *Klebsiella pneumoniae* at 16.3%. Using the same screening and confirmatory methodology, the rate of ESBL producers for *E. coli* and *Enterobacter* spp. was 4.0% and 9.5%, respectively. However, the NCCLS screening or confirmatory tests are not specific for ESBLs in the *Enterobacter* spp. and initial PCR molecular detection methods have determined the actual incidence of ESBL production in *Enterobacter* spp. may be closer to 3% based upon initial sequencing data. Further testing (PCR, iso-electric focusing and DNA sequencing) is being conducted to determine the relative rate of ESBL production from the *Enterobacter* spp. collected from this study.

Since the late 1980's when the first reports of vancomycin-resistant enterococci (VRE) appeared, geographic distribution and the importance of VRE as a nosocomial pathogen have increased worldwide. For example, the incidence of VRE in the United States has increased from 0.3% to 10.8% in intensive care units from 1989 to 1995 [5]. Reports of VRE incidence are difficult to ascertain since many reports focus on colonization rates. VRE incidence rates in Europe range from none in Croatia and Turkey to 1.5% in Germany [6, 7, 8, 9]. We found the overall incidence of VREF in Europe to be 9.2%, highest in Portugal (58.6%) and Austria (16.3%).

The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) varies in different European countries, in different cities in the same country, or even within a city or a hospital [9]. Current surveillance reports place MRSA rates as high as 50% in Europe [10]. The overall incidence of MRSA in this study is 34.6% with wide variation among the countries reporting a high of 88.5% in Portugal and a low of 2.0% in the Netherlands.

Conclusions

- Current incidence of ESBL producers is 9.6% for *E. coli* and *K. pneumoniae* in Europe.
- The incidence of ESBL in *Enterobacter* species is currently undefined. However, additional molecular studies are needed to further define ESBL in this species.
- The overall incidence of VREF in Europe is 9.2%. Portugal and Austria reported the highest incidence of VREF, 58.6% and 16.3%, respectively.
- MRSA comprise 34.6% of the European isolates studied with wide variation among countries.

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