

Regional Incidence of Extended Spectrum Beta-Lactamase (ESBL) Producing Enterobacteriaceae, Vancomycin Resistant *Enterococcus Faecium* (VREF), and Methicillin Resistant *Staphylococcus Aureus* (MRSA) from 36 Centers in 16 Countries: The Pan-European Antimicrobial Resistance Using Local Surveillance (PEARLS)

S.K. Bouchillon¹, B.M. Johnson¹, D.J. Hoban¹, J.L. Johnson¹, M.J. Dowzicky², D.H. Wu², K. Focht², P.A. Bradford³

¹Laboratories International for Microbiology Studies, Schaumburg, IL; ²Wyeth-Ayerst Pharmaceuticals, St. Davids, PA; ³Wyeth-Ayerst Research, Pearl River, NY

Revised Abstract

Background: The current incidence of ESBL producers in selected Enterobacteriaceae, VREF and MRSA were determined from 36 centers in 16 countries from Jan 2001 to August 2002. The PEARLS study will be useful in determining selective pressures that influence the increasing incidence of resistance. **Methods:** The 10,627 isolates were tested in a central reference laboratory using broth microdilution following manufacturer's instructions and NCCLS guidelines. **Results:** The overall incidence of VREF and MRSA was 8.3% and 32.2%, respectively. The ESBL rate for Enterobacteriaceae: *K.pneumoniae* (16.6%) plus *E.coli* (5.1%) was 10.3%. The highest rates of ESBL occurrence (*K.pneumoniae* plus *E. coli*) per country were Egypt, 40.9%; Greece, 33.2%; Saudi Arabia, 18.7%; Italy, 18.0%; South Africa, 17.3%; Lebanon, 15.7%; Turkey, 14.2%; Portugal, 9.8%; Spain, 6.8%; Belgium, 6.6%; Austria, 3.8%; Germany, 3.6%; Switzerland, 3.6%; The Netherlands, 2.0%; France, 0.3%. Portugal, 34/58 isolates (58.6%), exhibits an unusually high occurrence of VREF; no VREF's were recorded in Belgium, Egypt, France, Greece, Lebanon, Saudi Arabia, Slovenia, South Africa and Switzerland. MRSA rates varied with a high of 46/52 (88.5%) in Portugal to 0/25 (0%) in Saudi Arabia. **Conclusion:** The rates of these three determinants of resistance are consistent with current literature for VREF, MRSA and ESBL 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. This study attempts to set a baseline for the current incidence of ESBL producers in selected Enterobacteriaceae, VREF and MRSA in a multi-center, multi-country study. These data will be used to identify selective pressures and determinants affecting the incidence of drug resistance.

Materials and Methods

- Isolates were collected between February 2001 and August 2002 from 36 study centers in 16 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, Schaumburg, IL)

Antimicrobial Susceptibility Testing

- MICs were determined by the NCCLS recommended broth microdilution testing method.¹ The broth microdilution 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 mcg), cefotaxime/clavulanic acid (30/10mcg), and ceftazidime (30mcg), ceftazidime/clavulanic acid (30/10mcg). 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 manufactures guidelines (Oxoid) using the following ATCC strains: *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922.

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 5,881 selected isolates collected in 36 Centers from 16 Countries.

Organism	Phenotype	n	Total N	% Total
Enterobacteriaceae (<i>Kleb+Ecoli</i>)	ESBL ¹	438	4,252	10.3
<i>Escherichia coli</i>	ESBL ¹	118	2,326	5.1
<i>Klebsiella pneumoniae</i>	ESBL ¹	320	1,926	16.6
<i>Enterococcus faecium</i>	VREF	67	803	8.3
<i>Staphylococcus aureus</i>	MRSA	266	826	32.2

¹ Using the antimicrobial disk Phenotypic Confirmatory Tests for ESBL's as described for *Klebsiella pneumoniae* and *Escherichia coli* in NCCLS documents (M100-S11, Table 2A).

Table 2. Incidence (%) of Extended Spectrum Beta-Lactamase (ESBL) in 4,252 *Escherichia coli* and *Klebsiella pneumoniae* Isolates Categorized by Country, n/Total N (%).

Country	<i>E. coli</i> ESBL ¹	<i>K. pneumoniae</i> ESBL ¹	<i>E. coli</i> + <i>K. pneumoniae</i> ESBL ¹
Austria	3/154 (1.9)	7/106 (6.6)	10/260 (3.8)
Belgium	3/151 (2)	15/122 (12.3)	18/273 (6.6)
Egypt	6/14 (42.9)	3/8 (37.5)	9/22 (40.9)
France	0/171 (0)	1/151 (0.7)	1/322 (0.3)
Germany	4/272 (1.5)	14/229 (6.1)	18/501 (3.6)
Greece	29/123 (23.6)	40/85 (47.1)	69/208 (33.2)
Italy	30/302 (9.9)	77/294 (26.2)	107/596 (18)
Lebanon	8/50 (16)	3/20 (15)	11/70 (15.7)
Portugal	5/161 (3.1)	23/126 (18.3)	28/287 (9.8)
Saudi Arabia	16/81 (19.8)	12/69 (17.4)	28/150 (18.7)
Slovenia	0/70 (0)	16/69 (23.2)	16/139 (11.5)
South Africa	4/131 (3.1)	39/118 (33.1)	43/249 (17.3)
Spain	3/288 (1)	33/240 (13.8)	36/528 (6.8)
Switzerland	0/74 (0)	4/38 (10.5)	4/112 (3.6)
The Netherlands	4/153 (2.6)	2/142 (1.4)	6/295 (2)
Turkey	3/131 (2.3)	31/109 (28.4)	34/240 (14.2)
Total	118/2326 (5.1)	320/1926 (16.6)	438/4252 (10.3)

¹ Using the antimicrobial disk Phenotypic Confirmatory Tests for ESBL's as described for *Klebsiella pneumoniae* and *Escherichia coli* in NCCLS documents (M100-S11, Table 2A).

Figure 1. Overall Incidence (%) of Extended Spectrum Beta-Lactamase (ESBL) Producers in 4,252 *Escherichia coli* and *Klebsiella pneumoniae* Isolates Graphed in Ascending Order By Country

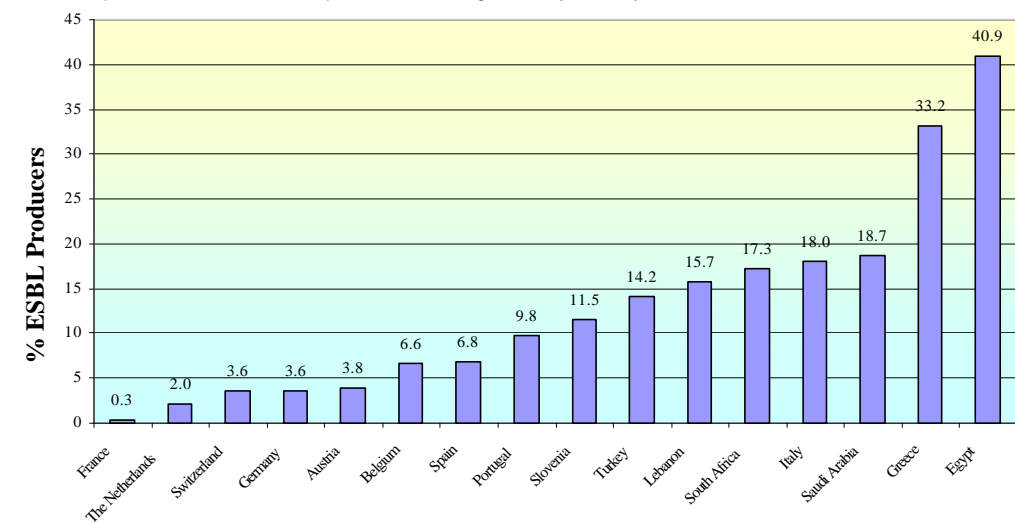


Table 3. Incidence (%) of Vancomycin-Resistant *Enterococcus faecium* (VREF) in 803 *Enterococcus faecium* Isolates from 36 Centers in 16 Countries Categorized by Country

Country	VREF	Total N	% VREF
Austria	9	61	14.8
Belgium	0	21	0.0
Egypt	0	2	0.0
France	0	75	0.0
Germany	9	112	8.0
Greece	0	8	0.0
Italy	8	200	4.0
Lebanon	0	2	0.0
Portugal	34	58	58.6
Saudi Arabia	0	3	0.0
Slovenia	0	42	0.0
South Africa	0	18	0.0
Spain	3	127	2.4
Switzerland	0	7	0.0
The Netherlands	3	52	5.8
Turkey	1	15	6.7
Total	67	803	8.3

Figure 2. Overall Incidence (%) of Vancomycin-Resistant *Enterococcus faecium* (VREF) in 803 *Enterococcus faecium* Isolates from 36 Centers in 16 Countries Graphed in Ascending Order By Country

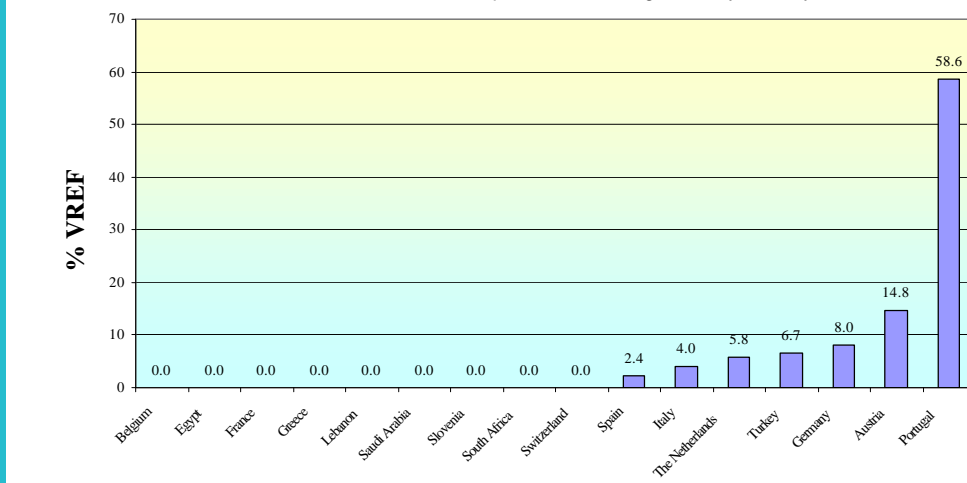
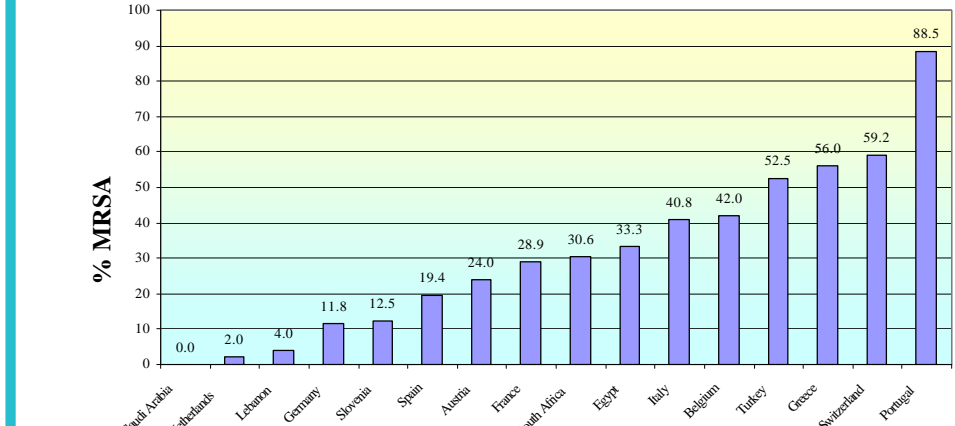


Table 4. Incidence (%) of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in 826 *Staphylococcus aureus* Isolates from 36 Centers in 16 Countries Categorized by Country

Country	MRSA	Total N	% MRSA
Austria	12	50	24.0
Belgium	21	50	42.0
Egypt	3	9	33.3
France	13	45	28.9
Germany	12	102	11.8
Greece	28	50	56.0
Italy	40	98	40.8
Lebanon	1	25	4.0
Portugal	46	52	88.5
Saudi Arabia	0	25	0.0
Slovenia	3	24	12.5
South Africa	15	49	30.6
Spain	21	108	19.4
Switzerland	29	49	59.2
The Netherlands	1	50	2.0
Turkey	21	40	52.5
Total	266	826	32.2

Figure 3. Overall Incidence (%) of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in 826 *Staphylococcus aureus* Isolates from 36 Centers in 16 Countries Graphed in Ascending Order By Country



Discussion

The incidence of ESBL producing Enterobacteriaceae, VRE and MRSA vary widely from region to region 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 (ceftazidime or cefotaxime with and without clavulanic acid) identified the ESBL rate for *Klebsiella pneumoniae* at 16.6%. Using the same screening and confirmatory methodology, the rate of ESBL producers for *E. coli* and *Enterobacter* spp. was 5.1% (118/2326) and 9.1% (190/2093), respectively. However, the NCCLS screening and 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 DNA sequencing data. Further testing (PCR, isoelectric focusing and DNA sequencing) is being conducted to determine the relative rate of ESBL production from the *Enterobacter* spp. collected from this study and will be presented in future publications.

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]. However, the actual incidence of VRE infection or isolation rates is difficult to ascertain since many reports focus on colonization and carrier rates. From previous publications 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 this study to be 8.3%, highest in Portugal (58.6%) and Austria (14.8%).

The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) also varies widely from country to country, city to city in the same country, or hospital to hospital within a city [9]. Current surveillance reports place MRSA rates as high as 50% in Europe [10]. The overall incidence of MRSA in this study is 32.2% with wide variation among the countries reporting a high of 88.5% in Portugal and a low of 0% in Saudi Arabia.

Conclusions

- Current incidence of ESBL producers is 10.3% for *E. coli* and *K. pneumoniae* in this multi-center, 16 country study.
- The overall incidence of VREF in this study is 8.3%. Portugal and Austria reported the highest incidence of VREF, 58.6% and 14.8%, respectively.
- MRSA comprise 32.2% of the isolates studied with wide variation among countries.
- The incidence of ESBL in *Enterobacter* species is currently undefined. Additional molecular tests are needed to further define ESBL production in this genera.

References

- National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Villanova, PA, USA: NCCLS, 1997: approved standard M7-A4.
- National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*. Villanova, PA, USA: NCCLS, 2001: approved standard M100-S11.
- Winkler PL, Canton R, Casellas JM, Legakis N. *Variations in the prevalence of strains expressing an extended-spectrum beta-lactamase phenotype and characterization of isolates from Europe, the Americas and the Western Pacific region*. Clin Infect Dis 2001, 32(Suppl 2):S84-103.
- Babini GS, Livermore DM. *Antimicrobial resistance amongst Klebsiella spp. Collected from intensive care units in Southern and Western Europe in 1997 - 1998*. J Antimicrob Chemother 2000, 45(2):183-9.
- Gaynes R, Edwards J, the NNIS system. *Nosocomial vancomycin-resistant enterococci in the United States, 1989-1995: the first 1000 isolates* [abstract]. Infect Control Hosp Epidemiol 1996, 17:18.
- Barisic Z, Punda-Polic V. *Antibiotic resistance among enterococcal strains isolated from clinical specimens*. Int J Antimicrob Agents 2000, 16(1):65-68.
- Ozkuyumcu C. *Resistant enterococci: prevalence and factors associated with colonization in a Turkish university hospital*. Acta Microbiol Pol 1999, 48(2):203-7.
- Reiner RR, Conrads G, Schlaeger JJ, Werner G, Witte W, Luticken R, Klare I. *Survey of antibiotic resistance among enterococci in North Rhine-Westphalia, Germany*. J Clin Microbiol 1999, 37(5):1638-41.
- Cookson B. *Aspects of the epidemiology of MRSA in Europe*. J Chemother 1995, 7(Suppl 3):93-8.
- Hoban DJ, Bouchillon SK, Johnson JL, Zhanel GG, Butler DL, Miller LA, Poupard JA; *Gemifloxacin Surveillance Study Research Group*. *Comparative in vitro potency of gemifloxacin and fluoroquinolones against recent European clinical isolates from a global surveillance study*. Eur J Clin Microbiol Infect Dis 2001, 20(11):814-9.

Acknowledgements

This study was sponsored by Wyeth-Ayerst Pharmaceuticals. We acknowledge our appreciation for the contributions of the following investigators: Prof. Franz Allerberger; Prof. Dr. Apostolos Georgopoulos; Prof. Marc Struelens; Prof. Herman Goossens; Dr. Sohair Abdul Latif; Dr. Jacqueline Nguyen; Dr. Daniel Talon; Dr. Helene Jean Pierre; Dr. Stefan Ziesing; Prof. Dr. Harald Seifert; Dr. Marianne Horn; Prof. Dr. Heinrich K Geiss; Prof. Helen Giannarelli; Prof. Paul Nikkaldis; Prof. Gian Carlo Schito; Prof. Giovanni Fadda; Dr. Francesco Luzzaro; Prof. Daniela Cirillo; Dr. George Araj; Dr. Jose Correia da Fonseca; Dr. Dario Costa; Dr. Ziad Memish; Dr. Manica Muller-Premru; Prof. H. Crewe-Brown; Prof. Lynne Liebowitz; Dr. Rogelio Martin; Dr. Miguel Gobernado; Dr. Emilia Cercenado; Dr. Javier Aznar; Prof. Jorge Garbino; Prof. Jan Verhoef; Prof. Dr. J.A.A. Hoogkamp-Korstanje; Prof. Dr. Serhat Unal; Dr. Volkan Korten; Dr. Mesut Yilmaz; Prof. Dr. Mehmet Ali Ozinel.