Chapter 3

Standardisation of *Streptococcus pneumoniae* and *Staphylococcus aureus* susceptibility data within EARSS

**Introduction**

In several countries of the European Union increased resistance of micro-organisms to antimicrobial agents is reported; the rise of methicillin-resistant *Staphylococcus aureus* infections, the occurrence of vancomycin resistant enterococci and the presence of penicillin-resistant *Streptococcus pneumoniae* cause severe problems. In a geographical context it seems that resistance problems become urgent especially in southern European countries. From an epidemiological and methodological standpoint the comparison of antimicrobial resistance from different countries is very difficult. Reasons for these difficulties are that:

1. Different antimicrobial agents are tested.
2. Different systems for antimicrobial susceptibility testing are used.
3. Different breakpoints for antimicrobial susceptibility are used.
4. Data from point prevalence studies are used for longitudinal comparisons; e.g. studies on antibiotic resistance performed in 1970 and 1990 are compared, in spite of differences in study conditions and methodology.
5. Only the resistant strains are tested.
6. Differences between the prevalence of resistant strains from local practices and university hospitals are not taken into account.

In order to obtain more comparable and validated data, the European Commission has funded a European Antimicrobial Resistance Surveillance System (EARSS). In this chapter we present how susceptibility data of *Staphylococcus aureus* and *Streptococcus pneumoniae* within EARSS are standardised in order to address difficulties as mentioned above.

**Methods**

During the feasibility phase of EARSS it is important to use a limited number of pathogenic bacterial species, in order to manage the set-up of the surveillance system. More than 400 laboratories have agreed to participate in this European surveillance network. EARSS depends on national surveillance data, so input of the participants of the different member states is essential. The methodology of the surveillance system was decided during the first plenary EARSS meeting with all national representatives (May 18-20, 1998). The first decision to be taken was which species are to be included under surveillance. Before the meeting a working group prepared a discussion paper with objective criteria for selection. The rationale of selection of a community acquired pathogen and a hospital-acquired (nosocomial) pathogen for the pilot phase of EARSS is summarised in the discussion paper:
1. Relevance for Public Health. Most participants believe that \textit{S. pneumoniae} and \textit{S. aureus} are the most relevant species. They are both proven pathogens, clinically relevant on population level for the community or the hospitalised population, have a high potential for spread in community and/or hospital setting and are known to acquire resistance against currently used and recommended antibiotics. Other relevant species are \textit{Campylobacter jejuni}, \textit{Haemophilus influenzae}, \textit{Streptococcus pyogenes}, \textit{Pseudomonas aeruginosa} and \textit{Escherichia coli}.

2. Political Interest. Most participants believe that especially \textit{S. aureus}, \textit{S. pneumoniae} and \textit{Enterococcus faecium/faecalis} are of interest to policy makers. These species are the ones most often attracting media and political attention. In addition, in relation to the present discussion on the use of fluoroquinolones in animals and humans, resistance in micro-organisms such as \textit{Salmonella typhimurium}, \textit{C. jejuni} and \textit{E. coli} are of interest.

3. Availability of quantitative data. The participants believe that quantitative resistance data in sufficient numbers are present for several species, including \textit{S. aureus}, \textit{S. pneumoniae}, \textit{H. influenzae} and \textit{E. coli}.

4. Reliability of data. For some species, such as \textit{E. coli} in urinary tract infections, sample bias can occur in resistance surveillance. Physicians only send isolated bacteria to the laboratories when they have patients with treatment failures. For \textit{S. aureus} and \textit{S. pneumoniae} blood isolates that always cause patients to be severely ill, irrespective of the susceptibility of the isolate, it is to be expected that clinicians in the hospital send all isolates for susceptibility testing.

5. Quality assurance. \textit{S. aureus}, \textit{S. pneumoniae}, \textit{E. faecium/faecalis}, \textit{E. coli}, \textit{P. aeruginosa} and \textit{H. influenzae} are common micro-organisms and are often included in quality assurance systems. Therefore susceptibility data for these species are normally quality-assured.

6. Interaction with other resistance surveillance systems. For some species such as \textit{Salmonella spp.}, \textit{Mycobacterium tuberculosis} and \textit{Neisseria gonorrhoea} other well-organised European surveillance systems are already in place, like Enternet (www.phls.co.uk/inter/enter-net/menu.htm) and EuroTB (www.eurotb.org).

**Results consensus meeting**

Having circulated the discussion paper on the rationale for selection of pathogens for comments before, consensus was reached relatively fast during the meeting. Realising that in the phase of establishing a surveillance programme not the most complex species should be selected, it was decided that \textit{S. pneumoniae} and \textit{S. aureus} are the most relevant species for the pilot phase in EARSS. In the future it is expected that EARSS will be a permanent surveillance system for the most public health relevant species and after the feasibility phase more species will be added.
In order to minimise sample bias, it was decided to test only *S. pneumoniae* isolates from blood and cerebral spinal fluid (CSF), and *S. aureus* isolates from blood for antimicrobial resistance. During the same meeting the protocols for antimicrobial testing of *S. aureus* and *S. pneumoniae* were developed.

**Protocol Staphylococcus aureus testing**

**Objective**
To study the (methicillin)-resistance of *S. aureus*, in blood isolates in hospitals in Europe.

**Case definition**
Resistance data on the first isolate only of each strain from the blood of each patient with a *S. aureus* infection (confirmed by a coagulase test). We exclude duplicate isolates of the same species from the same patient, and collect information only on the first isolate from each patient (“patient-isolate”).

**Test procedure**

1. Oxacillin screen plates (6 µg/ml according to NCCLS) or oxacillin disks (1 µg or 5 µg) will be used. When *S. aureus* is tested for oxacillin resistance a disk with a load of 1 µg oxacillin (NCCLS) is used; non-susceptibles are strains with a zone size of 10 mm or less (≤ 10 mm). When a disk with a load of 5 µg oxacillin (according to the French guidelines, SFM) for oxacillin susceptibility testing is used, non-susceptibles are isolates with a zone size of 19 mm or less (≤ 19 mm).

2. In the case of oxacillin non-susceptible *S. aureus*, the participating laboratories are asked additionally to determine the MIC for oxacillin (range of dilutions: 0.016-256) and MIC for vancomycin specifying the method used: agar dilution, microdilution or E-test (range of dilutions: 0.016 – 256). A participating country can decide whether the local laboratory will perform the second step of the protocol or that a ‘reference’ laboratory will collect the non-susceptible strains and perform the MIC for oxacillin and vancomycin.
Protocol *Streptococcus pneumoniae* testing

**Objective:**
To study the penicillin-resistance of *S. pneumoniae* blood- and CSF-isolates in Europe.

**Case definition**
Resistance data on the first isolate only from the blood or CSF of each patient with a *S. pneumoniae* infection (confirmed by an optochin test). We exclude duplicate isolates of the same species from the same patient, and collect information only on the first isolate from each patient ("patient-isolate").

**Test procedure**

1. For testing of *S. pneumoniae* an oxacillin disk (1 µg or 5 µg) will be used. When *S. pneumoniae* is tested, non-susceptible penicillin resistant *S. pneumoniae* are strains with a zone size of 20 mm or less (≤ 20 mm). An alternative in oxacillin susceptibility testing is a disk with a load of 5 µg oxacillin (according to the French guidelines, SFM). Non-susceptible penicillin resistant *S. pneumoniae* are isolates with a zone size of 26 mm or less (≤ 26 mm).

2. In the case of oxacillin non-susceptible *S. pneumoniae*, the participating laboratories are asked additionally to determine the MIC of penicillin, cefotaxime or ceftriaxone and ciprofloxacin, specifying the method used: agardilution, microdilution or E-test (range of dilutions: 0,016–256 (penicillin) or 0,002–32 (cefotaxime/ceftriaxone and ciprofloxacin)). A participating country can decide whether the local laboratory will perform the second step of the protocol or that a ‘reference’ laboratory will collect the non-susceptible strains and perform the MIC for penicillin, cefotaxime/ceftriaxone and ciprofloxacin.

**Discussion**
EARSS is designed in order to minimise epidemiological or microbiological difficulties as were summarised in the introduction:
1. **Different antimicrobial agents are tested.**

In EARSS, resistance for two species (*S. aureus* and *S. pneumoniae*) is tested against a restricted set of specified antimicrobials. The choice for oxacillin, instead of methicillin, for determination of MRSA (ORSA) is a practical one. Because methicillin is becoming less available in the near future, we think that oxacillin is a reliable alternative. For *S. pneumoniae*, testing of oxacillin, as a first step, in combination with a penicillin minimum inhibitory concentration (MIC) for non-susceptibles is now generally accepted.\(^5\) We believe that the introduction of a new generation of fluoroquinolones for the therapy of respiratory tract infections necessitates us to follow ciprofloxacin resistance in *S. pneumoniae*.

2. **Different systems for antimicrobial susceptibility testing are used.**

The protocols for *S. pneumoniae* and *S. aureus* are clearly defined. Next to a simple first line screening method, a second step, in which the MIC is determined, is included. Such a protocol combines easy accessibility with careful quantitative examination of antimicrobial resistance.

- For a reliable comparison of resistance against oxacillin in *S. aureus* oxacillin agar screen plates can be used.\(^6,7\) However, results from a survey among national coordinators illustrate that agar screen plates are only used in a few countries. Because one of the key features of EARSS is easy accessibility, the protocol will also accept data from the oxacillin disk diffusion test.\(^7,8\)

- The golden standard for confirmation of an MRSA is testing for the presence of the mecA-gene. However, when a participating laboratory is not able to perform a PCR, determination of a MIC for oxacillin (range of dilutions: 0.016-256) will be done to confirm that an MRSA is not false positive.

- Testing of MRSA for resistance against vancomycin is very relevant but under debate. Vancomycin intermediate resistant *S. aureus* (VISA) strains, which were first reported in Japan\(^9\), are often heterogeneously resistant. Only a very limited percentage of the total population of isolated bacteria is intermediately resistant.\(^9,10\) The presence of these VISA can be missed measuring a MIC under standard conditions. At this moment there is not an established protocol to test for VISA. We propose to test the MRSA for vancomycin using the E-test, with a standardised protocol which is also used testing the oxacillin MIC, realising that some intermediate VISA strains might be missed. The determination of the vancomycin MIC will preferably be done at a central ‘reference’ lab in each country. In case of finding a VISA strain, arrangements will be made for further analysis (e.g. sequence analysis).
3. **Different breakpoints for antimicrobial susceptibility are used.**

   Breakpoints are defined in the two protocols, according to US - National Committee for Clinical Laboratory Standards (NCCLS) or in some cases the Société Francaise de Microbiologie (SFM) guidelines.

   - For all *S. pneumoniae* and *S. aureus* isolates, we ask the participating laboratories to register the inhibition zone (in case of the disk method). The collection of zone diameters has an additional value in case medium and disk load are standardised. Firstly, zone diameters will give more insight in the distribution of *S. pneumoniae* or *S. aureus* strains with different susceptibilities to oxacillin, e.g. high resistance versus intermediate resistance.\(^{11}\) Secondly, the distribution of zone diameters may be used to study the quality of resistance data from different participating laboratories.\(^{12}\) It is acknowledged that laboratories in some participating countries are not able to collect zone diameters.

   - In the second step (MIC testing), the validity of categorising the strains as susceptible or non-susceptible, according to the SFM and the NCCLS guidelines, is evaluated. Correction of false positive (resistant) strains is possible by MIC testing.

   - Also a monthly testing of quality control strains assesses correct use of breakpoints for the categorisation of strains into susceptible and resistant.

4. **Data from point prevalence studies are used for longitudinal comparisons; e.g. studies on antibiotic resistance performed in 1970 and 1990 are compared, in spite of differences in study conditions and methodology.**

   For a longitudinal analysis on developments in resistance continuous data are essential. EARSS wants to provide continuous data that is generated according to a standardised protocol. Sudden increases or decreases in resistance percentages could be caused by changes in the surveillance method and should be closely monitored.

5. **Only the resistant strains are tested.**

   Selection of strains can easily occur in case of less invasive infections. For instance, antimicrobial susceptibility testing of Enterobacteriaceae from urinary tract infections depends on the response of the patient on the initial therapy. Resistance testing will be more likely performed when the patient returns after therapy failure. Resistance surveillance on basis of routine samples may overestimate the problem.\(^{13,14}\) In the EARSS pilot, sampling of the species is restricted to invasive isolates, which are routinely tested for antimicrobial susceptibility.
6. Resistance in local practices or general hospitals is compared to resistance in university hospitals.

In order to tackle this problem we asked the national co-ordinators to ensure reasonable coverage in their country. In case of the community-acquired pathogen, a coverage of more than 20% of the total national population is necessary. Participating laboratories are providing the national co-ordinator with information on the catchment population (the number of people living in the area they serve). In case of \textit{S. aureus} we believe that 20% of the total number of patient-days in every country is a minimum. Therefore, the participating laboratories provide the national co-ordinators with information on the number of patient-days of every hospital they serve. The national co-ordinator selects laboratories; not only laboratories that serve university hospitals but also laboratories, which serve small regional hospitals and general practitioners, are part of EARSS.

We believe that EARSS can evolve as a good framework to monitor antimicrobial resistance in the EU for the coming years. The first result of EARSS is that this system has activated several countries to establish or to update their national resistance surveillance system in order to follow national resistance patterns and to compare these to developments in Europe.

\textbf{Addendum}

At the third plenary EARSS meeting in November 2000 it was decided with all the national representatives to extend surveillance to three other species: \textit{E. coli}, \textit{E. faecium} and \textit{E. faecalis}. The protocol for testing for these species was agreed and most countries started data collection in January 2001.

The EARSS manual was updated accordingly and sent to the participating laboratories. Next to testing protocols the EARSS Manual 2001 provides an overview of the organisation and infrastructure of EARSS, and of data management.\textsuperscript{15} In annex it provides the data exchange format, updated isolate record forms, an updated laboratory/hospital questionnaire and a template Memorandum of Understanding between national EARSS representatives and participating laboratories.

In further chapters susceptibility results will be presented only for \textit{S. pneumoniae} and \textit{S. aureus}, for which data collection began in 1999.

\textbf{References}

2. Goldstein FW, Acar JF. Antimicrobial resistance among lower respiratory tract isolates of \textit{Streptococcus}


