

# Incidence Of Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae (ESBL), Vancomycin Resistant Enterococcus Faecium (VREF) And Methicillin Resistant Staphylococcus Aureus (MRSA) In France Through the Pan-European Antimicrobial Resistance Using Local Surveillance (PEARLS) Study

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## Revised Abstract

**Background:** ESBL isolates were first reported in France in 1986. Since that time, ESBL rates have steadily decreased in this country. At the same time, it appears that the VREF and MRSA rates are also decreasing in France. The PEARLS study was designed to determine the current levels of ESBL, VREF and MRSA in France and compare those values to rates obtained in prior studies during the 1990s. **Methods:** Two laboratories in France were asked to collect and submit the following isolates to a central laboratory: 75 *Escherichia coli* (EsC); 75 *Klebsiella pneumoniae* (KP); 50 *Enterococcus faecium* (EF); 50 *Enterobacter aerogenes* (EA); 50 *Enterobacter cloacae* (EC); 25 *Pseudomonas aeruginosa* (PA); 25 *Staphylococcus aureus* (SA). Isolates were submitted from January, 2001 through October, 2001. All isolates were analyzed by the central laboratory for ESBL, VREF and MRSA characteristics. Antimicrobial susceptibility testing was performed and MICs determined using broth microdilution. **Results:** The ESBL rates decreased from 3.2% in 1998 (C. De Champs) to 0.0% in this study, a three fold decrease over a three year period. The number of ESBL isolates of EsC in 1998 had been reported as low as 0.2%. The results of this study showed a decrease to 0.0%. The ESBL rate for EC has decreased from 6.7% in 1998 to 0.0%. In addition, the ESBL rate for KP was 0.0%, respectively. The MRSA activity appears to have decreased from 42% in 1992 to 37% in 1997 to 20.8% in this study. No VREFs were found at either of these institutions. **Conclusions:** The ESBL rates have been decreasing for the past decade. A continued surveillance program is required to monitor this situation as well as monitor the VREF and MRSA rates in France.

## 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 PEARLS study is an ongoing surveillance to examine the resistance determinants and patterns of common pathogens.

It has also been designed to determine the ESBL, VREF and MRSA rates in France and other countries. Over many years several studies have been conducted to determine these rates in France. The data presented in this paper shows and confirms trends in the ESBL, VREF and MRSA rates in France when compared to data reported from other studies. 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 Oct 2001 from 2 study centers in France.
- 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.<sup>1</sup> 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 tested for ESBL activity according to NCCLS guidelines<sup>2</sup> (table 2A, M100-S11)
- Preliminary ESBL activity was determined by screening cefotaxime, ceftazidime, and ceftriaxone with MICs  $\geq 1$  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  $\geq 5$ mm in the inhibition zone of the combination disc when compared to that of the cephalosporin alone: cefotaxime/clavulanic acid - cefotaxime  $\geq 5$  mm or ceftazidime/clavulanic acid - ceftazidime  $\geq 5$  mm.
- Quality control of antibiotic disks followed manufactures 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. Comparison of Extended Spectrum  $\beta$ -Lactamase (ESBL) Rates for *Escherichia coli* in France from 1990 to 2001.

| Study Results           | Year | "n" ESBL | Total "n" | %ESBL | Reference |
|-------------------------|------|----------|-----------|-------|-----------|
| <i>Escherichia coli</i> | 2001 | 0        | 147       | 0.0%  | PEARLS    |
|                         | 1997 | -        | -         | 0.5%  | 4         |
|                         | 1996 | -        | -         | 0.1%  | 3         |
|                         | 1991 | -        | -         | 0.9%  | 3         |
|                         | 1990 | -        | -         | 1.5%  | 3         |

Table 2. Comparison of Extended Spectrum  $\beta$ -Lactamase (ESBL) Rates in *Klebsiella pneumoniae* in France from 1991 to 2001.

| Study Results                | Year | %ESBL | "n" ESBL | Total "n" | Reference |
|------------------------------|------|-------|----------|-----------|-----------|
| <i>Klebsiella pneumoniae</i> | 2001 | 0.0%  | 0        | 92        | PEARLS    |
|                              | 1998 | 9.4%  | -        | -         | 3         |
|                              | 1997 | 15.0% | -        | -         | 4         |
|                              | 1996 | 17.7% | -        | -         | 3         |
|                              | 1994 | 22.2% | -        | -         | 3         |
|                              | 1991 | 9.6%  | -        | -         | 3         |

Figure 1. Comparison of Extended Spectrum  $\beta$ -Lactamase (ESBL) Rates for *Escherichia coli* in France from 1990 to 2001.

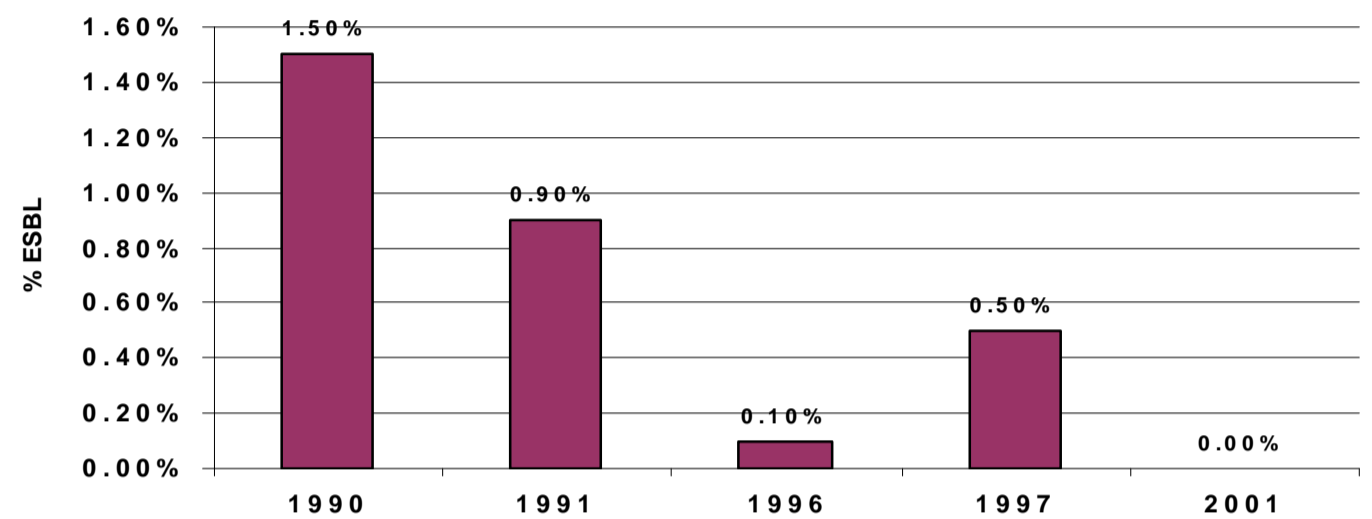


Figure 2. Comparison of Extended Spectrum  $\beta$ -Lactamase (ESBL) Rates in *Klebsiella pneumoniae* in France from 1991 to 2001.

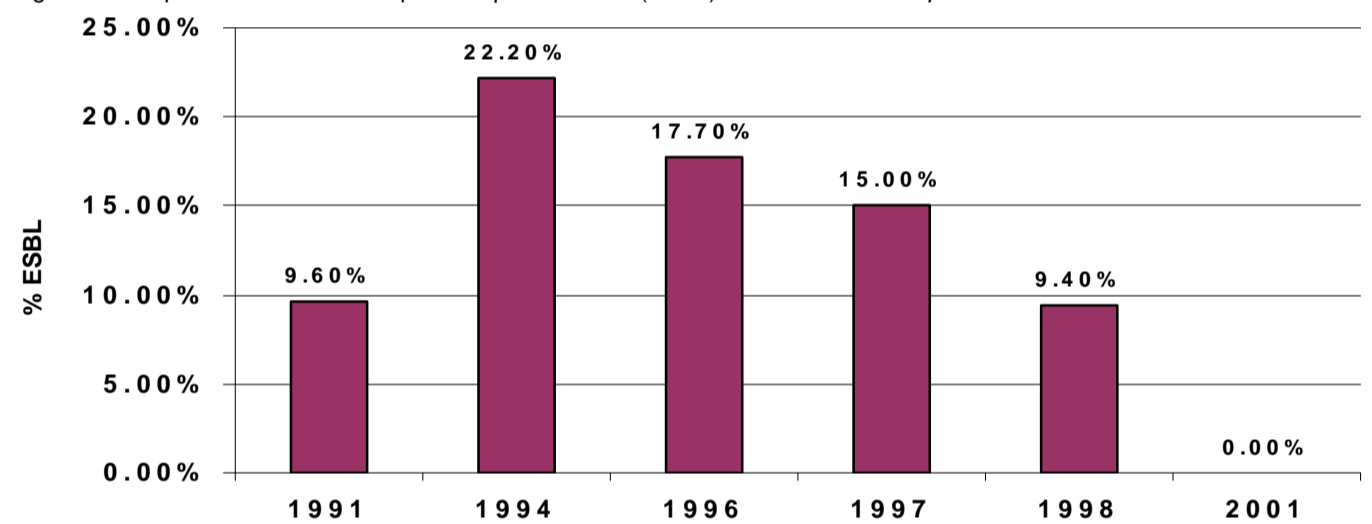


Table 3. Comparison of Methicillin-Resistant *Staphylococcus aureus* in France from 1992 to 2001.

| Study Results                       | Year | %MRSA | "n" MRSA | Total "n" | Reference |
|-------------------------------------|------|-------|----------|-----------|-----------|
| <i>Staphylococcus aureus</i> , MRSA | 2001 | 20.8% | 5        | 24        | PEARLS    |
|                                     | 1997 | 37.0% | -        | -         | 4         |
|                                     | 1992 | 42.0% | -        | -         | 4         |

Table 4. Comparison of Vancomycin Resistant *Enterococcus faecium* (VREF) Rates in France from Two Separate Studies 1993 to 2001.

| Study Results               | Year | "n" VREF | Total "n" | %VREF | Reference |
|-----------------------------|------|----------|-----------|-------|-----------|
| <i>Enterococcus faecium</i> | 2001 | 0        | 55        | 0.0%  | PEARLS    |
|                             | 1993 | -        | -         | 7.5%  | 4         |

## Discussion

The ESBL rates of *E. coli* in France have been very low for the past twelve years. Ninety-nine isolates were obtained from the two centers in France for the PEARLS study. None of these isolates exhibited ESBL activity. Only one study showed a slight increase from 1996 to 2001 [4].

The ESBL rates of *K. pneumoniae* in France have been decreasing since 1994[3, 4]. Eighty isolates were obtained from the two centers in France for the PEARLS study. None of these isolates exhibited ESBL activity.

The incidence of MRSA from the PEARLS study (20.8%) is consistent with the decreasing MRSA rates for France from a previous study [4]. Twenty-nine isolates of *Staphylococcus aureus* were obtained. Four of those isolates were methicillin resistant.

The VREF rates in France have not been as well published as the other three categories, however, the rate has decreased since 1993. Fifty-five isolates of *E. faecium* were collected for the PEARLS study and none exhibited vancomycin resistant activity.

*Enterobacter cloacae* and *Enterobacter aerogenes* were collected and screened for ESBL activity, however, the NCCLS screening and confirmatory tests are not specific for ESBLs in the *Enterobacter* spp. As a result, initial PCR molecular detection methods have determined an incidence of ESBL production for the *Enterobacter* spp. approaching 13% 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.

## Conclusions

- The ESBL rate for *E. coli* is remaining constant for the past twelve years with a high of 1.5% in 1990 [3] to a low of 0.0% in 2001.
- The ESBL rate for *K. pneumoniae* is steadily decreasing in France.
- Future studies are needed to monitor the ESBL, VREF and MRSA activity in France.

## References

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